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The influence of certain metal ions on the visible spectra of food dyes

A. V. JONES AND J. D. R. THOMAS

Summary. The visible absorption spectra of twenty-two food dyes have been plotted both in the free state and in the presence of added calcium, magnesium, aluminium, iron (II), iron (III), copper (II) and cobalt (II) ions at the pH values 3·0, 6·4, 7·4 and 12·5.

The frequencies of the absorption band maxima of the free dyes are briefly discussed in relation to colour and structure. While added metal ions do not, in general, have an appreciable effect on the spectrum of the dye, the transition metal ions studied frequently bring about a shift in the characteristic frequencies of the free-dye absorption maxima. Examples of where this effect is greatest are carmoisine in the presence of copper (II) at pH 6·4 and 7·4, and black PN in the presence of iron (II) at pH 12·5.

The possibility of dye complexes affecting iron metabolism is also briefly discussed.

Introduction

The essential requirements of a food dye have been listed by Minor (1962). Apart from the fact that the dye should not be injurious to health, it should also be fast to light and should withstand relatively high temperatures and variable conditions of acidity. Furthermore, it should not be affected by preservatives and other constituents of food.

The bleaching effect of sunlight on dyes is well known, and of the food dyes permitted in the United Kingdom (Her Majesty's Stationery Office, 1966), indigo carmine is particularly susceptible to such spoilage. On the other hand, a search of the literature reveals that relatively little attention has been given to a systematic study of the behaviour of food dyes under various conditions although, of course, a great deal is known, and can be predicted about the breakdown characteristics of individual dyes. A number of investigations on the metabolic fate of several food dyes have been carried out over recent years (Radomski & Diechmann, 1956; Koether, 1960; Daniel, 1962; Manchon, 1965), and with regard to their possible denaturing prior to ingestion, Lueck (1965) has studied the effect of heat, reducing agents and

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oxidizing agents on a selected range. Thus, black PN, cochineal red A, ponceau 6R and yellow 27175N were found to be unstable to boiling and sterilization under certain conditions of pH. Most of the eighteen dyes studied were affected by ascorbic acid and sodium sulphite.

While in Lueck's (1965) study 0·06% hydrogen peroxide was found to be without effect, it is well known that certain oxidizing agents denature food dyes, for example, amaranth and ponceau 4R are destroyed by excess of oxidant when they are used as indicators in oxidation titrations involving potassium bromate and potassium iodate (Belcher & Nutten, 1955; Vogel, 1962).

Azo dyes have been the centre of intense interest in the search for possible indicators suitable for use in complexometric titrations (Close & West, 1960a, b), but despite a similarity between many of the compounds investigated and the food dyes containing azo groupings, the investigations have not included the food dyes. The same is also true of investigations in the search for indicators among the triphenylmethane dyes (Brazier & Stephen, 1965).

In the light of the above there is a paucity of systematic information on the behaviour of food dyes in the presence of metal ions normally encountered in the practice of food technology and of food preparation. The present investigation has, therefore, been directed to an examination of the influence over a wide pH range of varying concentrations of calcium, magnesium, aluminium, cobalt (II), iron (II), iron (III) and copper (II) ions on the colours of most of the coal tar food dyes permitted in the United Kingdom, as well as some of those that have recently been deleted from the permitted list (Her Majesty's Stationery Office, 1957, 1966), that is, ponceau SX, yellow RY, yellow RFS and blue VRS.

Experimental

Materials

The dyes studied were of the 'Hexacol' range and were kindly donated by L. J. Pointing and Son Ltd, Hexham, Northumberland.

The metal ion-containing solutions were prepared from the appropriate AnalaR sulphate except for iron (III), when the alum was used, and calcium, where the solution was prepared by dissolving AnalaR calcium carbonate in the minimum quantity of hydrochloric acid.

Procedure

A range of solutions was prepared for each metal ion and each dye by mixing varying volumes of aqueous 0·0005 M metal ion-containing solution, 5 ml aqueous 0·0005 M dye solution, the appropriate volume of a suitable reagent to ensure the required conditions of pH, followed by de-ionized water to give a total volume of 50 ml. The absorption spectra of these solutions were recorded with a recording spectrophoto-

meter. Spectra of solutions containing 40 ml of the metal ion-containing solution but no dye were also recorded, but the absorption was minimal and, therefore, ignored.

Solutions of pH 3·0 were obtained by adjustment with about 5 ml glacial acetic acid; those of pH 12·5 by adjustment with about 5 ml diethylamine; those of pH 7·4 by adjustment with about 10 ml of a Sörensen type buffer solution prepared from 80·0 ml of 0·200 M disodium hydrogen phosphate and 20·0 ml of 0·0667 M potassium dihydrogen phosphate and, finally, solutions of pH 6·4 were obtained by adjustment with about 10 ml of a similar buffer solution prepared from 30·0 ml 0·200 M disodium hydrogen phosphate and 70·0 ml 0·0667 M potassium dihydrogen phosphate.

Results

Table 1 summarizes the frequencies (cm^{-1}) of the absorption band maxima observed between about 25,000 cm^{-1} (400 nm) and 12,500 cm^{-1} (800 nm) together with the optical densities for the free dyes (5 ml aqueous 0·0005 M dye solution made up to 50 ml as described above) and for the dyes in the presence of excess metal ions (5 ml aqueous 0·0005 M dye solution + 40 ml 0·0005 M metal ion-containing solution (35 ml at pH 6·4 and 7·4) made up to 50 ml as described above). Where the absorption band maxima of the free dye and of the dye in the presence of excess metal ions differ by a frequency of greater than 800 cm^{-1} (that is, a wavelength of about 20 nm at 500 nm), the figures recorded in the latter case are shown in italics in Table 1.

Variations in frequency of the absorption band maxima of the dye may be brought about by added metal ions due to complex formation. An estimate of the structure and stability of the resulting complex may be made by Job's (1928) method of continuous variation as used by Close & West (1960a, b). This kind of calculation involving a plot of the differences in optical densities between the complex and the estimated amounts of non-complexed dye against solution composition has been made in a limited number of cases and the results are summarized in Table 2.

Discussion

Features of the spectra of dye solutions

The principal characteristics of the absorption spectra of solutions of the dyes naturally follow a pattern according to the colour imparted by the solution. Thus, the yellow dyes show absorption bands in the region of 23,000 cm^{-1} (435 nm) to 25,000 cm^{-1} (400 nm), the orange dyes in the region of 21,000 cm^{-1} (476 nm) with a further band at 24,000 cm^{-1} (417 nm) to 25,000 cm^{-1} (400 nm), while the red dyes have absorption bands at 19,000 cm^{-1} (526 nm) to 20,000 cm^{-1} (500 nm). The differences observed in the absorption band maxima for dyes of the same colour match differences in hue, for example, the red dyes tending towards an orange hue have absorption bands nearer to 20,000 cm^{-1} (500 nm) while those of a pronounced deep red have their absorption bands nearer 19,000 cm^{-1} (526 nm).

TABLE 1. Absorption band maxima (in 10^{-3} cm^{-1}) and optical densities (in parentheses) at various conditions of pH and in the presence of certain metal ions

Dye	pH 3·0	pH 6·4	pH 7·4	pH 12·5	pH 3·0	pH 6·4	pH 7·4	pH 12·5
	← Free dye →				← Dye in the presence of excess copper (II) ions →			
1-Napthyl-azo-phenyl dyes								
Orange G	*25·0 (0·51)	*24·5 (0·50)	*25·0 (0·50)	25·2 (0·58)	*24·8 (0·51)	*24·4 (0·50)	*25·0 (0·49)	--
	20·8 (0·89)	20·8 (0·88)	21·0 (0·88)	20·2 (0·49)	20·8 (0·88)	20·8 (0·86)	21·0 (0·87)	*21·0 (0·62)*
Orange RN	†24·2 (0·56)	†23·8 (0·56)	24·8 (0·56)	23·0 (0·62)	†24·2 (0·50)	†23·8 (0·55)	*26·4 (0·58)*	--
	20·8 (0·87)	20·3 (0·87)	21·0 (0·87)	†20·0 (0·52)	20·7 (0·87)	20·3 (0·84)	21·2 (0·84)	†*20·8 (0·74)*
								17·6 (0·66)
Sunset Yellow FCF	*24·2 (0·61)	*23·8 (0·60)	*24·4 (0·61)	22·2 (0·74)	*24·4 (0·57)	*23·8 (0·63)	†24·8 (0·57)	—
	21·0 (0·91)	20·4 (0·89)	21·0 (0·91)	*20·0 (0·60)	21·0 (0·88)	20·6 (0·87)	20·8 (0·86)	22·2 (0·71)
Ponceau MX	25·8 (0·43)	*25·6 (0·45)	25·8 (0·39)	—	25·6 (0·44)	*25·0 (0·55)	26·0 (0·40)	—
	19·6 (0·88)	19·7 (0·88)	20·4 (0·87)	20·0 (0·69)	19·6 (0·88)	19·8 (0·85)	20·2 (0·86)	*21·6 (0·67)*
Chocolate Brown HT	21·6 (0·86)	21·2 (0·85)	21·6 (0·86)	21·2 (0·91)	21·6 (0·86)	21·4 (0·83)	21·6 (0·80)	21·2 (0·81)
1-Naphthyl-azo-1-naphthyl dyes								
Ponceau 4R	20·0 (0·99)	19·4 (0·88)	20·0 (1·00)	20·6 (0·93)	19·6 (1·00)	19·4 (0·85)	19·6 (1·00)	22·0 (0·95)
Amaranth	19·0 (0·92)	19·0 (0·93)	19·6 (0·91)	20·2 (0·79)	19·0 (0·92)	19·2 (0·83)	19·6 (0·85)	*21·0 (0·75)*
Fast Red E	19·6 (0·86)	19·7 (0·85)–	20·2 (0·84)	21·0 (0·72)	19·8 (0·86)	19·7 (0·79)	19·9 (0·75)	21·4 (0·69)
1-Naphthyl-azo-2-naphthyl dyes								
Carmoisine	†24·6 (0·60)	†24·4 (0·40)	—	—	†24·8 (0·61)	—	—	—
	19·4 (0·98)	19·2 (0·90)	19·6 (0·88)	19·6 (0·98)	19·4 (0·98)	*20·8 (0·66)*	*21·6 (0·63)*	19·6 (0·98)
Black PN	24·4 (0·64)	24·3 (0·66)	24·0 (0·65)	25·8 (0·72)	24·4 (0·64)	24·3 (0·66)	24·0 (0·56)	25·6 (0·73)
	17·6 (0·98)	17·2 (0·97)	17·2 (0·96)	17·6 (0·89)	17·6 (0·96)	17·2 (0·92)	17·6 (0·95)	17·6 (0·90)
2-Naphthyl-azo-phenyl dyes								
Ponceau SX	20·0 (0·92)	19·7 (0·91)	20·0 (0·89)	21·0 (0·84)	20·0 (0·91)	20·0 (0·36)	20·6 (0·70)	20·8 (0·86)
Red 10B	19·0 (0·92)	18·7 (0·90)	19·0 (0·91)	18·8 (0·89)	19·0 (0·92)	18·5 (0·91)	19·4 (0·91)	18·8 (0·91)
Red 2G	20·0 (0·95)	19·8 (0·95)	20·0 (0·95)	21·8 (0·80)	20·0 (0·95)	19·8 (0·95)	20·0 (0·92)	21·8 (0·77)
	19·0 (0·95)	18·7 (0·95)	19·0 (0·95)		19·0 (0·95)	18·7 (0·95)	19·0 (0·92)	
Red 6B	19·0 (0·93)	18·9 (0·94)	19·0 (0·90)	21·6 (0·83)	19·0 (0·94)	18·9 (0·94)	19·4 (0·89)	21·6 (0·83)
(a) Red FB	19·3 (0·49)	19·6 (0·49)	19·6 (0·49)	18·8 (0·48)	19·3 (0·50)	19·6 (0·45)	19·6 (0·36)	18·5 (0·49)
Phenyl-azo-phenyl dyes								
Yellow RY	23·0 (0·89)	22·7 (0·88)	23·0 (0·86)	22·0 (0·83)	23·0 (0·90)	22·7 (0·86)	23·0 (0·86)	*23·0 (0·78)*
Yellow RFS	24·0 (0·83)	23·3 (0·84)	25·0 (0·81)	24·0 (0·86)	24·0 (0·82)	23·2 (0·85)	24·8 (0·82)	24·0 (0·93)

Phenyl-azo-pyrazole dyes								
Yellow 2G	24·6 (0·91)	24·7 (0·91)	24·8 (0·89)	25·0 (0·90)	24·6 (0·91)	24·8 (0·90)	24·8 (0·88)	25·2 (0·88)
Tartrazine	23·0 (0·91)	23·3 (0·91)	23·8 (0·91)	25·0 (0·84)	23·2 (0·91)	23·3 (0·91)	23·6 (0·92)	25·0 (0·84)
Triphenyl-methanol anhydride dyes								
Blue VRS	24·4 (0·93)	24·0 (0·74)	24·0 (0·74)	24·4 (0·43)	24·4 (0·90)	24·0 (0·77)	24·4 (0·79)	24·4 (0·66)
	16·0 (0·99)	15·5 (1·00)	16·0 (1·00)	16·0 (0·97)	16·0 (0·98)	15·2 (1·00)	16·0 (1·00)	16·0 (1·00)
Green S	25·0 (0·50)	24·9 (0·47)	25·0 (0·54)	25·0 (0·64)	25·0 (0·52)	24·8 (0·52)	25·2 (0·56)	*26·6 (0·55)*
	22·8 (0·48)	22·5 (0·42)	22·8 (0·37)	—	22·8 (0·47)	22·2 (0·46)	22·8 (0·40)	—
	16·0 (1·00)	15·4 (0·90)	16·0 (1·00)	16·4 (0·99)	16·0 (0·98)	15·5 (1·00)	16·4 (1·00)	16·4 (0·99)
Violet BNP	†18·0 (0·98)	18·0 (0·96)	18·8 (0·96)	†18·4 (0·78)	†18·0 (0·98)	†18·0 (0·93)	18·4 (0·98)	—
	17·0 (0·98)	†16·9 (0·96)	17·8 (0·95)	17·0 (0·85)	16·8 (0·98)	16·8 (0·97)	†17·4 (0·98)	16·8 (0·10)
	← Dye in presence of excess iron (II) ions →				← Dye in presence of excess iron (III) ions →			
1-Naphthyl-azo-phenyl dyes								
Orange G	*25·0 (0·51)	—	*24·4 (0·49)	—	—	—	*23·8 (0·57)*	—
	20·8 (0·89)	20·8 (0·96)	20·8 (0·85)	*21·0 (0·60)*	20·8 (0·97)	20·8 (0·92)	20·6 (0·89)	20·8 (0·62)
Orange RN	†24·4 (0·58)	*24·8 (0·60)*	25·2 (0·59)	†*24·0 (0·78)*	24·4 (0·60)	24·7 (0·52)	24·7 (0·53)	23·0 (0·79)
	20·5 (0·89)	20·4 (0·87)	20·8 (0·84)	—	20·4 (0·89)	20·6 (0·94)	20·4 (0·85)	*19·8 (0·56)
Sunset Yellow FCF	*24·0 (0·65)	†23·9 (0·57)	*24·0 (0·62)	**24·0 (0·94)*	*23·8 (0·60)	*23·8 (0·63)	*24·0 (0·56)	22·5 (0·78)
	20·6 (0·91)	20·6 (0·83)	20·8 (0·91)	—	20·6 (0·89)	20·6 (0·89)	20·4 (0·26)	*20·0 (0·66)
Ponceau MX	25·6 (0·43)	—	25·8 (0·47)	—	*25·3 (0·96)	—	—	—
	19·8 (0·78)	19·8 (0·95)	20·0 (0·85)	20·2 (0·74)	19·6 (0·88)	19·7 (0·91)	19·7 (0·89)	19·6 (0·74)
Chocolate Brown HT	21·4 (0·85)	21·3 (0·88)	22·0 (0·86)	21·4 (0·90)	21·3 (0·88)	21·4 (0·85)	21·3 (0·81)	21·3 (0·92)
1-Naphthyl-azo-1-naphthyl dyes								
Ponceau 4R	19·6 (0·99)	19·8 (0·82)	20·0 (1·00)	**23·0 (0·96)*	19·4 (0·88)	19·4 (0·89)	19·6 (0·84)	20·6 (0·65)
Amaranth	19·0 (0·92)	19·0 (0·92)	19·6 (0·87)	20·4 (0·81)	*24·4 (0·38)* 18·9 (0·95)	*20·0 (0·94)*	19·3 (0·93)	20·7 (0·96)
Fast Red E	20·0 (0·84)	19·7 (0·82)	19·9 (0·80)	†*25·0 (0·84)*	19·2 (0·86)	19·6 (0·81)	19·6 (0·98)	20·6 (0·79)
1-Naphthyl-azo-2-naphthyl dyes								
Carmoisine	*24·4 (0·60)	—	—	—	25·0 (0·53)	—	—	—
	19·3 (0·98)	19·2 (0·87)	20·0 (0·86)	19·6 (0·98)	18·8 (0·92)	19·2 (0·94)	19·2 (0·88)	20·2 (0·98)
Black PN	24·4 (0·64)	—	24·0 (0·60)	†*24·4 (0·84)*	24·4 (0·67)	24·3 (0·67)	24·3 (0·60)	25·3 (0·67)
	17·4 (0·98)	17·4 (0·98)	17·6 (0·96)	†*20·6 (0·83)*	17·2 (0·98)	17·2 (0·97)	17·2 (0·95)	17·2 (0·91)

TABLE 1 (*Continued*)

Dye	pH 3·0	pH 6·4	pH 7·4	pH 12·5	pH 3·0	pH 6·4	pH 7·4	pH 12·5
2-Naphthyl-azo-phenyl dyes								
Ponceau SX	20·0 (0·84)	19·6 (0·90)	20·2 (0·87)	21·4 (0·93)	19·7 (0·91)	19·7 (0·89)	19·8 (0·92)	20·6 (0·87)
Red 10B	19·2 (0·93)	18·9 (0·89)	19·0 (0·88)	19·0 (0·91)	*24·0 (0·48) 18·5 (0·92)	18·7 (0·90)	18·7 (0·91)	19·0 (0·95)
Red 2G	20·0 (0·95) 19·0 (0·95)	19·5 (0·95) 18·7 (0·95)	20·0 (0·93) 19·0 (0·93)	21·9 (0·84)	20·5 (0·96) 18·5 (0·96)	19·6 (0·95)	19·5 (0·92) 18·7 (0·92)	*20·0 (0·87)*
Red 6B	19·0 (0·94)	18·9 (0·92)	19·4 (0·93)	†*22·8 (0·93)*	18·5 (0·96)	18·9 (0·94)	19·0 (0·91)	21·0 (0·85)
(a) Red FB	19·5 (0·47)	†*20·6 (0·69)*	19·6 (0·55)	*20·4 (0·66)*	19·7 (0·53)	19·6 (0·52)	19·6 (0·52)	†*20·4 (0·66)*
Phenyl-azo phenyl dyes								
Yellow RY	22·8 (0·89)	†*25·5 (0·97)*	22·8 (0·82)	*23·8 (0·89)*	23·3 (0·83) *19·6 (0·80)*	22·6 (0·89)	22·5 (0·89)	†*26·0 (0·95)*
Yellow RFS	23·6 (0·77)	*24·9 (0·93)*	24·8 (0·81)	*24·8 (0·95)*	*22·9 (0·87)*	*24·4 (0·89)*	24·7 (0·71)	—
Phenyl-azo-pyrazole dyes								
Yellow 2G	24·4 (0·91)	—	24·8 (0·88)	*25·8 (0·96)*	†24·9 (0·95)	*25·5 (0·92)*	25·0 (0·92)	†*26·3 (0·97)*
Tartrazine	23·6 (0·90)	23·2 (0·90)	23·6 (0·87)	†*23·0 (0·99)*	22·7 (0·90)	23·8 (0·98)	23·2 (0·92)	†25·2 (0·92)
Tri-phenyl-methanol anhydride dyes								
Blue VRS	24·4 (0·93) 15·6 (0·95)	24·3 (0·92) 15·8 (1·00)	24·6 (0·68) 16·0 (1·00)	*26·6 (0·96) 16·0 (1·00)	24·3 (0·95) 15·4 (0·97)	*23·0 (0·79)* 15·3 (1·00)	24·2 (0·75) 15·3 (1·00)	*24·0 (0·91) 15·6 (0·90)
Green S	24·0 (0·45) 22·8 (0·43) 16·0 (0·92)	— — 15·5 (0·99)	25·2 (0·56) *22·8 (0·48) 16·0 (0·99)	— — 16·4 (0·97)	— *22·7 (0·56) 15·6 (1·00)	— — 15·4 (1·00)	24·9 (0·57) *23·0 (0·42) 15·9 (1·00)	— — 16·0 (0·99)
Violet BNP	†18·0 (0·98) 17·0 (0·98)	— 18·3(0·90)	18·4 (0·96) †17·6 (0·96)	†18·4 (0·62) 17·0 (0·69)	†18·0 (0·98) 16·7 (0·90)	18·0 (0·92) 16·7 (0·98)	18·1 (0·99) †16·7 (0·98)	*17·4 (0·88)* 16·5 (0·90)
	← Dye in the presence of excess cobalt (II) ions →				← Dye in the presence of excess aluminium (III) ions →			
1-Naphthyl-azo-phenyl dyes								
Orange G	*24·7 (0·67) 20·8 (0·87)	*23·8 (0·50) 20·8 (0·88)	*25·2 (0·50) 21·0 (0·89)	25·0 (0·66) 20·3 (0·56)	*24·7 (0·67) 20·8 (0·87)	*24·0 (0·53) 20·8 (0·88)	*25·0 (0·51) 21·2 (0·89)	25·0 (0·55) 20·0 (0·51)
Orange RN	24·4 (0·38) 20·4 (0·88)	†23·8 (0·56) †20·3 (0·87)	25·0 (0·56) 21·2 (0·87)	22·8 (0·72) *19·8 (0·56)	24·4 (0·38) 20·4 (0·88)	†23·8 (0·56) 20·3 (0·87)	24·6 (0·57) 21·2 (0·88)	22·7 (0·66) †19·8 (0·56)

Sunset Yellow FCF	*23·8 (0·58) 20·62 (0·89)	*23·8 (0·60) 20·4 (0·89)	*24·6 (0·59) 21·2 (0·91)	22·0 (0·76) *20·0 (0·66)	*23·8 (0·58) 20·6 (0·89)	*23·8 (0·60) 20·4 (0·89)	*24·6 (0·63) 21·4 (0·91)	22·1 (0·70) *20·0 (0·60)
Ponceau MX	19·6 (0·88)	19·7 (0·88)	20·0 (0·95)	*23·7 (0·40)* 19·3 (0·59)	19·6 (0·88)	19·8 (0·88)	20·2 (0·88)	19·5 (0·68)
Chocolate Brown HT	21·3 (0·85)	21·2 (0·85)	21·6 (0·86)	21·2 (0·87)	21·3 (0·85)	21·2 (0·85)	21·8 (0·84)	21·2 (0·85)
1 Naphthyl-azo-1-naphthyl dyes								
Ponceau 4R	19·4 (0·88)	19·4 (0·88)	20·0 (1·00)	20·0 (0·62)	19·4 (0·87)	19·4 (0·88)	20·0 (1·00)	20·0 (0·62)
Amaranth	18·9 (0·92)	19·0 (0·93)	19·6 (0·91)	20·0 (0·67)	18·9 (0·92)	18·9 (0·93)	19·6 (0·91)	19·5 (0·73)
Fast Red E	19·2 (0·87)	19·7 (0·85)	20·2 (0·84)	20·8 (0·71)	19·2 (0·87)	19·9 (0·85)	19·6 (0·80)	20·8 (0·71)
1-Naphthyl-azo-2-naphthyl dyes								
Carmoisine	*24·0 (0·40) 18·8 (0·92)	†24·4 (0·40) 19·2 (0·90)	— 19·8 (0·84)	— 19·6 (1·00)	*24·0 (0·40) 18·8 (0·92)	†24·4 (0·40) 19·2 (0·92)	— 19·6 (0·98)	— 19·0 (0·89)
Black PN	24·3 (0·65) 17·2 (0·98)	24·3 (0·66) 17·2 (0·97)	23·8 (0·66) 17·4 (0·95)	25·3 (0·78) 17·3 (0·91)	24·3 (0·63) 17·2 (0·98)	24·3 (0·66) 17·2 (0·97)	24·4 (0·66) 17·8 (0·96)	25·4 (0·74) 17·2 (0·90)
2-Naphthyl-azo-phenyl dyes								
Ponceau SX	19·7 (0·91)	19·7 (0·91)	20·0 (0·87)	20·6 (0·86)	19·7 (0·91)	19·7 (0·91)	20·2 (0·91)	20·6 (0·86)
Red 10B	*23·6 (0·34) 19·0 (0·92)	23·6 (0·40) 18·5 (0·91)	19·4 (0·89)	19·0 (0·80)	23·6 (0·34) 19·0 (0·92)	18·5 (0·91)	19·0 (0·91)	18·5 (0·89)
Red 2G	20·0 (0·96) 18·5 (0·96)	20·0 (0·95) 19·0 (0·95)	20·6 (0·95) 19·0 (0·95)	21·7 (0·86)	20·0 (0·96) 18·5 (0·96)	18·5 (0·95) 20·0 (0·95)	20·0 (0·95) 19·0 (0·95)	21·7 (0·76)
Red 6B	18·5 (0·96)	18·9 (0·94)	19·6 (0·93)	20·8 (0·82)	18·5 (0·96)	18·9 (0·94)	19·6 (0·94)	20·8 (0·78)
(a) Red FB	19·3 (0·49)	19·6 (0·49)	19·6 (0·45)	18·0 (0·50)	19·3 (0·49)	19·6 (0·49)	19·6 (0·49)	18·8 (0·49)
Phenyl-azo-phenyl dyes								
Yellow RY	24·4 (0·75)	22·7 (0·88)	23·0 (0·86)	24·0 (0·88)	24·0 (0·79)	22·5 (0·89)	22·8 (0·86)	24·3 (0·85)
Yellow RFS	24·4 (0·70) *19·6 (0·62)	23·2 (0·85)	25·0 (0·84)	24·4 (0·78)	24·4 (0·70)	24·3 (0·88)	24·8 (0·84)	24·4 (0·78)
Phenyl-azo-pyrazole dyes								
Yellow 2G	24·4 (0·90)	24·7 (0·91)	25·0 (0·97)	24·8 (0·91)	24·4 (0·90)	24·7 (0·91)	25·0 (0·89)	25·0 (0·88)
Tartrazine	23·0 (0·90)	23·3 (0·91)	23·4 (0·91)	24·8 (0·90)	22·7 (0·90)	23·3 (0·92)	23·6 (0·91)	24·9 (0·87)
Tri-phenyl-methanol anhydride dyes								
Blue VRS	23·9 (0·82) 15·4 (0·98)	24·0 (0·75) 15·3 (1·00)	24·4 (0·79) 16·0 (1·00)	24·2 (0·80) 15·1 (1·00)	24·4 (0·91) 15·8 (0·98)	24·1 (0·74) 15·3 (1·00)	24·6 (0·79) 16·4 (1·00)	24·0 (0·75) 15·1 (0·97)

TABLE 1 (*Continued*)

Dye	pH 3·0	pH 6·4	pH 7·4	pH 12·5	pH 3·0	pH 6·4	pH 7·4	pH 12·5
Green S	24·9 (0·50) 22·3 (0·44) 15·6 (1·00)	25·0 (0·50) 22·3 (0·44) 15·4 (0·99)	25·2 (0·55) 22·8 (0·45) 16·2 (1·00)	24·9 (0·72) — 16·0 (0·99)	24·8 (0·54) *23·0 (0·45) 15·4 (1·00)	24·9 (0·50) 22·4 (0·45) 15·6 (0·99)	25·0 (0·52) — 16·0 (1·00)	24·9 (0·64) — 15·9 (0·99)
Violet BNP	*23·6 (0·16) 16·8 (0·98)	18·0 (0·96) †16·4 (0·96)	18·4 (0·96) †17·2 (0·95)	16·7 (0·15) —	*23·5 (0·15) 17·0 (0·77)	18·4 (0·96) †16·8 (0·96)	18·8 (0·97) †18·0 (0·95)	16·4 (0·82) —
	← Dye in the presence of excess calcium ions →				← Dye in the presence of excess magnesium ions →			
1-Naphthyl-azo-phenyl dyes								
Orange G	*25·0 (0·50) 20·8 (0·90)	*23·9 (0·50) 20·7 (0·88)	*24·8 (0·51) 21·0 (0·88)	25·4 (0·59) 20·2 (0·52)	*25·0 (0·52) 20·7 (0·90)	*23·9 (0·50) 20·6 (0·88)	*24·8 (0·50) 21·0 (0·88)	25·4 (0·59) 20·4 (0·52)
Orange RN	†24·2 (0·56) 20·5 (0·88)	†23·8 (0·56) 20·3 (0·87)	†24·6 (0·56) 21·4 (0·88)	23·3 (0·60) †20·5 (0·51)	†24·4 (0·57) 20·8 (0·87)	†23·8 (0·56) 20·3 (0·87)	†24·6 (0·56) 21·6 (0·88)	23·0 (0·61) †20·4 (0·54)
Sunset Yellow FCF	*24·4 (0·63) 21·0 (0·91)	*23·8 (0·60) 20·4 (0·89)	*24·0 (0·61) 20·8 (0·91)	— 22·2 (0·76)	*24·4 (0·63) 20·8 (0·91)	*23·8 (0·60) 20·4 (0·89)	*24·2 (0·61) 20·8 (0·91)	— 20·2 (0·74)
Ponceau MX	20·0 (0·88)	19·7 (0·87)	20·0 (0·87)	20·0 (0·64)	19·8 (0·89)	19·6 (0·87)	20·2 (0·95)	19·9 (0·70)
Chocolate Brown HT	21·6 (0·86)	21·2 (0·85)	22·0 (0·85)	21·2 (0·87)	21·3 (0·85)	21·2 (0·85)	21·6 (0·85)	21·2 (0·85)
1-Naphthyl-azo-1-naphthyl dyes								
Ponceau 4R	19·4 (1·00)	19·4 (0·88)	20·0 (1·00)	20·6 (0·95)	19·6 (0·98)	19·4 (0·88)	20·0 (1·00)	20·8 (0·94)
Amaranth	19·0 (0·92)	19·0 (0·93)	19·8 (0·91)	20·4 (0·76)	19·4 (0·91)	18·7 (0·93)	19·6 (0·91)	20·0 (0·77)
Fast Red E	19·6 (0·87)	19·7 (0·83)	20·4 (0·84)	21·0 (0·73)	19·8 (0·85)	19·7 (0·85)	20·0 (0·84)	21·2 (0·71)
1-Naphthyl-azo-2-naphthyl dyes								
Carmoisine	†24·6 (0·60) 19·4 (0·98)	†24·4 (0·40) 19·2 (0·90)	— 19·8 (0·87)	— 19·8 (0·97)	†24·4 (0·59) 19·2 (0·98)	†24·4 (0·40) 19·2 (0·90)	— 19·6 (0·90)	— 19·6 (0·96)
Black PN	24·4 (0·63) 17·6 (0·98)	24·3 (0·66) 17·2 (0·97)	24·0 (0·66) 17·6 (0·96)	— 17·6 (0·91)	24·5 (0·63) 17·9 (0·98)	24·3 (0·66) 17·2 (0·97)	24·0 (0·63) 17·4 (0·96)	25·6 (0·80) 17·6 (0·95)
2-Naphthyl-azo-phenyl dyes								
Ponceau SX	19·8 (0·91)	19·7 (0·91)	20·4 (0·91)	20·8 (0·85)	20·0 (0·91)	19·7 (0·91)	20·0 (0·91)	20·8 (0·85)
Red 10B	19·2 (0·92)	18·4 (0·91)	19·4 (0·91)	19·0 (0·90)	19·0 (0·91)	24·8 (0·39) 18·8 (0·91)	19·6 (0·91)	19·0 (0·90)
Red 2G	20·0 (0·95) 19·0 (0·95)	20·0 (0·95) 19·0 (0·95)	20·0 (0·95) 19·0 (0·95)	21·6 (0·78)	20·0 (0·95) 19·0 (0·95)	20·0 (0·95) 19·0 (0·95)	20·0 (0·95) 19·0 (0·95)	21·8 (0·77)

Red 6B	19·0 (0·94)	18·9 (0·94)	19·4 (0·94)	21·6 (0·75)	18·8 (0·94)	18·9 (0·94)	19·4 (0·94)	21·0 (0·76)
(a) Red FB	19·6 (0·49)	19·6 (0·49)	19·6 (0·49)	18·8 (0·48)	19·6 (0·49)	19·6 (0·49)	19·6 (0·49)	18·8 (0·48)
Phenyl-azo-phenyl dyes								
Yellow RY	22·6 (0·90)	22·5 (0·89)	23·2 (0·86)	22·4 (0·81)	22·6 (0·90)	22·6 (0·89)	23·2 (0·86)	22·0 (0·80)
Yellow RFS	24·0 (0·78)	23·0 (0·84)	25·0 (0·81)	24·0 (0·87)	23·6 (0·78)	23·3 (0·85)	24·8 (0·81)	23·8 (0·95)
Phenyl-azo-pyrazole dyes								
Yellow 2G	24·8 (0·91)	24·7 (0·91)	24·6 (0·90)	25·0 (0·88)	24·6 (0·91)	24·7 (0·91)	24·8 (0·90)	25·2 (0·96)
Tartrazine	22·4 (0·90)	23·3 (0·91)	23·6 (0·91)	25·0 (0·86)	22·9 (0·91)	23·8 (0·92)	23·8 (0·91)	25·0 (0·91)
Tri-phenyl-methanol-anhydride dyes								
Blue VRS	24·6 (0·91)	24·0 (0·74)	24·4 (0·68)	24·2 (0·73)	24·4 (0·90)	24·4 (0·68)	24·4 (0·68)	24·4 (0·73)
	16·0 (0·98)	15·8 (1·00)	16·0 (1·00)	15·8 (1·00)	15·9 (0·98)	16·0 (1·00)	16·0 (1·00)	16·0 (0·98)
Green S	25·0 (0·54)	24·9 (0·50)	25·0 (0·52)	25·6 (0·57)	25·0 (0·44)	25·0 (0·52) †22·7 (0·38)	25·0 (0·53)	25·0 (0·53)
	23·0 (0·49)	22·2 (0·44)	†22·7 (0·38)	—	22·6 (0·49)	—	†22·8 (0·35)	—
	16·0 (1·00)	15·2 (0·99)	16·1 (0·98)	16·2 (0·97)	16·0 (0·93)	16·1 (0·98)	16·1 (1·00)	16·4 (1·00)
Violet BNP	†18·0 (0·98)	18·0 (0·96)	18·4 (0·96)	—	†18·0 (0·98)	18·0 (0·96)	18·4 (0·96)	—
	17·0 (0·98)	†16·7 (0·96)	†17·2 (0·95)	17·0 (0·18)	16·4 (0·97)	†16·7 (0·96)	†17·2 (0·95)	17·0 (0·17)

(a) The data for Red FB were obtained with solutions diluted, in all cases, by a factor of five.

Data in italics relate to absorption band maxima in the presence of metal ions removed by 800 cm^{-1}, or more, from those observed for the free dyes.

* Inflexion. † Shoulder.

TABLE 2. Stability constants of selected metal–dye complexes

pH	Metal	Dye	Metal–dye ratio in complex	Stability constant of complex
12·5	Iron(II)	Ponceau 4R	2 : 1	6×10^{11}
12·5	Iron(III)	Amaranth	1 : 1	3×10^{6}
7·4	Copper(II)	Carmoisine	1 : 2	2×10^{11}
12·5	Copper(II)	Yellow RY	2 : 1	8×10^{11}

The four dyes that have absorption bands further towards the red end of the spectrum possess, as expected, bluish characteristics in their colours. Thus, violet BNP and black PN have absorption maxima at around 17,000 cm^{-1} (588 nm) while blue VRS and green S have their maxima near 16,000 cm^{-1} (625 nm).

The colours and, hence, absorption spectra of the dyes studied, fall into a pattern according to structure of the dye molecule. Thus, a yellow coloration is characteristic of the phenyl-azo-phenyl and the phenyl-azo-pyrazole dyes. On the other hand, 1- and 2-naphthyl-azo dyes, with molecules of more enhanced bathochromic characteristics, may generally be distinguished by their red colours.

There are, however, several exceptions to the generality of the red character for the naphthyl-azo dyes. For example, the orange dyes, namely orange G, orange RN and sunset yellow FCF, which belong to the phenyl-azo-naphthyl group, do not appear to carry substituents of potential bathochromic character as do the other dyes belonging to this group. Another exception is black PN which brings out the bathochromic characteristics of the extended conjugation brought about by the favourable position of its extra azo grouping and absorbs at lower frequencies than the red dyes. Chocolate brown HT also has two azo linkages, but due to their unfavourable position, an extended conjugation is not possible and hence, with its two apparently independent phenyl-azo-naphthyl halves, its absorption maximum at around 21,200 cm^{-1} (472 nm) is close to that observed for the orange dyes.

The triphenylmethanol anhydride dyes, namely, blue VRS, green S and violet BNP are characteristic of their class and all show absorptions well on the low frequency side of those of the red dyes.

Except at pH 12·5, the effect of pH on the frequency of the absorption band is not great. At pH 12·5, however, there is a distinct tendency for the absorption band to be shifted to a slightly different frequency. For example, this is evidenced by the orange colour of red 6B and red 2G at this pH.

The only other feature of the spectra of the dye solutions that calls for comment is that with the exception of red FB, the optical densities of the bands responsible for the colours do not vary appreciably (only by a factor of two or three) in passing from

one dye to the next. However, there is a tendency for the optical densities of solutions at pH 12·5 to be less than those for solutions at other pH values. This is particularly true of violet BNP, a feature that is characteristic of triphenylmethanol anhydride dyes under alkaline conditions when they have basic (or positive) auxochromic groups. The optical densities of the red FB solutions are appreciably greater, due possibly to the presence of the benzothiazole grouping in this 2-naphthyl-azo-phenyl dye.

The effect of metal ions on the spectra of dye solutions

Table 1, and the trends noted above, reveal that there are differences of 1000 cm^{-1} (25 nm at 500 nm) or more, in the absorption band maxima of the main colour bands. For a pronounced change of colour to be observed visually, a shift of at least this magnitude is apparently required in the position of a maximum of an absorption band of a dye solution in the presence of metal ions when compared with that in the absence of metal ions. Towards this end, the maxima of absorption bands of the dye solutions in the presence of metal ions are italicized in Table 1 in cases where these differ by more than 800 cm^{-1} (20 nm at 500 nm) from those of the free dye solution.

Several of the dyestuffs used as metallochromic indicators in EDTA titrations belong to the *o-o'*-dihydroxy group of azo dyes (Barnard, Broad & Flashka, 1956), and under suitable conditions, give well-defined colour changes at the titration end-points. These colour changes are due to changes in the electronic configuration brought about by chelation arising from the favourable position of the *o-o'*-hydroxy groups. However, only a limited number of *ortho*-monohydroxy azo dyes have applications as metallochromic indicators and these, for example, the sodium salt of 3-(4-sulphophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (SPADNS), normally have two hydroxy groups suitably disposed to form a ring by chelation (Barnard, *et al.*, 1956). All, except four, of the azo dyes included in the present investigation have one hydroxy group in a position *ortho* to the azo linkage. Of the remainder, chocolate brown HT has two hydroxy groups—*ortho* to the same end of the azo linkage; yellow 2G also has two hydroxy groups, but neither are in a suitable position for chelation, as is the case of the monohydroxy group of tartrazine. Yellow RFS is in the unique position of possessing not even a single hydroxy group.

At best, chelation of the food dyes with metal ions is possible on a more limited scale than that indicated above for the metallochromic indicators with the result that the consequent changes in electronic configuration are on a more restricted scale. In confirmation of this, it may be seen from Table 1 that appreciable changes in the absorption spectra [shifts of greater than 800 cm^{-1} (20 nm at 500 nm) in the position of the maximum of the absorption band], and hence of colour of the dyes brought about by metal ions are not, by any means, the rule. Lesser changes are, as might be expected, more frequent and certain dyes, for example, ponceau SX, give only small changes. Again, with added calcium or magnesium ions, there is a negligible effect.

This is to be expected since these ions do not usually have a strong affinity towards complex formation. Aluminium ions, on the other hand, do cause a few changes in the absorption spectra, more especially for red 10B, red 2G, yellow RFS, yellow RY and violet BNP.

The larger changes in the absorption spectra of the dyes are brought about by the transition metal ions examined. Changes brought about by copper (II) and iron (II) tend to be hypsochromic while those of iron (III) are more variable. In fact, the most pronounced colour change observed visually is that brought about by copper (II) on carmoisine (normally red) at pH 6·4, and especially at pH 7·4 when the colour is orange. This corresponds to a shift in the absorption band maximum of 1200 cm^{-1} (29 nm) and 2000 cm^{-1} (47 nm) at the respective pH values. Copper (II) ions are also responsible for a less pronounced visual colour change over the normal colour of the free dye (orange red → orange) in ponceau MX at pH 12·5. This is characterized by a shift of 1600 cm^{-1} (37 nm) in the maximum of the absorption band of the dye. A further example is the red colour exhibited by black PN in the presence of iron (II) ions at pH 12·5, a shift of 3000 cm^{-1} (83 nm) away from the bluish purple absorption at 17,600 cm^{-1} (568 nm).

It is interesting to note that the yellow dyes frequently show changes of frequency in their absorption maxima with the metal ions, despite the fact that with the exception of yellow RY, they do not possess suitably disposed groups for chelation.

Dyes have had to be monitored individually for their potential as indicators in complexometric titrations (Close & West, 1960a, b; Brazier & Stephen, 1965). The present investigation might form such a monitoring and suggests that carmoisine might be a suitable indicator for copper (II). Indeed, its 1 : 2 (metal–dye) complex stability constant of the order of 10^{11} (Table 2) would serve to confirm this. However, while the dye functioned at the predicted end-point in the titration of copper (II) with EDTA, it was considered to be inferior to the other excellent indicators now available.

Even though phosphates play an active part in forming complexes with metal ions, they are also a common constituent of foodstuffs and for this reason phosphate buffer solutions were selected for the neutral pH values of 6·4 and 7·4. Under these conditions, the dye competed with the phosphate for the metal ions, but despite this, shifts were observed in the frequencies of the absorption maxima of a number of dyes in the presence of metal ions (Table 1). With the excessive iron used to obtain the data of Table 1 at these pH values, and also at pH 12·5, there is a tendency for the ultraviolet absorptions to spread into the visible region (due to a slight cloudiness through slight precipitate formation) but nevertheless, the frequencies of the colour causing absorption maxima can, in the majority of cases, easily be distinguished. The cloudiness is very much less in evidence for the iron concentrations used to obtain the data for the calculation of the stability constants shown in Table 2.

An interesting facet of this work is the possible effect of food dyes on iron metabolism.

It is believed that iron in the +2 oxidation state is the form more effectively utilized by the body and that towards this end, iron (III) is reduced to the +2 oxidation state before diffusion in the mucosal cell (Saltman, 1965). Since, it appears that iron in both the +2 and +3 oxidation states are available to the body, the question arises of whether the food dyes affect iron metabolism. Some of the dyes clearly form complexes with iron and since the formation of biological iron chelates is claimed to be important in iron metabolism (Charley *et al.*, 1963a, b), it is interesting to have some indication of the stability of the iron–dye complexes. As can be seen from Table 2, the stability constant for the 1 : 1 complex with amaranth at pH 12·5 is of the order of 10^6, while the 2 : 1 (metal–dye) complex with ponceau 4R, at the same pH has a stability constant of around 10^{11}. These figures relate to the more alkaline pHs. Conditions in the human body are more acid with the pH of human saliva at around 7·4, the stomach being distinctly acid, and, finally, a pH of 6·5–7 being characteristic of the lumen of the intestine which is the region normally associated with iron absorption. However, the present investigation cannot throw any light on how far these iron–dye complexes compete with complexes of iron with materials, such as sugars and other polyhydroxy compounds, which are claimed to be highly significant in iron metabolism (Charley *et al.*, 1963a, b; Saltman, 1965).

The triphenylmethanol anhydride dyes also do not show an appreciable change of frequency in absorption band maxima in the presence of metal ions, although changes in hue are frequently apparent. Here again, this time in the presence of metal ions, a pH of 12·5 is sufficiently alkaline for the basic (or positive) auxochromic groups of violet BNP to have an influence, thus causing fading and, of course, the extreme fall in optical density noted above for the free dye.

Conclusion

Traces of metal ions do not, in general, have an appreciable effect on the colour of coal-tar food dyes; indeed extreme alkaline conditions have the more pronounced effect. There is, however, the question of the possible role of the dyes in influencing iron metabolism and it is suggested that further enquiry on this point is desirable.

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Reactions in food systems: negative temperature coefficients and other abnormal temperature effects

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Summary. The rate of a chemical reaction in a simple system normally increases when the temperature is raised, but there are a number of apparent exceptions to this rule when more complex systems are used. When the rate of production or disappearance of a single component in a multi-component system is considered there are a number of examples of interest to the food scientist and technologist where the apparent rate of reaction decreases when the temperature is raised. Examples of this phenomenon, involving fats, proteins, carbohydrates and vitamins are introduced, and the circumstances under which rates of reactions can show a negative temperature coefficient are discussed. It is concluded that this type of effect can usually be related to the fact that the change in rate of reaction is being measured in a system where conditions other than temperature (e.g. rates of concurrent reactions, concentrations of reactants, phase conditions) are being allowed to vary and the rate is being compared *in different systems* as well as at different temperatures. It is pointed out that accelerated storage tests at elevated temperatures may give misleading results under these circumstances and that cool or cold storage of foodstuffs may not necessarily be beneficial in all respects.

Introduction

It is a matter of general experience that simple chemical reactions in homogeneous solution increase in speed as the reaction temperature is raised, and this relationship is quantified in the van t'Hoff and Arrhenius equations. As a rough guide the rate of reaction is often taken as being doubled for every 10°C rise in temperature. In the case of individual reactions in more complex systems or in non-homogeneous systems there are often very significant departures from this rate of increase. For example, colour production (presumably due to one of the Maillard-type reaction sequences which are so important in non-enzymic browning of foodstuffs during processing and storage) doubles in speed for each 3·3°C rise in temperature in dehydrated potatoes (Ross, 1948). This corresponds to an eight-fold increase in rate for each 10°C rise in temperature.

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The purpose of this paper is to consider some instances of wide departure from this guide which have been encountered in the field of food science and technology; in particular the reactions under consideration are those which have a negative temperature coefficient, i.e. the rate of reaction increases as the temperature is reduced. The examples quoted are intended to illustrate the factors which can be involved – they are not intended to form a comprehensive list of all instances which have been recorded. Energy considerations do, of course, demand that a temperature coefficient can only be negative over a restricted temperature interval: as the temperature is reduced the rate at which molecules achieve the activation energy necessary for a particular chemical reaction will also be reduced, but it is of some interest to identify the factors which may be involved in providing the combination of circumstances in which a reaction can show a negative temperature coefficient within even a restricted temperature interval.

It should be emphasized that in the present context, when considering the relationship between temperature and the rate of an individual reaction, the term 'temperature coefficient' is being used to refer to the rate of change with temperature of the reaction rate, without making any correction for changes in the system (other than temperature) which may also affect the rate of reaction.

The reactions which will be considered are: (a) the oxidation of unsaturated fats and oils in the presence of certain amines and other compounds, (b) oxidized flavour in milk fat, (c) green discoloration in certain carotenized hydrogenated fats, (d) the production of free fatty acids in certain hydrogenated fats, (e) oxidation in irradiated foods, (f) the staling of bread and other baked goods (g) protein denaturation (superchilled cod), (h) vitamin losses in various foods, (i) certain enzymic reactions, and (j) various chemical reactions in ice.

The temperature intervals over which these effects are observable are all in the range to which foodstuffs might be subjected during processing and storage in normal modern commercial and domestic practice.

Examples

(*a*) *Oxidation of fats in the presence of amines and other compounds*

Harris & Olcott (1966) have shown that the development of oxidation in menhaden oil was inhibited by 1·8% tri-octylamine for over a month at 70°C, whereas the additive was ineffective at temperatures of 60°C or less. It is, of course, normal to find that autoxidation of fats, alone or with added antioxidants, proceeds more rapidly as the temperature is raised; in Olcott's studies the oxidation rate drops sharply once the temperature is raised above 60°C.

The explanation for this unexpected temperature dependence is that trioctylamine itself is relatively ineffective as an inhibitor of menhaden oil oxidation, but that at 70°C a reaction between the amine and hydroperoxides (produced in the initial stages of oxidation) leads to the formation of di-octyl hydroxylamine. It has been shown that this compound (like its C_{10} and C_{12} analogues) is a very effective antioxidant. Thus in

squalene, the induction period at 50°C is 1 day with 50 μM trioctylamine/g, whilst with 0·5 μM di-octyl hydroxylamine/g it is 3 days. Apparently the hydroxylamine is some 300 times more effective than the amine as an antioxidant.

The reason for the decrease in oxidation rate as the temperature is raised above 60°C is, therefore, probably due to the high-temperature formation of a reaction inhibitor. Presumably the reaction leading to the formation of hydroxylamines by this route has a very marked positive temperature dependence and at lower temperatures does not occur sufficiently rapidly to affect the oxidation rate.

In studies of the effect of temperature on the oxidation of corn and safflower oils containing added pro-oxidants, Anderson & Huntley (1964) obtained results which are as yet unexplained. They found that with certain di-carbonylic compounds (maltol, isomaltol, cyclotene and kojic acid) added at 100 ppm, the rate of oxidation in the oil was considerably enhanced at 57°C as compared to that in controls with no antioxidants. However, at 99°C, dicarbonyls had no pro-oxidant effect. Whilst there is as yet no explanation of this phenomenon, it has certain implications in the context of methods for evaluating the oxidation stability of oils; clearly, with oils containing pro-oxidants of this type, the A.O.M. test of stability at 99°C will give a result which may be quite misleading as a guide to stability at temperatures of 57°C or less. The results obtained at high temperatures with fats containing tertiary amines, as in Harris' and Olcott's studies, may be similarly misleading.

In a rather different context, Banks (1950) and other workers (Lea, Parr and Carpenter, 1958; March *et al.*, 1961) showed that autoxidation of the fats in dehydrated herrings and herring meal proceeds faster at lower temperatures than at higher temperatures. Thus Lea *et al.* (1958) found the rate faster at 10°C than at 37°C, Banks (1950) found it faster at 0°C than at 25°C and March *et al.* (1961) found it faster at −20°C than at +25·5°C. Lea (1962) suggests that concentration effects on freezing, increased adsorption of oxygen at low temperatures or the development at higher temperatures of browning reaction products with antioxidant activity may explain these results. This last suggestion is, in terms of the general mechanism involved, similar to that recently proposed by Harris & Olcott (1966), i.e. production at higher temperatures of a reaction inhibitor.

The implication of this type of explanation is that a period of high-temperature treatment should improve the subsequent storage performance (in terms of autoxidation) at lower temperatures and it seems significant that Banks found that herrings dried at 80°C and above had a greatly improved resistance to autoxidation during storage at 10°C as compared to herrings dried at 70°C or less. The nature of the antioxidants formed during dehydration at the higher temperatures is not known; they may be non-enzymic browning products, as suggested by Lea (1962), but the sharp temperature dependence of their production at around 70°C is similar to that found by Harris & Olcott (1966) and this might be an indication that the antioxidants are being formed by an amine–hdyroperoxide interaction.

(*b*) *Oxidized flavour in milk*

It was reported many years ago (Tracy, 1931) that fluid milk is less susceptible to development of oxidized flavour when stored at 20°C as compared to 4°C. Dunkley & Franke (1967) show that off-flavour tends to be greater in liquid milk stored at 0°C than at 8°C, and Bell (1939) showed condensed milk to be more susceptible to development of oxidized flavour at −17°C than at −7°C. The nature of the compounds responsible for the development of this flavour defect and the factors controlling their production have been the subject of extensive study in recent years. This work has been summarized by Parks (1965) and refers to work demonstrating the involvement of oxygen, light, copper and ascorbic acid in the development of oxidized flavour; tocopherols are also involved (Erickson, Dunkley & Ronning, 1963). Whilst the mechanism by which it is produced is not yet understood in detail, it has been concluded (Day, 1965) that the flavour is due to a mixture of carbonyl compounds, particularly unsaturated aldehydes. Decreases in the intensity of flavour may be due to further oxidation of the flavoured compounds to relatively tasteless compounds (Greenbank, 1940; Lillard & Day, 1964), and the condensation of aldehydes with amines (Montgomery & Day, 1965). It may be that in milk some of these reactions are sufficiently rapid at room temperature to keep the concentration of the highly flavoured components at sub-threshold levels, but that at lower temperatures these reactions are slower and noticeable concentrations accumulate. If this is the case, the negative temperature coefficient of the formation of oxidized flavour in milk is due to the difference between the temperature coefficients of the reactions leading to formation and removal of the highly flavoured carbonyl compounds.

(*c*) *Green discoloration of certain fats containing* β*-carotene*

Recent studies by McWeeny (1968) amplify the original observations by Hayes & Steele (1964) of green discoloration in β-carotene/hardened palm kernel oil (HPKO) which was more rapid at +3°C than at +25°C. It has been shown that green discoloration occurred rapidly in twenty-three samples of hardened coconut or palm kernel oil containing added β-carotene and that in each case the rate at which this occurred showed a marked temperature dependence. In most cases the rate of discoloration was very low at room temperature, but rapid in the region of +6°C to −6°C. In many cases the rate of reaction increased by more than fifty-fold as the temperature was reduced by some 12–14°C; several samples became green within 1 week at +6°C, but were still yellow after 1 year at room temperature (18–20°C). In two recent publications Luck (1966a, b) has reported some observations on butter oils which developed a green discoloration during extended storage at −12°C, whilst similar samples at +2°C were unchanged in colour.

These green discolorations seem to have much in common and appear to involve oxidation of β-carotene to epoxide derivatives. The rate of development of the green colour in β-carotene/HPKO is not retarded by phenolic antioxidants, but many

amines inhibit it very effectively. These characteristics suggest the involvement of a mechanism based upon the oxidation of β-carotene by peroxyacids; it is known that this reaction results in the formation of epoxides (Karrer & Jucker, 1945) and that it proceeds by an ionic mechanism (Swern, 1953) which is unlikely to be inhibited by phenolic antioxidants. It has been suggested that one explanation of the unexpected temperature dependence of this colour change might be that the peroxyacids involved are very thermolabile and that at ambient temperatures they undergo thermal decomposition rather than cause oxidation of β-carotene; at a lower temperature thermal decomposition is slower and consequently oxidation of β-carotene can proceed to a greater extent. This suggestion is supported by the observation that if the carotenized fat is stored at 30°C for 2 days prior to storage at +6°C, then development of the green discoloration is greatly retarded.

If the hypothesis is correct, then the mechanism contrasts with that suggested in section (a). In one case the negative temperature coefficients are due to high temperature production of a reaction inhibitor, in the other case it is due to high temperature decomposition of a reaction intermediate, but in both cases the net effect is a very sharp decrease in reaction as the temperature is raised by 10–15°C.

(*d*) *Formation of free fatty acids in certain stored fats*

In a recent publication Imamura *et al.* (1967) report on the changes occurring during storage of various confectioners fats containing coconut oil for periods up to 12 months. After 2 months at −20°C, 5°C and 30°C the samples stored at 5°C developed a peculiar rancid odour, an increased acid value and an increased acidity in the steam distillate; there was no change in the samples at −20°C and 30°C. The changes increased in fats with increased coconut oil content and with increase in the degree of hydrogenation. The peroxide values did not increase, but the sample stored at 30°C showed an elevated carbonyl value.

These observations are similar in some respects to those in contemporary work by Hearne (1966, unpublished data) on fats based on hardened groundnut oil and 1 : 1 blends of this fat with hardened palm, palm kernel or coconut oils. These fats all developed high acidities during low temperature storage in sealed cans for 3 years at −10°C and 0°C as compared to samples in unheated store in U.K. (mean temperature 10°C). In all cases the free fatty acid content was higher at −10°C than at ambient temperature and in the case of hardened groundnut oil the acidity was higher at 0°C than at either of the other two temperatures.

The mechanism by which the free acidity is generated is not yet known, but it seems clear that in the hardened groundnut oil the acidity arose from hydrolysis of the fat rather than oxidation; the free fatty acid chain-length distribution was the same in the free fatty acid fraction as in the total fatty acids (Roberts, 1966 personal communication). It might be suggested that the agent responsible for catalysing the triglyceride

hydrolysis is thermolabile and hence is able to promote more hydrolysis at lower temperatures, but there is as yet no direct evidence of this.

(*e*) *Oxidation in irradiated foods*

In experiments reported some years ago Hannan (1955) showed that when butter fat, saturated with air and water, is irradiated with high-energy electrons at 0°C or below and then immediately stored at temperatures in the region of −20°C to −30°C, the fat rapidly develops a high peroxide value. The reaction rate falls off rapidly at higher or lower storage temperatures.

When storage temperatures between +20°C and −30°C are considered the net rate of formation of peroxides shows a negative temperature coefficient throughout the range; at −30°C the rate is approximately seven times as fast as at +20°C over a 5-day storage period after irradiation at −70°C.

Hannan points out that these observations can be explained in terms of the formation during irradiation of free radicals which are relatively stable at low temperatures, but are destroyed at higher temperatures. It is considered that these radicals can react with oxygen to cause peroxide formation; the amount of peroxide formed is of the correct order of magnitude to correspond to a 1 : 1 interaction between oxygen and the primary free radicals formed by irradiation. If the fat is warmed for a short period (15 min to 3 hr) at 20°C between irradiation and storage, then subsequent peroxide formation does not occur; this behaviour lends support to the theory that the peroxides are derived from a thermolabile precursor.

In this respect, explanation of temperature dependence of the formation of green discoloration in carotenized fats (McWeeny) relies upon a mechanism which is analogous to that proposed by Hannan for the post-irradiation development of peroxides in fats. In each case the abnormal temperature dependence of the reaction is attributed to the fact that the thermal decomposition of a reactant increases with temperature at a rate which is faster than that shown by the reaction which leads to the products which are found at lower temperatures. In each case the overall effect is a reduction in the rate of the latter reaction as the temperature is raised.

In work with irradiated meats there have been reports of undesirable effects associated with cold storage following irradiation. Thus Chang, Younathan & Watts (1961) found that the TBA values in radiation sterilized roast beef slices stored at room temperatures were consistently lower than those in similar samples stored at −26°C. In studies with cooked ground beef Greene & Watts (1966) found that irradiation at room temperature inhibited lipid oxidation and noted that the TBA values of irradiated meat stored at 25°C were consistently lower than in samples stored at 7°C. The authors suggest that at the higher temperature the TBA reactive material is being removed by reaction with amines and that, as an additional means of retarding the increase in TBA values, the products of this reaction are acting as antioxidants.

(f) The staling of bread

Using crumb firmness as an index of staling in bread it has been shown by many workers that bread stales much faster as its temperature is lowered towards the freezing point and that commercially significant changes in firmness can be caused by moderate changes in storage temperature. Meisner (1953) found large differences in the rate of decline of softness at temperatures of $-1°$, 4°, 24° and 43°C. Similarly, Pence & Standridge (1955) showed that the rate of increase in crumb firmness became progressively more rapid as the temperature was reduced from 30°C, via 23°, 12° and 8°C, to 1°C; firming was somewhat slower at $-7°$C than at 1°C. The differences were quite marked and, for instance, the results of Pence & Standridge (1955) show that at 23°C 6 days are required for bread to attain a firmness which was reached in 1 day at 1°C. These authors also showed that although bread became firmer during freezing and thawing the subsequent rate of firming was slower than in unfrozen bread stored at the same temperature and that, with the treatment they employed, the breads showed equal firmness after 48 hr storage at 23°C. Subsequently the bread which had been frozen and thawed before storage was softer than the unfrozen bread. It is pointed out that commercial freezing of bread need involve no penalty in respect of firmness – but only if care is taken to freeze the bread as soon as possible after baking and to ensure that during the freezing and thawing processes the bread does not spend any extended time at temperatures in the region of 1°C.

Pence & Standridge (1955) also point out that at the higher storage temperatures the firmness: time graph is almost linear, whilst at the lower temperatures it is markedly curvilinear. This observation has been confirmed and amplified by Cornford, Axford & Elton (1964), who showed that regardless of the storage temperature (providing this is above the freezing point) the firmness tends to approach the same limiting value, given sufficient time. This is taken by the authors to show that the storage temperature does not affect the final state of the product, in terms of firmness; it merely affects the rate at which it is achieved. They show that in the temperature range which they studied the relationships between elastic modulus, time and temperature in bread crumb are those which would be expected if an increase in crystallinity of the crumb was the process causing the increase in elastic modulus. The results are compatible with the view that firmness is related to the degree of crystallization of the starch gel and that as the temperature falls progressively below the melting point of the crystalline phase then the probability increases that a nucleus will develop into a grain and then grow at a steady rate. In a recent publication which supports this view (Axford & Colwell, 1967), differential thermal analyses on stale and fresh bread show the presence of a 'phase' in stale bread which is absent from fresh bread.

The way in which firming of bread increases in rate as the temperature is reduced towards a certain value seems to be related to the way in which the rate of crystallization in a high polymer system is usually slow just below the melting point of the crystals

and becomes progressively faster with increased supercooling, reaches a maximum and then decreases as molecular mobility is reduced.

(g) Protein denaturation in fish

When stored in the frozen state, fish muscle proteins gradually alter in character and become less desirable foodstuffs, due to their increasingly tough and fibrous texture.

It has been reported by Love & Elerian (1964) that in cod frozen at −29°C and then stored at temperatures in the range −0·5°C to −4·0°C the rate of protein denaturation is maximal at −1·5°C, i.e. as the storage temperature is reduced from −0·5°C to −1·5°C the rate of reaction increases. Again, this represents a reaction having a negative temperature coefficient.

The full explanation of this unusual effect has not yet been elucidated, but present hypotheses on the subject (Anderson, Steinberg & King, 1964; Love & Elerian, 1964; Love, 1966a) appear to be based upon the fact that, in concentrated salt solution, phospholipid hydrolysis (itself maximal at −4°C) releases fatty acids which interact strongly with proteins and denature them. This denaturation is thought to be responsible for the tough, fibrous texture noted in fish subjected to freezing to −29°C and subsequent storage at around −1·5°C. Love & Elerian (1964) regard it as probable that the tissue salts are sufficiently concentrated to cause maximal denaturation throughout the range from −4°C to −29°C and that the overriding factor governing the rate of denaturation is the storage temperature. When temperatures between −4° and 0°C are considered, another effect begins to over-ride that of temperature; at −2°C the tissue water is 52·4% frozen, whilst at −1°C only 8% is frozen and consequently there is a very rapid reduction in the salt concentration during this temperature rise of 1°C. It is thought that this dilution of the dissolved salt results in the concentration becoming less than that required for the optimal denaturation rate. Since this reduction in rate occurs over a very small temperature change it is not surprising that it should outweigh any slight acceleration of the denaturation rate due to a simple temperature effect, and that as a result the reaction should show a negative temperature coefficient in this temperature range.

Whilst considering this effect it is worthwhile noting that in cod which has been 'superchilled' to −1·5°C (i.e. without hard-freezing to a low temperature first), the muscle remains tender, and that this presents a dramatic difference when compared with similar fish hard-frozen before storage at −1·5°C (Love, 1966b). The 'superchilled' muscle contains far less ice than the previously hard-frozen muscle and consequently the salt concentration in the aqueous phase is much less; it is thought that under these conditions the sodium chloride concentration will be around 3–6% and it has been shown that at this concentration the cod actomyosin is dissolved most effectively. It has recently been concluded (Love, 1967) that superchilling causes some of the structural

protein to be dissolved out of the muscle fibrils and that after depletion by this process the myofibrils do not bind together side to side (as in fish muscle frozen at low temperatures) and that as a result the product does not toughen.

The effects of adjusting the temperature to $-1{\cdot}5°C$ in both hard-frozen and 'super-chilled' cod are attributable in each case, at least in part, to the concentration effects which occur when a tissue is partially frozen. The fact that opposing effects are found at different degrees of concentration is quite remarkable. In combination with the observations that one of the effects shows a negative temperature coefficient over at least a small range, this makes the investigation of the behaviour of fish muscle a fascinating field of study on a matter of considerable commercial significance.

(h) Vitamin losses in various foods

There have been a number of cases reported in which large losses in the vitamin content of foods have occurred at reduced temperatures. The examples quoted below are by no means comprehensive, but serve to illustrate the variety of vitamins and commodities in which such losses might be encountered.

Bunnel *et al.* (1965) report in their studies on the stability of tocopherol in fried foods that there are large losses of tocopherol in potato chips ('crisps') and 'french-fried' potatoes at $-12°C$. The authors comment on the effect of the consumption of deep-fried convenience foods, as compared to similar freshly prepared foods, on the dietary intake of tocopherol. The rate of loss does not exhibit a negative temperature coefficent, but it is abnormal in that it is only some 10% greater at room temperature than at $-12°C$; for a temperature variation of some 30°C one might not be suprised by a ten-fold variation in the rate of change in a normal system. Bunnell *et al.* (1965) suggest that fatty hydroperoxide formation is not prevented at low temperatures, and that instead of decomposing to give rancid smelling products, as they do at normal temperatures, these hydroperoxides react with tocopherol to cause the large losses which they observe. Zaehringer, Rickard & Lehrer (1963) record considerable losses in tocopherol in pork chops during storage at $-18°C$, but the authors do not compare these losses with those at other temperatures.

In studies on the development of a green discoloration in a sample of hydrogenated groundnut oil, containing added β-carotene and vitamin A (and in certain other fats with similar additives) Hayes & Steele (1964) showed that at room temperature one of their fats had lost only 20% of its β-carotene and retained the full initial content of vitamin A after 2 years of storage. By contrast, a similar length of storage at $-9°C$ led to complete loss of β-carotene and 40–60% loss of vitamin A. The authors show that the rate of decomposition of β-carotene at various temperatures decreased in the order $+3°C$, $-6°C$, $+25°C$, $-35°C$ and suggest that this abnormal temperature effect is due to concentration of a catalyst in the diminishing liquid phase of the fat as the temperature is reduced.

(*i*) *Certain enzymic reactions*

It is, of course, well known that enzyme reactions and microbiological processes have a reaction rate which is maximal at a certain temperature – the occurrence of a negative temperature coefficient above this temperature is sufficiently familiar as to be regarded as normal. However, there is another aspect to the occurrence of negative temperature coefficients in association with enzymic reactions and foodstuffs which might be mentioned. Two examples will be referred to – although there are other similar cases reported. Both these examples involve discoloration of potatoes.

Firstly, the pre-cooking discoloration of potatoes known as 'black spot' has been studied by Mondy, Gedde-Dahl & Owens Mobley (1966). These workers have demonstrated a correlation between cytochrome oxidase and polyphenoloxidase activities, the phenolic content and the subsequent incidence of 'black spot' in potatoes held at temperatures of 40° and 50°F.

As compared to the situation at 50°F, enzyme activity in potatoes at 40°F is restricted and appears to allow the accumulation of higher concentrations of phenolic substances. It is suggested that the increased incidence of 'black spot' in these potatoes when bruised, subsequently, whilst being held at higher temperatures is due to the production of pigmented materials by the action of the enzymes on this relatively high concentration of phenols. The effect of the normal reduction in enzyme activity as the temperature is lowered, is manifested at a later stage as an acceleration of a deteriorative reaction in potatoes previously stored at the lower temperature.

Secondly, in a rather similar type of reaction mechanism (Ross, Hilborn & Jenness, 1945) the incidence of non-enzymic browning in processed potatoes can be related to the storage history of the unprocessed potato and its effect upon the reducing sugar content. At lower storage temperatures (below about 42°F) the reducing sugar content is raised, possibly due to a simple reduction in enzymic activity, or, alternatively, due to a shift in the 'equilibrium' concentration of reducing sugar in the reducing sugar $\rightleftharpoons$ polysaccharide system owing to a difference between the temperature coefficients of the 'forward' and 'back' reactions. Whichever of these two mechanisms is responsible, the overall effect is the same, viz. at the lower storage temperature reducing sugars accumulate and after processing these potatoes (as crisps or as an air-dried product) are more susceptible to non-enzymic browning (in which reducing sugars are a major reactant).

In both these instances, a reduction in storage temperature accelerates the rate of deteriorative reactions at a later stage, but a short period of 'conditioning' at a higher temperature can reduce the elevated concentration of the reactive substance to a more normal level. The value of conditioning (at 60–70°F) has been demonstrated in the production of potato crisps and air-dried potato strips where non-enzymic browning must be minimized (Ross, 1948; Gooding, Duckworth & Harries, 1956).

Quite apart from this type of effect and the negative temperature coefficient normally found at relatively high temperatures in enzymic and microbiological processes,

there is the possibility of negative temperature coefficients occurring at relatively low temperatures.

In a recent publication Hugh Smith (1967) demonstrates that fungal rotting in carrots is slower to develop at 34°F than at 32° or 36°F. This effect is only noticeable during storage for periods greater than 2 months. No precise explanation for the negative temperature coefficient between 32° and 34°F has been proposed, but Hugh Smith offers the comment that 'since growth of fungi would be expected to be retarded by lowered temperature (as it apparently is between 34°F and 36°F in carrots), it may be inferred that some change of metabolic balance in the surface tissues of the root renders them more susceptible to invasion'.

(*j*) *Various chemical reactions in ice*

There have been a number of studies in recent years on the effect of temperature on the rates of reactions in ice. In several cases an acceleration is observed and whilst none of these reactions have been studied specifically with foodstuffs in mind, they are sufficiently diverse to be of potential significance in food science and technology. The reactions include hydrolysis, acid catalysed hydrolysis, base-catalysed hydrolysis, transfer reactions and ultraviolet photochemical reactions.

A number of factors come into play in raising the rates of reaction as the temperature is reduced. Firstly, of course, in going from a completely liquid system to a near-solid system there can be a marked concentration of the solutes. This can often be sufficient to cause an increase in reaction rate which is greater than the reduction in rate due to the lower temperature. However, this is not an adequate explanation of all the effects observed, some of which are mentioned below, along with suggested reasons for this.

Grant, Clark & Alburn (1961), Alburn & Grant (1965) and Grant & Alburn (1965) show that in some systems reaction is greater at -18°C than at -8°C, whereas the reverse is true in other cases. It is suggested (i) that phase changes in ice crystals between 0°C and -30°C may be involved, (ii) that ice surface catalysis may be important, and (iii) that the dielectric constant may be an important feature in favouring nucleophile associations. They do not feel that the concentration which occurs on freezing can be a full explanation of all the observed facts. In particular they point out that the accelerated reaction in ice is inhibited only by certain substances and that the effect of these substances is much greater in ice than at 1°C. Bruice & Butler (1964) also conclude that concentration effects are only part of the explanation; they show that in their systems the increase in reaction rate is only 10% of that which would be expected if concentration effects were solely responsible.

Another factor which has been suggested as an explanation in some of the reactions studied is the high mobility of protons in ice as compared to water. This was investigated by Eigen & De Maeyer (1958), and this phenomenon has been suggested as being involved in the reversal of the effects of ultraviolet irradiation of 2′-deoxyuridine

at −20°C (Prusoff, 1963). This reaction (the formation of 5-hydro, 6-hydroxy uridine) is reversed by acids at room temperature, but can be reversed at −20°C in much less acid conditions. It is suggested that the high proton mobility in ice leads to a much higher rate of reaction than would be expected from the same acidity in water. Alburn & Grant (1965) suggest that this same effect may be responsible for some of the effects they note in the hydroxaminolysis of various amides. The effective increase in acidity due to enhanced proton mobility in ice may be a reaction of some significance in a variety of contexts in the food industry and might be worthy of further investigation from this standpoint.

Whilst considering the ways in which reaction rates may be increased by freezing the system it is necessary to bear in mind the possibility of other effects. Tappel (1966) points out, in respect of an enzyme reaction that the rate of freezing can affect the extent to which solutes are concentrated; if freezing is slow, concentration effects can be large, but if freezing is rapid the system may be much more homogeneous and little concentration effect may be noted. Tappel also refers to the effect of temperatures on enzyme conformation which may affect the reaction rate; presumably other conformation changes might alter the reaction rate in chemical systems containing either proteins or carbohydrates and might result in either an acceleration or retardation of the reaction rate. One other factor which might also be relevant, particularly in hydrolysis reactions, is referred to by Butler & Bruice (1964). They point out that in frozen systems the concentration of water itself may become a limiting factor and explain the acceleration of certain reactions in the presence of added salts as being due to their effect in increasing the size of the liquid phase and hence the amount of water available.

Discussion

In the examples quoted, there are instances where the factors responsible for abnormal effects of temperature on reaction rates are unknown, or at best inadequately understood. On the other hand, well substantiated explanations are available for some of the observed effects and apparently sound hypotheses have been advanced in explanation of others. These explanations are widely diverse and illustrate the wide range of circumstances in which abnormal temperature effects, particularly negative temperature coefficients, can be encountered, i.e. they may arise as a result of:

(i) Production, by a competing reaction at higher temperatures, of an inhibitor for the 'normal' reaction.

(ii) Thermal destruction, by a competing reaction at higher temperatures, of a substance which in the 'normal' reaction is a reactant, a reaction intermediate or catalyst.

(iii) A decrease at higher temperatures in the rate at which crystallization occurs.

(iv) An increase in reaction, following a period of storage at lower temperatures, due to a decrease in enzymic activity at the lower temperatures which allowed a reactant to accumulate.

(v) An increase in reaction, following a period of storage at lower temperatures, due to a shift in the 'equilibrium' point of a chemical or enzymic system and a consequent increase in the concentration of a reactant.

(vi) Concentration processes in frozen and partly-frozen systems which may: (a) cause a general increase in reaction rates on freezing, (b) affect the salt concentration of the aqueous phase and hence the solubilization of protein, and (c) affect the ionic strength of the aqueous phase and hence the rate of protein denaturation.

(vii) The dielectric constant of ice being more favourable than that of water towards nucleophile associations.

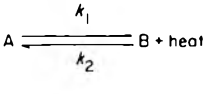
(viii) The enhanced mobility of protons, causing an increase in acid-catalysed reactions in ice as compared to water.

(ix) A change in the composition of the equilibrium mixture of possible conformational structures of a reactant resulting in an increase in the concentration of a particular conformation in a sugar, protein or enzyme system.

This list of mechanisms which may be involved is, doubtless, incomplete – yet it provides an indication of the wide variety of factors which may operate in producing an abnormal temperature coefficient. On thermodynamic grounds, these effects can operate over only a restricted temperature interval, but it is, perhaps, well to bear in mind that in the complex systems existing in foodstuffs the possibility of encountering an abnormal effect of temperature on a reaction rate is by no means remote.

In a food system where physical as well as chemical changes can be important, the environment in which a particular reaction occurs can be widely different from one temperature to another and inevitably this must affect the rate – temperature relationship. Furthermore, in considering the rate at which an individual reaction will occur it should be borne in mind that the normal rate–temperature relationships can be expected only when there are no other reactions taking place which involve the production or utilization of any reactant, intermediate, catalyst or product participating in the reaction under consideration. In the complex systems found in most foodstuffs these conditions are seldom encountered and inevitably there must be some departures from ideal behaviour when competing reactions cannot be excluded.

In attempting any general classification of the effects which may lead to a negative temperature coefficient it is important to distinguish between effects upon the true rate of reaction and effects, in reversible reactions, upon the *net* rate of reaction, i.e. on the relative rates of the 'forward' and 'back' reactions and hence on the composition of the equilibrium mixture. Taking a simple exothermic transformation as an example:



it is clear that as the temperature is reduced the concentration of B in the equilibrium mixture will increase (le Chatelier's principle) – although the individual rate constants (k_1 and k_2) and the true rates of the 'forward' and 'back' reactions will all be reduced. The 'forward' and 'back' reactions will, of course, be affected to different extents and, depending upon the way in which the reaction rate of A ⟶ B is determined this may show an apparent negative temperature coefficient.

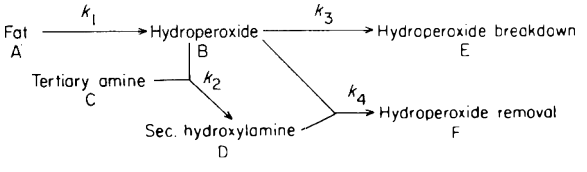
It is not difficult to envisage that similar situations can arise when considering an individual reaction in a multi-step sequence:

e.g. $$A \xrightarrow{k_1} B \xrightarrow{k_2} C,$$

where the variation, with temperature, of the net rate of formation of B will depend upon the relative temperature coefficients of k_1 and k_2. Similar considerations come into play where alternative reactions exist:

e.g. $$A \xrightarrow{k_1} B \begin{cases} \xrightarrow{k_2} C \\ \xrightarrow{k_3} D \end{cases} \quad \text{or} \quad A \xrightarrow{k_1} B \xrightarrow{k_2} C, \quad A \xrightarrow{k_3} D$$

More complicated systems must often exist in foodstuffs and, for instance, the work of Harris and Olcott appears to be on a system which might be shown as:



Depending upon the relative temperature coefficients of k_1, k_2, k_3 and k_4 the temperature dependence of the *net* rate of formation of B in these systems may be either positive or negative. It seems that the net rate of formation of 'B' can show a negative temperature coefficient when a related reaction (e.g. reversal of formation of B; alternative reaction of A; further reaction of B) shows a *marked positive* temperature coefficient. This type of situation occurs frequently in the examples which have been quoted.

In all the cases in which a chemical change is considered it seems likely that the reason for the abnormal relationship between temperature and reaction rate is due to one of a variety of situations in which an entirely normal temperature–rate relationship is over-shadowed by the influence of some other temperature-dependent effect which changes the system in which the reaction is being studied. The true temperature coefficient refers to the rate of change in the reaction rate in a system in which temperature is the only parameter which is allowed to vary. In all the cases considered above this condition has not been satisfied; in some cases the rate of concurrent reaction has been allowed to vary (e.g. when the rate of an alternative, opposing, competing or consecutive reaction has changed), in other cases the concentration of reactants have been allowed to vary (e.g. when solubility changes, liquid–solid ratio changes or con-

formational changes have occurred), in others the environment of the reaction has been allowed to vary (e.g. changes in phase, dielectric constant, or reactive surfaces). Clearly, under these circumstances the reaction is not being studied in systems which are identical, apart from temperature, and a comparison of reaction rates will not give a true measure of the temperature coefficient.

In view of the considerations outlined above, the circumstances in which a negative temperature coefficient may exist are apparently not too difficult to envisage and whilst a reduction in temperature will usually give a reduction in the rate at which a particular change occurs in a food, this must not be taken entirely for granted. Indeed, as the range of temperatures involved in food processing and storage expands (it now extends over almost 400°C), it seems increasingly likely that within this range there will be instances where individual reactions show a negative temperature coefficient over a restricted temperature interval. Possibly the chances of encountering this phenomenon in food processing and storage should receive more systematic consideration than they have in the past.

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The temperature dependence of the lethal rate in sterilization calculations

M. C. JONES

Summary. Two common methods of calculating thermal sterilization times are compared and shown to give significantly different results for *Clostridium botulinum* at 140°C.

Introduction

Thermal sterilization is widely used in the food industry to destroy harmful organisms. Many modern techniques use sterilization temperatures greater than those used in traditional canning processes, and much care and experience is required to calculate the processing time necessary to effect sterilization at the higher temperatures. Often the sterilizing temperature is outside the range of temperature used in the basic bacteriological studies of the rate of destruction of organisms by heat, and it is then important to use a method of calculation which does not underestimate the processing time required.

List of symbols used

A = Constant in Arrhenius equation (min^{-1}).
C_1, C_2 = Constants.
E = Energy of activation for spore destruction (cal/g mole).
F = Lethality of sterilizing process (min) (defined in the text.)
k = Specific reaction rate for spore destruction (min^{-1}).
$\mathcal{N}$ = Number of spores at time t_R.
$\mathcal{N}_0$ = Number of spores at $t = 0$.
R = Universal gas constant (cal/g mole °K).
t = Time (min).
t_R = Total sterilizing time (min).
t_{R_1} = Total sterilizing time calculated by Esty & Meyer (1922) method.
t_{R_2} = Total sterilizing time calculated by Deindoerfer & Humphrey (1959) method.
T = Temperature (°K).
T_A = Temperature at which Esty & Meyer (1922) and Deindoerfer & Humphrey (1959) methods agree (°K).
z = Experimental factor in Esty & Meyer (1922) method (°K).

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Two methods of calculating sterilization times

Two common methods are available for expressing analytically the lethality of a sterilizing process in which the sterilizing temperature varies with time. The first, extensively used by Ball & Olson (1957), and recently by Klostergaard (1965), is based on the classical experimental results of Esty & Meyer (1922). In this method the lethality of a process, F (defined below), is related to the processing temperature, T°K by the empirical equation:

$$F = t_R\, 10^{(T-394{\cdot}3)/z}, \tag{1}$$

where F is the equivalent time, in minutes, at 250°F (394·3°K) required to cause a specified destruction of selected organism, usually the reduction of *Cl. botulinum* by a factor of 10^{12}. The temperature T and the temperature difference z appear in the usual form of equation (1) as °F, in which case the constant is 250°F. For convenience later, T and z are in °K in equation (1).

For a process in which the temperature varies with time the lethality can be calculated by using a modified form of equation (1):

$$F = \int_0^{t_R} 10^{(T-394{\cdot}3)/z}\, dt.$$

The problem is then to specify the sterilizing temperature as $T = f(t)$, and to determine the value of F in terms of t_R by integration.

The second method of calculation, used by Deindoerfer & Humphrey (1959) and recently by Richards (1965), expresses the rate of destruction of an organism by pseudo first order reaction kinetics, and the rate dependence on temperature by the Arrhenius Equation. By this method the integrated form of the rate equation:

$$\ln\,(\mathcal{N}_0/\mathcal{N}) = \int_0^{t_R} k\,dt,$$

and the Arrhenius Equation:

$$k = A.\exp\,(-E/RT)$$

are combined to give

$$\ln\,(\mathcal{N}_0/\mathcal{N}) = \int_0^{t_R} A.\exp\,(-E/RT)\,dt \tag{2}$$

from which $\ln\,(\mathcal{N}/\mathcal{N}_0)$, the logarithmic reduction in the population of the selected organism is calculated by integration, using $T = f(t)$.

The factors A and E in equation (2) must be experimentally determined.

Clearly, the two methods are similar. We inquire here whether they are identical, and if not, which is preferable.

Comparison of the two methods

If the sterilizing temperature T is constant, equation (2) becomes:

$$\ln(\mathcal{N}_0/\mathcal{N}) = t_R.A.\exp(-E/RT). \tag{3}$$

If the two methods are identical equations (1) and (3) must give the same value of t_R, the sterilizing time at a constant temperature, when the same value of T is used in each equation. Thus, eliminating t_R between equations (1) and (3):

$$\begin{aligned} F.A.\exp(-E/RT) &= \ln(\mathcal{N}_0/\mathcal{N})\ 10^{(T-394{\cdot}3)/z} \\ &= \ln(\mathcal{N}_0/\mathcal{N})\exp\ 2{\cdot}303(T-394{\cdot}3)/z. \end{aligned} \tag{4}$$

The method of Deindorfer & Humphrey (1959) assumes that the term A is independent of temperature. Consequently, equation (4) is of the form:

$$C_1\exp\ (-E/RT) = C_2\ \exp\ 2{\cdot}303\ (T-394{\cdot}3)/z$$

which can be re-written as:

$$2{\cdot}303\ \frac{(T-394{\cdot}3)}{z} + \frac{E}{RT} = \text{constant}. \tag{5}$$

Equation (5) clearly cannot be obeyed when E and z are temperature-independent, and therefore the two methods do not correspond accurately. The size of the discrepancy between the two methods is shown by the following calculations:

Let t_{R_1} be the retorting time predicted by the Ball & Olson (1957) method for sterilization at the constant temperature T, and t_{R_2} the retorting time by the Deindoerfer & Humphrey (1959) method.

Then, from equations (1) and (3),

$$\frac{t_{R_1}}{t_{R_2}} = \frac{F}{10^{(T-394{\cdot}3)/z}} \cdot \frac{A\ \exp\ (-E/RT)}{\ln(\mathcal{N}_0/\mathcal{N})}. \tag{6}$$

Although the two methods do not correspond accurately, there must be at least one temperature for which $t_{R_1} = t_{R_2}$. Call this temperature T_A. Then, at $T = T_A$:

$$\frac{t_{R_1}}{t_{R_2}} = 1 = \frac{F}{10^{(T-394{\cdot}3)/z}} \cdot \frac{A\ \exp\ (-E/RT_A)}{\ln(\mathcal{N}_0/\mathcal{N})},$$

i.e.

$$\frac{FA}{\ln (\mathcal{N}_0/\mathcal{N})} = \frac{10^{(T_A - 394\cdot3)/z}}{\exp(-E/RT_A)}. \tag{7}$$

When equation (7) is substituted into equation (6), there results: on simplification:

$$\frac{t_{R_1}}{t_{R_2}} = \exp\left[(T - T_A)\left(\frac{E}{RTT_A} - \frac{2\cdot303}{z}\right)\right]. \tag{8}$$

In order to use equation (8), experimental values of E and z are required. Table 1 contains published values for three organisms, *Cl. botulinum*, PA 3679 and *Bacillus stearothermophilus*. In the case of *Cl. botulinum* we have included two values of z, (a) $z = 10°$ from Esty & Meyer (1922), and (b) $z = 8\cdot33$°K, from the data used by Levine (1956). (a) is more commonly used. Table 2 shows values of the ratio (t_{R_1}/t_{R_2}) calculated from equation (8) in the temperature range 100–140°C. $T_A = 100$°C in the calculations for Table 2. The choice of T_A is arbitrary, but it can be shown that any value of T_A in the range 100–140°C results in a similar value of the ratio $(t_{R_1}/t_{R_2})_{100°} \div (t_{R_1}/t_{R_2})_T$.

Table 1. E and z values for three organisms

Organism	E (cal/g mole)	Reference	z (°K)	Reference
Cl. botulinum				
(a)	82,200	a	10	d
(b)			8·33	e
PA 3679	72,500	a	10	e
B. stearothermophilus	67,700	b, c	11	f

(a) Levine (1956), (b) Deindoerfer (1957), (c) Klostergaard (1965), (d) Ball & Olsen (1957), (e) Stumbo, Murphy & Cochran (1950), (f) Knock (1954).

Table 2. Relative sterilization times by the two methods

Organism	$\frac{(t_{R_1})}{(t_{R_2})}$ 100°C	$\frac{(t_{R_1})}{(t_{R_2})}$ 110°C	$\frac{(t_{R_1})}{(t_{R_2})}$ 120°C	$\frac{(t_{R_1})}{(t_{R_2})}$ 130°C	$\frac{(t_{R_1})}{(t_{R_2})}$ 140°C
Cl. botulinum					
(a)	1	1·82	2·89	3·99	4·6
(b)	1	1·14	1·12	0·96	0·73
PA 3679	1	1·30	1·47	1·50	1·34
B. stearothermophilus	1	1·34	1·58	1·69	1·61

Numerical examples comparing the two methods

The value of (t_{R_1}/t_{R_2}) in Table 2 show that the two methods of calculating the sterilizing time are not even approximately equivalent, and that for *Cl. botulinum* the sterilizing time calculated by one method may be only one-fifth of the time calculated by the other method, using the commonly accepted value of $z = 10°K$.

A difference of a factor of 5 would be an extreme case, however. The following examples illustrate the magnitude of the difference between the two methods for possible practical cases.

Example 1

A manufacturer has been sterilizing a food product by a heat treatment of 5 min at 120°C (393·2°K). He wishes to introduce a new process in which the sterilization temperature is 140°C (413·2°K). Assuming that the sterilization involves the destruction of *Cl. botulinum* and that the Ball & Olson (1957) and the Deindoerfer & Humphrey (1959) methods agree at 120°C, what will be the sterilizing time at 140°C by the two methods?

(1) Ball & Olson (1957)

(a) *Taking* $z = 10°K$:

$$t_{R_{120}} 10^{(393·2 - 394·3)/z} = t_{R_{140}} 10^{(413·2 - 394·3)/z}$$

$$t_{R_{140}} = 5 \times 10^{(393·2 - 413·2)/10}$$

$$= 0·050 \text{ min } (3·0 \text{ sec}).$$

(b) *Taking* $z = 8·33°K$:

$$t_{R_{140}} = 0·019 \text{ min}$$

(2) Deindoerfer & Humphrey (1959)

The two methods are assumed to give the same t_R value at 120°C. Table 1 can be converted to this condition (i.e. $T_A = 120°C$) by dividing each row by the appropriate entry in the (t_{R_2}/t_{R_2}) 120° column. Thus for *Cl. botulinum* at 140°C:

(a) *If* $z = 10°K$:

$$\frac{(t_{R_1})}{(t_{R_2})} = \frac{4·55}{2·89}$$

$$t_{R_2} = t_{R_1} \times \frac{2·89}{4·55} = 0·30 \text{ min } (1·8 \text{ sec}).$$

When $z = 10°$ the Ball & Olson (1957) method predicts a processing time that is 40% *greater* than that predicted by the Deindoerfer & Humphrey (1959) method.

(b) *If* $z = 8{\cdot}33°K$:

$$t_{R_2} = t_{R_1} \times \frac{1{\cdot}12}{0{\cdot}73} = 0{\cdot}029 \text{ min } (1{\cdot}7 \text{ sec}).$$

Taking $z = 8{\cdot}33°$, the Ball & Olson (1957) method predicts a sterilizing time that is 35% *less* than that calculated by the Deindoerfer & Humphrey (1959) method.

Example 2

The feed to an industrial fermenter is sterilized by heating for 90 min at 120°C (393·2°K). This time and temperature are adequate to destroy the most resistive organism in the feed, *B. stearothermophilus*. It is proposed to sterilize the feed by a new technique in which its temperature it raised rapidly to 140°C (413·2°K).

(1) What should be the holding time at the new temperature to destroy *B. stearothermophilus*? (2) To avoid interruption to the plant output during modification to the sterilizing equipment the feed is sterilized in a low pressure retort at 110°C (383·2°K). What should be the retorting time at 110°C?

(1) Ball & Olson (1957) method:

(a) *At* 140°C $\quad t_{R_{140}} \cdot 10^{(413{\cdot}2 - 394{\cdot}3)/z} = t_{R_{120}} \cdot 10^{(393{\cdot}2 - 394{\cdot}3)/z}$

For *B. stearothermophilus*, $z = 11°K$

$$t_{R_{140}} = 90 \times 10^{(393{\cdot}2 - 413{\cdot}2)/11} \text{ min}$$

$$= 1{\cdot}36 \text{ min.}$$

(b) *At* 110°C $\quad t_{R_{110}} = 90 \times 10^{(393{\cdot}2 - 383{\cdot}2)/11}$ min

$$= 736 \text{ min.}$$

(2) Deindoerfer & Humphrey (1959) method:

Assume that the two methods agree at 120°C. For *B. stearothermophilus* from Table 2,

(a) *At* 140°C

$$\frac{t_{R_1}}{t_{R_2}} = \frac{1{\cdot}61}{1{\cdot}58}$$

$$t_{R_2} = 1{\cdot}36 \times \frac{1{\cdot}58}{1{\cdot}61}$$

$$= 1{\cdot}34 \text{ min.}$$

(b) *At* 110°C

$$\frac{t_{R_1}}{t_{R_2}} = \frac{1{\cdot}34}{1{\cdot}58}$$

$$t_{R_2} = 736 \times \frac{1{\cdot}58}{1{\cdot}34}$$

$$= 868 \text{ min.}$$

In this example the two methods agree closely for an increase in temperature from 120° to 140°C, but there is a difference of 15% between the times predicted by the two methods when the temperature decreases to 110°C. This discrepancy is not serious in view of other uncertainties.

It should be emphasized that, in practice, the calculation of sterilization times is more complicated than these examples suggest. The temperature of the material to be sterilized varies with time in a way that may be difficult to describe analytically. In our examples we have used constant temperatures for clarity and simplicity.

Discussion

In the temperature range 110–140°C the methods of Ball & Olson (1957) and of Deindoerfer & Humphrey (1959) agree reasonably for sterilizations in which *B. stearothermophilus* is the important organism. In the case of sterilization involving *Cl. botulinum* there is a significant discrepancy between the predictions of the two methods, and the discrepancy is itself sensitive to the z value used for *Cl. botulinum*.

In the literature, the Ball & Olson (1957) method is most frequently applied to *Cl. botulinum* sterilizations, especially in food preservation, whilst the Deindoerfer & Humphrey (1959) method has, to my knowledge, been applied to *B. stearothermophilus* sterilizations only. This may explain why the incompatibility of the two methods has not been observed previously.

It is of interest to note that the experimental data which Levine (1956) used to calculate the E value for *Cl. botulinum* in Table 1 also yields the z value of 8·33°K used in Table 2. This means that the same experimental data may be used to predict two different temperature effects on the lethal rate, that based on the E value being incorporated in the Deindoerfer & Humphrey (1959) method, and that based on the z value appearing in the Ball & Olson (1957) method. The reason for this anomaly can be traced to an assumption made by Levine (expressed as his equation 7, Levine, 1956) which, effectively, converted to the experimentally observed temperature dependence of the lethal rate to a temperature dependence of the form contained in (our) equation (3).

In deciding which temperature dependence, i.e. which method, is preferable, we should, from a practical standpoint, use safety as the criterion. According to the calculations of example 1, when we use a z value of 10°K for *Cl. botulinum* the Ball & Olson (1957) method predicts a longer sterilizing time and, therefore, is safer, but the Deindoerfer & Humphrey (1959) method is safer when we take $z = 8{\cdot}33$°K. Because the value $z = 10$°K is most commonly used for *Cl. botulinum*, the method of Ball & Olson (1957) would seem to be preferable, but the problem of the discrepancy remains, and it is clear that we need more experimental evidence, particularly at high temperatures, before we can confidently perform sterilization calculations.

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Specific fungi as the causative agents of the sporadic disintegration of sulphited strawberries

J. C. DAKIN AND J. TAMPION

Summary. Fresh strawberries were inoculated with specific strawberry rotting fungi. When lesions of $\frac{1}{4}-\frac{1}{2}$ in. diameter had developed on the fruits, they were added to sulphite-preserved strawberries to constitute 25% of the whole contents. Periodically the pulps were examined and breakdown estimated on a four point scale. Of the ten species of fungi used only *Rhizopus stolonifer*, *R. sexualis* and *Mucor* sp. induced breakdown of the fruit to a puree-like consistency during storage. Two fungi failed to produce macerating activity and five failed to grow on the fruit before casual contaminants had outgrown them. *Botrytis cinerea*, the most common strawberry rotting fungus, did not cause breakdown. It is concluded that *Rhizopus* sp. and *Mucor* sp. are mainly responsible for the breakdown of sulphite-preserved strawberries and that the prevention of this trouble necessitates the exclusion of infected fruit. Practical recommendations for achieving this object are suggested. It is stressed that a major outbreak of this form of spoilage is still possible.

Introduction

In order to ensure a continuous supply of fruit throughout the year for jam manufacture, quantities of strawberries are preserved with sulphur dioxide and stored, until required, in wooden casks. Such fruit will normally remain in a satisfactory condition for at least a year but occasionally sporadic outbreaks of spoilage are encountered in which the fruit deteriorates in texture and may eventually disintegrate completely to a puree. Such pulp, although giving an organoleptically sound product after processing, is of limited value since it is considered that the presence of whole fruit, or at least recognizable pieces of tissue, is an essential requisite of a good retail jam.

This form of spoilage is extremely sporadic in its occurrence and variable in its degree of severity. Complete breakdown of the fruit may be reached at any time from a month to a year after sulphiting. Partial breakdown is often deceptive for the fruit may still appear to be sound in the barrel yet subsequently break down during the jam making process. Pulps partially damaged in this way may be more common than is generally

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appreciated for it is possible to differentiate between them and those made from overripe fruit, or those lacking in the appropriate quantity of added firming agent (calcium). Fully disintegrated pulps are, however, definitely characteristic of the spoilage under discussion.

The problem of the breakdown of sulphited strawberries has been under consideration for some time at this Association, although the agent of spoilage has eluded discovery until the present study. From the beginning it was thought likely that the trouble would be attributable to fungi either growing on the fruit, or in the storage containers whilst empty, prior to the pulp being introduced (Pandhi, 1953).

It was known that certain fungi could produce pectinolytic enzymes, in particular polygalacturonase, capable of destroying the pectin present in plant tissue. However, repeated efforts to detect the presence of polygalacturonase in disintegrated pulps failed, as did attempts to correlate breakdown with a high mould count. Pulp made from mouldy fruit (infectants not identified) also remained intact. Later work (Dakin & Tampion, 1965) showed that factors such as mechanical damage of the fruit, overripeness, low sulphur dioxide content, and delay in sulphiting (excluding microbially spoiled fruit), although adversely affecting the quality or wholeness of the fruit, were not primary causes of breakdown. The addition of commercial pectic enzyme preparations at activity levels normally considered to damage other fruit tissues (Bell, Etchells & Jones, 1955) had little effect on sulphited strawberries, although massive additions resulted in breakdown. Whilst polygalacturonase could not be detected in disintegrated pulps, the continued presence of the macerating factor in some pulps was demonstrated by adding their supernatant liquid to sound sulphited strawberries, which subsequently disintegrated. Controls, in which the supernatant liquid was first heated, remained sound, thereby indicating that the macerating factor was probably an enzyme.

A separate investigation by Staden (1964) in Holland, into the breakdown of sulphited strawberries indicated that an enzyme was responsible for the trouble, since breakdown could be prevented by an appropriate heat treatment, and that the strawberry itself was likely to be the producer of the enzyme.

A problem which would appear to be closely analogous to the breakdown of sulphited strawberries, concerns the softening of sulphited cherries. This fruit is mainly used in the preparation of maraschino and glacé cherries, and most of the American requirements are now grown in the States of Washington and Oregon. Here, the 1957 processed crop suffered extensive damage, softening not becoming apparent until several weeks after sulphiting, and in all a total of 11,800 barrels were lost, valued at over half a million dollars (Steele & Yang, 1960). Investigations by Weigand (1957) had previously shown that breakdown was associated with the presence of pectin splitting enzymes. Steele & Yang (1960) then found that cherries suffering from 'cats claw' disease (claw-like marks on the fruit) had a high polygalacturonase activity and that when 10% of infected berries were included with sound fruit, softening was apparent in 10 days. The polygalacturonase activity of these brines decreased rapidly until it was

not detectable after approximately 3 weeks. This activity could be inhibited initially with alkyl aryl sulphonate at a level of 250 ppm and softening of the fruit correspondingly prevented. It was further observed that the period of breakdown of the fruit paralleled the time of polygalacturonase activity and ceased once it had disappeared.

Previous work had already indicated that it was unlikely that the softening of sulphited cherries was associated with all cherry-rotting micro-organisms. Those responsible for 'cats claw' disease were clearly implicated but it was thought that other rots might also induce softening. Lewis, Pierson & Powers (1963) therefore undertook an investigation designed to indicate the responsible micro-organisms. Of a number of fungi examined, only three – namely *Cytospora leucostoma*, *Aspergillus niger* and *Penicillium expansum* – proved capable of softening cherries in the presence of sulphite. It was subsequently concluded that since the two last-mentioned fungi rotted a constant proportion of the cherry crop each year, whilst spoilage was sporadic and varied greatly in incidence between the years, only *Cytospora leucostoma* whose distribution pattern was correspondingly sporadic could be the primary agent of spoilage. These authors, however, did not find that polygalacturonase activity decreased in the brine, and also found that brine from softened cherries would subsequently soften sound cherries when placed upon them.

Failure to obtain any indication of the causative agent of the breakdown of sulphited strawberries during the present investigation raised the question of specific fungi being responsible. The difficulty of undertaking this study lay in the comparatively minor information which existed on the identity of the micro-organisms responsible for the spoilage of British-grown strawberries. The most informative paper was that by Lowings (1956) on fungal infections of Kentish strawberry fruits in 1955. This, however, only covered one year and was confined to the crop from Kent. It did not, therefore, provide an indication of the overall pattern of spoilage in the country or how it varied from season to season. However, on the basis of the information provided in this paper, and on advice received from Dr R. J. W. Byrde of the Long Ashton Research Station, a collection of fungi was assembled, mainly from the Commonwealth Mycological Institute and the Ditton Laboratory, all cultures being known to have been associated with the rotting of strawberries. Fungi were also isolated in the laboratory from rotten strawberries, from a number of localities, and included in the collection.

Materials and methods

Cultures

Fungi were isolated from spoiled fruit, or were received as pure cultures from other organizations, and were maintained on malt extract agar (Oxoid). The origin of the culture or the source of the rotted fruit from which it was isolated is given in Table 1.

Treatment of the fruit

Good quality strawberries, in a just-ripe state, of the cultivar Cambridge Favourite

were obtained from the East Malling Research Station. The calyces of the fruits were removed with care, and the fruit then placed in a single layer in clean aluminium trays, approximately ½ lb. of fruit per tray.

Two trays of fruit were then inoculated with one culture, this being achieved by scratching the individual fruits with a platinum wire contaminated with the appropriate culture. The trays were then stored at laboratory temperature (approximately 20°C), care being taken to reduce aerial contamination by means of an overall polythene cover, whilst not restricting air circulation unduly.

Growth of the culture, at the point of infection, was allowed to proceed until lesions of ¼–½ in. diameter had developed on the majority of fruits in a tray. Any fruits showing signs of casual infection, i.e. atypical growth or growth not originating at the point of infection, were discarded. Excessively over or under-spoiled fruits were also discarded.

When spoilage had developed to the required extent in a tray, the fruits were added to freshly sulphited strawberries in a 2-lb. Kilner jar, so that the diseased fruit constituted 25% of the whole. Duplicate jars were always prepared. The sulphited fruit had been prepared from sound strawberries on the day of inoculation, the quantity of sulphurous acid (6% solution) added being sufficient to give a final sulphur dioxide content of 2000 ppm. The jars were stored at approximately 20°C for a period of 6 months. At monthly intervals the degree of breakdown of the fruit was visually estimated using a scale where 0 represented no disintegration, and 1, 2 and 3, equalled one-third, two-thirds and complete disintegration, respectively.

The technique used to infect the fruit was normally successful. However, cultures spoiled the fruits at varying rates, so that the fruit infected by some had reached the required state of spoilage much earlier than others. The time between inoculation and sulphiting of the fruit is recorded in Table 1. Control samples were prepared daily, i.e. sound fruit of the same age as the infected fruit was added to sulphited fruit, in order to ensure that physiological changes in the fruit were not confused with disintegration arising from the activity of the respective infectant.

In order to demonstate whether the degree of spoilage of the fruit influenced the rate of subsequent breakdown, a subsidiary experiment was run in conjunction with the main experiment. In this a number of trays of fruit were infected with *Rhizopus stolonifer*. Trays of fruit were sulphited as before, but at daily intervals, with appropriate controls, until the remaining fruit was mostly spoiled and inedible. The resultant pulps were examined and assessed for degree of breakdown at monthly intervals over a 6-month period of storage.

Results

The figures given in Table 1 represent the average breakdown for the duplicate samples prepared. In nearly all cases breakdown proceeded at the same rate in each duplicate.

Although the inoculation technique was successful not all the cultures tested spoiled

TABLE 1. Breakdown of sulphite-preserved strawberries following innoculation with specific moulds.

Mould	Origin of culture or spoiled fruit	Time between inoculation and sulphiting (days)	Degree of breakdown*					
			1 month	2 months	3 months	4 months	5 months	6 months
Rhizopus stolonifer (1)	C.M.I.	2	2	3	3	3	3	3
Rhizopus stolonifer (2)	C.M.I.	3	2	3	3	3	3	3
Rhizopus sexualis (1)	C.M.I.	3	1	2	3	3	3	3
Rhizopus sexualis (2)	C.M.I.	4	1	2	2	3	3	3
Rhizopus sexualis (3)	C.M.I.	3	1	2	2	2	3	3
Mucor sp. (1)	D.L.	3	1	3	3	3	3	3
Mucor sp. (2)	D.L.	3	1	2	3	3	3	3
Mucor sp. (3)	D.L.	3	1	2	3	3	3	3
Mucor sp. (4)	S.H.R.I.	4	1	1	2	2	3	3
Botrytis cinerea	E.M.R.S.	3	0	0	0	0	0	0
Cladosporium herbarum	C.M.I.	4	0	0	0	0	0	0
Control	–	3	0	0	0	0	0	0
Rhizopus stolonifer (1)	C.M.I.	1	0	1	2	2	3	3
Rhizopus stolonifer (1)	C.M.I.	2	2	2	3	3	3	3
Rhizopus stolonifer (1)	C.M.I.	3	2	2	3	3	3	3

C.M.I. Commonwealth Mycological Institute, Kew; D.L. Ditton Laboratory, Kent; S.H.R.I. Scottish Horticultural Research Institute, Invergowrie; E.M.R.S. East Malling Research Station, Kent.

* 0 = No breakdown; 1 = one-third disintegrated; 2 = two-thirds disintegrated; 3 = completely disintegrated.

the fruit sufficiently quickly to reach the required degree of spoilage before casual contaminants had taken such hold that further study was invalidated. Isolates representing the following fungi fell into this category: *Aspergillus malignus*, *Mucor hiemalis*, *Trichoderma viride*, *Gloeosporium* sp. and *Rhizoctonia solani*.

Discussion

The results clearly demonstrate that when strawberries infected with *Rhizopus stolonifer*, *R. sexualis* and *Mucor* sp. are included in sulphited strawberry pulps all the individual fruits subsequently disintegrate to a puree-like mass over a period of a few months storage. In these experiments the quantity of infected fruit represented 25% of the whole and was not badly spoiled, the extent of rot per berry not exceeding one lesion of $\frac{1}{4}$–$\frac{1}{2}$ in. in diameter. Consequently the experiment was not unrealistic in relation to what may happen in practice when strawberries are being sulphited for jam manufacture. It is, however, also clear that the inclusion of far less than 25% partially spoiled fruit could cause breakdown or at least seriously affect the texture quality of the fruit over an average period of storage.

The results from sulphiting fruit at successive stages of spoilage by *Rhizopus stolonifer*, the most active fungus in producing breakdown, indicate that appreciable macerating activity is developed before there is much visible sign of mould growth on the fruit, and that allowing the rot to develop further does not produce a corresponding increase in macerating potential. This observation is in accordance with that by Meloche & Fabian (1955) who found that appreciable macerating activity was liberated by moulds before the colonies were visible to the eye. Whilst the present results are insufficient to establish details of the quantity of rot per fruit, or the percentage of infected fruit necessary to damage a pulp, it would not seem unreasonable to suggest from these findings that the inclusion of 5% of fruit with rots only just becoming visible (say covering a surface area of $\frac{1}{8}$ in. diameter) might be sufficient to spoil the texture of all the fruit in a pulp after a few months storage, or induce complete breakdown within a year.

The observation that breakdown proceeds progressively over at least 6 months, agrees with the findings of Lewis *et al.* (1963) that the macerating enzymes are not unduly inactivated by the presence of sulphite and will persist in a pulp for a considerable period of time.

Concerning the identity of the fungi found to be capable of inducing breakdown, of those tested only *Rhizopus stolonifer*, *R. sexualis* and *Mucor* sp. seem to be capable of causing this trouble in practice. Of the other strawberry pathogens included all grew too slowly on the fruit for their presence to be of practical significance, or they did not produce macerating activity. Nevertheless, it cannot be claimed that representative cultures of all the pathogens likely to cause strawberry rots were included in these tests and furthermore, cultures maintained in collections often lose their virulence. Again, if early infection of fruit occurs in the field, sufficient time might be available for growth

to develop. It is, therefore, possible in practice that fungi other than those actually found to induce breakdown here, may on occasion be responsible for this trouble.

One of the most interesting observations concerned the inability of *Botrytis cinerea* to induce breakdown. This at once explains why so many previous attempts to connect breakdown with fungal activity have failed. *Botrytis* is the most common cause of strawberry rot and consequently Howard mould counts on strawberries will mainly reflect its presence and that of *Sphaerotheca humuli*, a non-rotting, surface growing mould which Lowings (1956) found to contribute largely to the mould count on strawberries. Neither of these fungi cause breakdown but since their hyphae will normally be those observed and counted during the mould count, any correlation between the count and breakdown will be obscured. Similarly when a sample of naturally spoiled fruit is sulphited, breakdown will not normally occur because the spoilage agent will usually be *Botrytis*.

The non-involvement of *Botrytis* in breakdown also offers an explanation of the sporadic appearance of the trouble both on an annual and local basis, and also why breakdown appears to be lessening in frequency during recent years. The dominance of *Botrytis* over other strawberry rotting fungi has probably increased of late years so that Byrde (1967 personal communication) thinks that, in cool wet seasons at least, it is probably ten times more common than any other strawberry rotting fungus. *Rhizopus* and *Mucor*, in that order of occurrence, then follow. These views contrast with results obtained by Lowings (1956) who found that *Mucor piriformis* was more prevalent than *Botrytis* although it was thought at the time that the season was atypical. The reason for the recent increased dominance of *Botrytis* is not certain although the succession of cool damp summers is likely to be the principal cause. *Rhizopus* on the other hand is favoured by hot summers and hence it becomes obvious why breakdown varies so considerably from season to season and, of course, between localities. In seasons favouring *Botrytis*, breakdown will be minimal but in hot seasons an increase is likely. Weather can also be intensively local in Britain and hence the extremely sporadic occurrence of breakdown is hardly surprising.

Suggestions for the prevention of breakdown of sulphited strawberries have taken two forms. Staden (1964) suggests the heat treatment of all fruit (55°C for 15 min) prior to sulphiting. Hinton & Pandhi (1958) recommend policies designed for the general elimination of mould growth. The former suggestion is based on the belief that the macerating enzyme is produced by the fruit itself. If this was true then all sulphited strawberries should break down. Since they do not, and as these present studies indicate specific strawberry rotting fungi as the causative agents, general heat treatments would appear to be unnecessary primarily on the grounds that most of the strawberries sulphited would have been so treated quite unnecessarily.

The authors, therefore, suggest that measures designed to eliminate or reduce fungal contamination of the product are still the most valid means of control. It is clear from the results, however, that more emphasis should be placed on the use of mould-free

fruit, and this inevitably means good quality freshly picked strawberries. Provided such fruit is obtained and sulphited on the same day of picking then trouble from breakdown should not develop. On the other hand if low grade fruit is used, or delays between picking and sulphiting exceed 12 hr, the danger of breakdown or at least deterioration of texture quality, will continue to be present.

Practical recommendations for controlling breakdown

The establishment by the present study that the breakdown of sulphited strawberries results from the infection of the fruit by specific fungi enables past recommendations for its avoidance to be re-assessed and for a more precise preventative policy to be suggested. Past measures (Hinton & Pandhi, 1958) were aimed at the general prevention of contamination of the product with moulds. This policy is still correct but there should now be a change of emphasis towards the prevention of fungal infection of the fruit and the elimination of infected fruit from the product. The following suggestions are, therefore, directed towards the attainment of this objective.

(1) The production of sound healthy fruit inevitably involves the use of a thorough and carefully thought out spraying programme. This, of course, is an agricultural matter but provided crops are grown under contract then at least a watching brief can be held over this aspect. Manufacturers should at least assure themselves that the grower understands and is equipped for this task.

(2) Having produced a clean fruit crop the problem is then to ensure that it reaches the pulping stage in this state. The time between picking and processing is very critical and should be as short as possible. Inevitably infection of a percentage of the fruits will have occurred during growth and picking, and such infection particularly by *Rhizopus* and *Mucor* will spread with considerable speed until the fruit is sulphited. It will be seen from the results that one day after infection was sufficient for significant macerating activity to be developed by *Rhizopus*.

Ideally the fruit should be plugged and sulphited on the field but certainly picking and sulphiting should take place on the same day. If delay is unavoidable then chilled storage is most advisable. Judging from the samples of broken-down pulp which the authors received, spoilage is more common in Holland than in this country. If this is true, it could arise from the Dutch practice of putting most of their fruit through auctions, with the inevitable delays permitting fungal growth, and hence subsequent damage to the pulp.

(3) Strawberries when they have just become ripe are less easily damaged by picking than when they have become fully or over-ripe. Furthermore, there has been less time for the just-ripe fruit to become infected or for that infection to develop. For this reason the fruit should be picked as soon as it is ripe and the crop should be gone over at least three times a week. Slightly under-ripe fruit is to be preferred to over-ripe fruit on these grounds.

(4) Unavoidably, some of the fruit coming in from the field, and after a short delay

in their containers, will be infected with *Rhizopus* and *Mucor*. The elimination of this fruit from the pulp is essential and the workers employed in plugging must be instructed to discard every fruit with even the smallest sign of fungal growth. This involves trouble on their part and hence a scheme for bonus payment for discarded fruit, i.e. on a weight basis, is suggested. It should be remembered that macerating activity is secreted very early in the growth of a fungal colony.

(5) It will be clear by now that the past practices of using end-of-season fruit and fruit unfit for the retail market are not consistent with the above aims. End-of-season fruit is frequently over-ripe, because the crop is not being picked over regularly, and usually treated carelessly because of its low value. Unmarketable fruit again may be subject to delays whilst buyers wait for lower prices. These sources of fruit are, therefore, not suitable for pulp manufacture but they are perfectly acceptable for the manufacture of fresh fruit jam. *In this respect the situation requires the reversal of past practice in that the best of the crop and the high quality fruit should now be sulphited whilst the second grade material should be reserved for fresh fruit jam manufacture.*

(6) The above considerations all relate to getting the fruit in a sound healthy state to the sulphiting stage. Such efforts will be wasted, however, if the sulphiting procedure and after-care of the product are neglected. Firstly, as stressed by Hinton & Pandhi (1958) all utensils and barrels must be clean. The use of inner plastic containers is advisable. Any spilled strawberry juice or fragments will certainly support the growth of *Rhizopus* and *Mucor* and maybe other fungal producers of macerating activity. Secondly, during the sulphiting procedure the solution should be added portion-wise as the barrel is gradually filled, to assist in the quick and even distribution of the preservative throughout the whole barrel. Also to further this end, once the barrel is full it should be headed up and rolled to help in mixing the contents. In this way, sulphite free pockets of strawberries are avoided in which fungal infection could soon develop if left undisturbed.

The Dutch recommendations of Staden (1964) for heating the fruit (55°C for 15 min) before sulphiting are clearly a positive method for preventing breakdown. It is, however, felt that the procedure would be troublesome in practice and lead to delays in sulphiting when, of course, build-up in fungal growth and macerating activity might be greater than that which the heat proceess could destroy. Furthermore, it is not thought that the supposition of these workers that the strawberry is the producer of the macerating enzyme is correct. Consequently, if heat treatments became general, heat would often be applied where it was not needed. It is, therefore, felt that the better approach to this problem is the elimination by all possible means of fruit infected with the fungi capable of causing breakdown, except perhaps in the case of poor quality strawberries when heat might be used as an alternative to discarding the fruit.

In conclusion, it is necessary to consider the question of the likelihood of future outbreaks of spoilage. There has undoubtedly been a decline in this trouble during the past fifteen or so years. This is no doubt attributable to some of the new fungicides and

the generally improved methods of horticulture resulting in a better and cleaner fruit crop. Some of this decline, however, may be linked with the weather. There has been a series of cool damp summers which favour the growth of *Botrytis* in relation to *Rhizopus* and *Mucor*. A hot summer could result in a very considerable increase in the incidence of rots by the two last-mentioned fungi, when of course, breakdown might become correspondingly intensified. Furthermore, there is evidence that both *Rhizopus* and *Mucor* (Byrde, 1967 personal communication) are less easily controlled by the newer fungicides than is *Botrytis* and hence should the weather favour the spread of these fungi, their control may not be easy.

A major outbreak of breakdown of sulphited strawberries is thus a distinct possibility in the future and an incident similar to that of the 1954 Oregon and Washington cherry crop cannot be entirely discounted. However, if manufacturers rigorously apply the recommendations made here, the impact of such a calamity would be greatly lessened, and in general an improvement in the texture quality of most sulphited strawberries could be expected.

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Moniliella acetoabutans: some further characteristics and industrial significance

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Summary. The distribution of *Moniliella acetoabutans* and its behaviour as a spoilage agent of acetic acid preserves, is discussed. The ability of this fungus to tolerate and utilize acetic acid as a nutrient source places it in a unique category. Its characteristics according to the yeast classification of Lodder and Kreger-van Rij are given. The levels of sulphur dioxide, methyl and propyl *para*-hydroxybenzoates, and sorbic acid which will inhibit this mould at pH 3·3, in the presence of 1·0% acetic acid, have been determined. Sulphur dioxide at 100 ppm, the maximum legal limit, inhibits the mould but its value in practice would be doubtful because of its tendency to combine with aldehydes and so become microbially inactive. Sorbic acid offers a means of controlling spoilage by this mould, were it to be legally permitted.

Introduction

This note records some additional observations on *Moniliella acetoabutans*, proposed by Stolk & Dakin (1966), and discusses briefly the industrial significance of this mould as a food spoilage agent.

Distribution

To the present time, *M. acetoabutans* has only been recovered from spoiled acetic acid preserves and when found, it normally exists as the sole agent of spoilage. In relation to the whole pattern of microbial spoilage of these foods in Great Britain, this mould is a rare contaminant and only ten separate instances have been encountered by the authors in 15 years. Nevertheless such spoilage, when it occurs, may be severe and result in considerable loss in production, and it would appear that the incidence of cases is increasing.

Almost invariably spoilage is confined to products which have been previously stored in bulk in the finished state for some time prior to final packing and retail distribution, which implies that the bulk storage vessels are the most likely sources of contamination. The types of preserves spoiled by *M. acetoabutans* and the number of

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known instances of each are as follows: brown fruit sauce, four; mint sauce, three; sweet pickle, one; gherkins, one; malt vinegar, one. There is no apparent pattern in the geographical distribution of these spoilage outbreaks and indeed Etchells (see Stolk & Dakin, 1966) has isolated the mould from spoiled pickles in America. It also seems very likely that the pickle spoilage mould isolated in America by Faville & Fabian (1949) and identified as *Geotrichum candidum* was in fact *M. acetoabutans*.

Form of spoilage

M. acetobutans grows at the product–air interface as a raised white mycelial mat, with a velvet-like surface, which later may turn dark brown or black owing to the production of chlamydospores. The growth has tensile strength which readily distinguishes it from yeast on superficial examination. A fruity or ester-like odour is often present, reminiscent of ethyl acetate. In the spoiled retail vinegar examined, continuous surface growth resulted in a tube of spent mycelium, of the same diameter as the bottle neck, gradually descending into the contents, which eventually became completely filled with hyphae. This intriguing form of growth is also occasionally exhibited by acetobacter when spoiling vinegar.

The microbial spoilage of acetic acid preserves can normally be prevented by ensuring that the acetic acid content of the product exceeds 3·6%, calculated as a percentage of the volatile constituents (Dakin, 1962). Unfortunately *M. acetoabutans* tolerates an acetic acid content considerably greater than this figure, provided oxygen is available, and spoilage has been found in products with acid contents in excess of 5%.

The acetic acid tolerance of *M. acetoabutans* is its most unusual characteristic. Microorganisms capable of tolerating more than 1% acetic acid are extremely rare and it would appear that only two species of yeast (Dakin & Day, 1958), four lactobacilli, certain acetobacter and this mould are capable of so doing. Other moulds are found occasionally on acetic acid preserves but in all the samples so far examined the product had first been colonized by a yeast upon which the mould subsequently grew. *M. acetoabutans* thus seems to be unique amongst moulds in being able to spoil these products directly. This mould utilizes acetic acid as a nutrient source and it has been observed by the authors to lower the acetic acid content of vinegar from more than 4% to 0·1% in 4 months at room temperature. The utilization of acetic acid is the reason for the specific epithet *acetoabutans* for 'abutor' means to use a thing which is consumed in the use.

Additional characteristics

Because of the similarity of *Moniliella* to *Trichosporon*, *M. acetoabutans* was examined and described according to the yeast classification and methods of Lodder & Kreger-van Rij (1952).

Growth in malt extract

After 3 days at 20°C a thick loose pellicle, with a few dry areas of mycelium and

pseudomycelium. Single free cells which may be blastospores—round to oval (3·5–6·6) × (4·5–7·8) μ; or pseudomycelial cells—cylindrical 5·5 × (15–20) μ. Hyaline chlamydospores, 7·5 μ, which may be showing the first signs of brown pigmentation, may be formed terminally on the hyphae or on free pseudomycelial cells.

After 1 month at 20°C, the pellicle occupies half the liquid contents and consists of mycelium and pseudomycelium, 4 μ in diameter, with terminal and intercalary hyaline or brown chlamydospores. Arthrospores, 5·5 × 7·5 μ may be present.

Streak culture on malt agar

The description conforms to the original (Stolk & Dakin, 1966).

Slide culture

After 3 days at 20°C, abundant hyaline mycelial and pseudomycelial growth at the outer edges of the cover slip, diminishing to no growth at the centre. The germination of the blastospores by protrusion of a tube from the spore is very distinctive in the region of restricted growth.

Sporulation

No spore (sexual) formation has been observed on potato and carrot wedges.

Fermentation:	Glucose +	Maltose +
	Galactose –	Lactose –
	Sucrose +	
Assimilation:	Glucose +	Maltose +
	Galactose –	Lactose –
	Sucrose +	

Assimilation of potassium nitrate: Negative. This medium induced a rapid and profuse formation of black chlamydospores.

Ethanol as sole source of carbon: Slow growth with the formation of a thin pellicle.

Inhibition by preservatives

The concentrations of certain food preservatives which will inhibit *M. acetoabutans* in low acetic acid content preserves were determined in media resembling such foods in relation to pH and acetic acid content.

Method

Agar slopes of the following composition were prepared (% w/w) in 1-oz. McCartney bottles; malt extract, 1·5; yeast extract, 0·2; mycological peptone, 0·3; acetic acid, 1·0; agar, 2·5 (pH was 3·3).

Methyl and propyl *para*-hydroxybenzoates, sorbic acid and sulphur dioxide were incorporated respectively in the slopes to provide a range of concentrations. The two

hydroxybenzoates and sorbic acid were added at levels up to 1000 ppm in successive stages of 100 ppm, prior to sterilizing by steaming for 10 min. Sulphur dioxide was added aseptically after sterilization, in the form of a solution of potassium metabisulphite equivalent to 10,000 ppm to give slopes containing 50, 100, 150 and 200 ppm, respectively.

The slopes were streaked separately with five isolates of *M. acetoabutans*, incubated at 20°C and periodically examined for signs of growth over a period of 3 months.

Results

The figures in Table 1 show the highest level of preservative at which some of the isolates grew, and the lowest level of preservative at which all five isolates were still inhibited, after the full period of incubation.

TABLE 1. Inhibition of *M. acetoabutans* by preservatives

Preservative	Not inhibited by:	Inhibited by:
Sulphur dioxide	50 ppm	100 ppm
Methyl *para*-hydroxybenzoate	1000 ppm	–
Propyl *para*-hydroxybenzoate	200 ppm	300 ppm
Sorbic acid	300 ppm	400 ppm

Discussion

The unusually high acetic acid tolerance of *M. acetoabutans* makes it a potential hazard to the pickle and sauce manufacturing industry. The addition of acetic acid to these foods in excess of that normally assumed to ensure microbial stability, namely 3·6% expressed as a percentage of the volatile constituents, is not acceptable on organoleptic grounds and hence this offers no means of control. The Preservatives in Food Regulations (1962) permit the addition of 100 ppm sulphur dioxide or 250 ppm methyl or propyl *para*-hydroxybenzoate to pickles and sauces. The above results indicate that this level of either of the hydroxybenzoates would be insufficient to ensure the protection of the food against this mould. The permitted level of sulphur dioxide was, however, sufficient to prevent growth in the medium used experimentally. The difficulty of using relatively low levels of sulphur dioxide as a preservative lies in its tendency to combine with sugars or other aldehydes whence it becomes microbially inactive. In the medium used above only 17% of the sulphur dioxide present became combined, whilst in many acetic acid preserves 75% or more may be so rendered inactive (Dakin, 1963). Sulphur dioxide would not, therefore, necessarily inhibit *M. acetoabutans* in these foods.

Sorbic acid is not at present permitted in pickles and sauces but should any future review of the preservative regulations so allow its use, then the levels normally con-

sidered to be acceptable in food—namely 800–1000 ppm—would appear from the above results to offer complete protection against this mould. This would be of some value in an industry where traditional methods of production often render bacteriological control difficult.

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The dehydration of foods in edible oil *in vacuo*

I. Stability of the drying medium

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Summary. Arachis oil, which was used as the medium for dehydration, showed considerable resistance to autoxidation when heated alone under the conditions employed in the drying of foodstuffs. The processing of cabbage, lean horse meat and herring had the effect of increasing the stability of the drying medium. The oil which adheres to the surface of the products was even more stabilized. Continuous use of the oil did not result in a rise in its content of free fatty acids. Neither the drying medium nor the adherent oil became rancid after storage for many weeks, although susceptibility to autoxidation was increased. The synthetic antioxidants BHT and sesamol, added to the arachis oil before heating, acted synergistically with the natural antioxidants of the foodstuffs, and gave rise to a striking improvement in the stability of the oil. The net protein utilization of herring dried in arachis oil *in vacuo* (73) was as high as that of freeze-dried herring (70).

Introduction

A method for the drying of foodstuffs by heating in edible oils *in vacuo* was first described by Platt & Heard (1944). Details of the process are given in a British Patent Specification (Platt & Heard, 1944). A similar procedure was patented by Zimmermann (1951) in the United States, and the use of a vacuum-oil process for the dehydration of meat in New Zealand has been reported by Vere-Jones (1957).

The method has many attractive features: the apparatus required is simple, drying is effected quickly at a relatively low temperature, and the dried material is easily reconstituted. Furthermore, the product retains its nutritive value, protein quality being little affected during dehydration, and vitamin losses small (Pellett, 1958).

The suggestion that dehydration in edible oil under vacuum might be a convenient and practicable method for the preservation of food in the developing countries (Platt, Heard & Pellett, 1958) prompted the present investigation.

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The principle of the method is that the foodstuff, suitably prepared, is immersed in an edible oil or fat at about 80–85°C, and the water removed under reduced pressure. Dehydration takes approximately 30 min. A small amount of oil adheres to the product.

One aspect of the method which, so far, has received no attention, is the risk of autoxidation of the dehydrating medium. This could present a major problem in deterioration if the dried material were stored for any length of time, with the attendant danger of rancidity on prolonged exposure to oxygen.

In the present investigation, a study was made of the effect of drying different foodstuffs and the use of antioxidants on the stability of the oil used as the dehydrating medium after processing and during storage, with a view to predicting the shelf-life of the products. The effect of processing on the biological value of a protein-rich food was also studied. Herring was used for this experiment since it is valued as a source of high quality protein and also contains a considerable quantity of highly unsaturated fat.

The susceptibility to autoxidation of the lipids of the dried product will be the subject of a future report.

Experimental

Materials

As the dehydrating medium, arachis oil was chosen, as it is relatively cheap and abundant, has a pleasant taste and a good natural protection against deterioration.

Three foodstuffs of very different composition and texture were dried, viz. cabbage (a leafy vegetable), horse flesh (a lean meat) and herring (an oily fish).

The antioxidants employed were butylated hydroxy toluene (BHT) and sesamol.

Procedure

A quantity of the oil (approximately 3 litres) was heated fifteen times under the conditions to be employed in subsequent experiments.

Each experiment involved fifteen dehydrations using the same batch of oil for successive loads of the foodstuff. In all experiments in which an antioxidant was used, the addition was carried out as follows: a quantity of the antioxidant calculated to give 0·05% (w/w) in the oil was dissolved in 10 ml of absolute alcohol. This solution was added to the hot oil and mixed with it thoroughly prior to the immersion of the foodstuff. It should be pointed out that the relatively high concentration of antioxidant was chosen primarily for experimental and comparative purposes. The reason for using synthetic sesamol, an antioxidant not commonly employed in the food industry, was to measure its effectiveness, in comparison with BHT, in this particular method of dehydration; sesame oil, which contains sesamol in free and bound form, might be used alone, or in combination with other vegetable oils, as a dehydrating medium.

Samples of the oil from the dehydration vessel and corresponding samples of the dehydrated products were taken after the first, fifth, tenth and fifteenth dehydrations. The oil samples (hereafter referred to as 'dehydrating oil') were centrifuged to remove

any particles of food, and stored in glass-stoppered tubes. The dried foodstuffs were stored in screw-capped glass bottles. All samples were kept in darkness at 25°C, along with fresh samples of the various batches of arachis oil used in the experiments. The oil adhering to the surface of the products was removed by shaking the material gently with an appropriate volume of peroxide-free A.R. diethyl ether. After centrifugation the oil (hereafter referred to as 'adherent oil') was freed from solvent by heating on a water bath at 50°C under reduced pressure. At regular weekly intervals, aliquots of the stored fresh oil, the dehydrating oil and the adherent oil extracted at that time, were submitted to various tests and analyses.

Measurement of susceptibility to autoxidation

The length of the induction period which precedes the rapid autoxidation of fats and oils was used as a criterion for estimating the susceptibility of the oil samples to rancidity, and for measuring the effectiveness of the antioxidants (Olcott & Einset, 1957). The various oil samples were incubated in a constant temperature draught oven at 70°C. The end of the induction period, estimated to the nearest half day, was detected by an acceleration in the increase in weight, and arbitrarily chosen to be the time which had elapsed from the start of the experiment until the samples had gained 0·2% in weight. At this point, all samples had begun to gain weight rapidly, and were rancid in odour.

Gas–liquid chromatography

Linoleic acid is a major constituent fatty acid of arachis oil, and is the component likely to decrease in amount if autoxidation occurs. For this reason, gas–liquid chromatographic analysis of the oil used for dehydration was undertaken, and interest was centred on the linoleic acid content of the oil after different treatments.

Samples taken after the first and fifteenth dehydrations only were analysed. The oil was saponified by refluxing for 2 hr with 0·5 N KOH in methanol. A few crystals of quinol were added to minimize oxidation. Non-saponifiable material was removed by washing once with petroleum spirit (boiling point 60–80°C), and the fatty acids extracted with petroleum spirit after acidification with 5 N H_2SO_4. Methyl esters were prepared by refluxing for 1 hr with 2% (v/v) H_2SO_4 in methanol. Gas–liquid chromatography was carried out on an argon gas chromatograph incorporating a strontium detector (W. G. Pye & Co. Ltd, Cambridge). The methyl esters were separated at 185°C on a column packed with 15% polyethylene glycol adipate on 100–120 mesh Celite. For identification of all fatty acids, retention times, relative to methyl stearate, were compared with those of pure reference compounds or with published values (Farquhar *et al.*, 1959). Measurement of peak areas on the chromatograms by triangulation gave the percentage of each component fatty acid in the lipid sample.

Lipids were extracted from fresh horse meat and herring with a 2:1 mixture of chloroform and methanol, and the extract washed with 0·05% $CaCl_2$ (Folch, Lees & Sloane-

Stanley, 1957). Aliquots of the lipid extracts were rapidly evaporated to dryness on a water bath under reduced pressure, and submitted to gas chromatographic analysis.

Determination of free fatty acids

The free fatty acid content of a fresh sample of arachis oil, and of oil used for fifteen successive dehydrations of cabbage and meat, was measured by the method of the Association of Official Agricultural Chemists (1955).

Measurement of protein quality

The net protein utilization (NPU) of a diet made with herring dried in oil under vacuum was compared with that of a diet made with herring freeze-dried by the method of Payne, Miller & Platt (1961). NPU was determined by the shortened method of Miller & Bender (1955). Nitrogen was estimated by a macro-Kjeldahl method with metallic mercury as a catalyst.

Results and discussion

Stability of arachis oil

Accelerated tests indicated that the repeated heating of arachis oil, with exposure to atmospheric oxygen at intervals between the heating operations, resulted in a progressive decrease in stability (Table 1). The effect of storage, on the other hand, was slight, samples kept for 7 weeks showing little change in the induction period.

TABLE 1. Effect of repeated heating, under vacuum, and of storage, on the stability of arachis oil

Treatment	Induction period (days)				
	Before storage	After 2 weeks	After 4 weeks	After 7 weeks	After 16 months
Unheated	6·5	7·5	7·0	7·5	3·0
First heating	6·0	7·0	6·0	6·0	3·5
Fifth heating	4·5	4·0	3·5	3·0	1·5
Tenth heating	3·5	3·0	3·0	3·0	1·5
Fifteenth heating	2·5	2·5	2·5	2·0	1·0

No alteration in the linoleic acid content of the oil could be detected, even after repeated heating and storage for a period of 8 weeks. The presence in arachis oil of a substantial amount of α-tocopherol (Deuel, 1951a) and some amino acids (Subramanian & Ramakrishnan, 1966) may account for its marked resistance to autoxidation. These constituents may assume greater importance when the oil is used in the

dehydration of foodstuffs, particularly if antioxidants are used. Amino acids have been shown to have a synergistic effect on phenolic type antioxidants, to which group both BHT and sesamol belong (Deuel, 1951b).

Dehydration of cabbage

The drying of cabbage imparted a greater stability to the oil used in dehydration (Table 2). Although the values for induction period showed similar changes with repeated heating and storage to those shown by the oil heated alone, they were consistently higher.

TABLE 2. Dehydration of cabbage: effect on the stability of the dehydrating oil

Treatment	Induction period (days)							
	Before storage	After 3 weeks		After 6 weeks		After 9 weeks		After 8 months
	Dehydrating oil	Dehydrating oil	Adherent oil	Dehydrating oil	Adherent oil	Dehydrating oil	Adherent oil	Dehydrating oil
Unheated	8·0	7·5	–	7·5	–	6·0	–	2·5
First dehydration	7·0	7·5	8·5	7·0	8·0	6·5	7·0	4·0
Fifth dehydration	6·5	6·5	6·5	6·0	6·0	4·5	4·5	3·0
Tenth dehydration	5·5	5·0	7·0	5·0	6·0	4·0	4·5	2·5
Fifteenth dehydration	5·0	5·0	6·0	5·0	5·0	3·5	4·0	2·5

This small improvement in stability might be attributed to the presence in cabbage of natural antioxidants; leafy vegetables have been shown to contain significant amounts of α-tocopherol (Deuel, 1951a).

The adherent oil, washed from the surface of the dried cabbage, was found to have an even greater resistance to autoxidation than had the corresponding samples of dehydrating oil, again suggesting that antioxidant material had been transferred from the product to the drying medium.

Dehydration of meat

With the drying of horse meat, the dehydrating oil developed a dark red-brown colour indicating that it had become contaminated with haem compounds which abound in muscle tissue. Haematin proteins are well known as powerful catalysts of the oxidation of unsaturated fatty acids (Tappel, 1955); it has, in fact, been claimed

that haem compounds represent the most important class of autoxidation catalysts (Watts, 1954). It was surprising, therefore, to find that the samples of the drying medium were no more susceptible to oxidation than the unheated sample of arachis oil (Table 3). Even after fifteen successive drying operations, the induction period of the oil was not significantly altered.

TABLE 3. Dehydration of meat: effect of antioxidants on the stability of the dehydrating oil

Treatment	Induction period (days)		
	Without additive	BHT	Sesamol
Control*	6·0	6·5	8·0
First dehydration	6·0	11·0	9·5
Fifth dehydration	5·5	8·0	9·5
Tenth dehydration	5·0	8·5	9·5
Fifteenth dehydration	5·5	7·0	9·5

* Control value determined before addition of antioxidants.

It has previously been noted (Lips, 1951) that fats recovered from fried or roasted meats have a marked resistance to oxidation, although the colour is darker, and the odour and flavour stronger. The conditions used in the present experiments resemble a frying operation, with limited exposure to atmospheric oxygen. It has also been shown that stabilization of fat can result from heating in contact with protein and carbohydrate (Evans *et al.*, 1958), and that antioxidants are produced in meat by prolonged cooking at temperatures above 100°C (Zipser & Watts, 1961).

It is possible that antioxidants were formed in the meat in the present study, although the temperature employed was no more than 85°C, and that these contributed to the stabilization of the drying medium. The addition of BHT or sesamol, at a concentration of 0·05%, to the oil prior to dehydration proved equally effective in improving its stability. All samples, in general, retained a satisfactory stability on storage (Tables 4, 5 and 6). Susceptibility to oxidation of the oil samples containing no additive was slightly increased after storage for 7 weeks. Those to which BHT or sesamol had been added, on the other hand, showed no such change with time.

The oil adhering to the dried product behaved rather differently from the drying medium in that greater stability was achieved as the number of dehydrations was increased. Both antioxidants had a striking effect on the induction period of the adherent oil. In this case, however, BHT was found to be more effective than sesamol and, with BHT only, resistance to autoxidation appeared to increase on storage.

The possibility of the production of material with antioxidant properties from protein and carbohydrate has already been suggested. BHT might well act synergistically

TABLE 4. Dehydration of meat: effect of antioxidants on the stability of the dehydrating oil (samples stored for 2 weeks)

Treatment	Induction period (days)			
	Dehydrating oil		Adherent oil	
	Without additive	BHT	Without additive	BHT
Control*	5·5	7·5	–	–
First dehydration	5·0	10·0	3·0	21·5
Fifth dehydration	5·0	10·0	4·0	–
Tenth dehydration	5·0	12·0	–	13·5
Fifteenth dehydration	5·0	11·0	13·5	15·5

* Control value determined before addition of antioxidants.

TABLE 5. Dehydration of meat: effect of antioxidants on the stability of the dehydrating oil (samples stored for 4 weeks)

Treatment	Induction period (days)					
	Dehydrating oil			Adherent oil		
	Without additive	BHT	Sesamol	Without additive	BHT	Sesamol
Control*	5·0	7·0	8·0	–	–	–
First dehydration	5·0	8·5	11·5	3·5	24·0	11·5
Fifth dehydration	5·0	8·5	10·0	5·5	15·0	11·5
Tenth dehydration	4·5	9·5	10·0	–	15·0	13·5
Fifteenth dehydration	5·5	9·0	9·0	9·5	15·5	14·5

*Control value determined before addition of antioxidants.

with such material, thereby affording an even greater protection to the adherent oil, and hence to the product, against deterioration.

Dehydration of fish

Herring was chosen as the third material for dehydration, as it was expected to pose another type of problem in the drying of foodstuffs by the vacuum-oil process. Herring may contain as much as 20% fat which liquifies at the temperature of dehydration, and is particularly rich in the readily oxidized polyunsaturated fatty acids.

TABLE 6. Dehydration of meat: effect of antioxidants on the stability of the dehydrating oil (samples stored for 7 weeks)

Treatment	Induction period (days)					
	Dehydrating oil			Adherent oil		
	Without additive	BHT	Sesamol	Without additive	BHT	Sesamol
Control*	5·0	7·5	7·5	–	–	–
First dehydration	4·0	10·5	10·5	3·0	27·0	8·5
Fifth dehydration	4·0	11·5	10·5	4·5	18·5	8·0
Tenth dehydration	3·5	10·5	9·0	–	16·5	11·0
Fifteenth dehydration	4·5	12·5	9·5	8·5	19·5	10·5

*Control value determined before addition of antioxidants.

It was observed that during dehydration, the quantity of oil in the dehydration vessel was increasing after each operation, and at the end of fifteen dehydrations, the amount of oil was noticeably greater than at the beginning. Some of the herring lipids had obviously been transferred to the dehydrating medium during the drying process. This transfer was confirmed by gas–liquid chromatography (Table 7). Analysis of the dehydrating oil corresponding to the fifteenth dehydration revealed the presence of some unsaturated fatty acids, characteristic of herring lipids, which were absent from fresh arachis oil. Consequently it was expected that the keeping properties of the oil would be adversely affected.

The results of the tests, presented in Table 8, show the effect of dehydration and of the antioxidants added prior to dehydration, on the stability of the oil. In contrast to the behaviour of arachis oil heated alone under the conditions of the experiments (Table 1), no decrease in stability of the drying medium occurred. All samples, without antioxidants, were found to have an induction period similar to that of the control.

The most obvious explanation of this finding is that herring oil has a relatively high content of α-tocopherol (Einset, Olcott & Stansby, 1957). The release of α-tocopherol from successive batches of herring appears to have been sufficient to protect the oil from the adverse effects of repeated heating. Furthermore, this protection was extended over a storage period of 9 weeks (Tables 9, 10 and 11).

The addition of antioxidants to the oil had a beneficial influence on stability, sesamol appearing slightly superior to BHT in its effectiveness. The oil, during storage, lost its stability to a small extent only, due presumably to the antioxidants having been partly consumed. After storage for 11 months, however, enough remained to

TABLE 7. Fatty acid composition of arachis oil and total herring lipids

Fatty acid	Percentage of total fatty acids		
	Fresh arachis oil	Herring lipids	Arachis oil after fifteen dehydrations
14 : 0	tr	8·6	1·9
16 : 0	7·8	14·4	10·C
16 : 1	—	6·0	1·6
17 : 0	—	1·5	—
17 : 1	—	0·6	—
18 : 0	2·8	0·6	2·4
18 : 1	64·9	8·7	55·8
18 : 2	20·2	1·2	14·4
18 : 3	tr	1·6	0·6
18 : 4	—	8·2	0·9
20 : 0	1·1	—	1·2
20 : 1	1·0	11·4	3·6
22 : 0	2·2	—	1·4
22 : 1	—	18·0	4·5
20 : 5	—	8·3	1·7
22 : 5	—	4·5	—
22 : 6	—	6·2	—

The fatty acids are designated $x : y$, where x gives the number of carbon atoms and y the number of double bonds in the molecule. —, The fatty acid was not detected; tr, the fatty acid was present but represented less than 0·2% of the total fatty acids.

TABLE 8. Dehydration of herring: effect of antioxidants on the stability of the dehydrating oil

Treatment	Induction period (days)		
	Without additive	BHT	Sesamol
Control*	7·0	9·0	9·0
First dehydration	6·5	11·0	9·0
Fifth dehydration	7·5	10·5	10·5
Tenth dehydration	8·0	11·0	14·0
Fifteenth dehydration	7·0	10·5	14·5

*Control value determined before addition of antioxidants.

TABLE 9. Dehydration of herring: effect of antioxidants on the stability of the dehydrating oil (samples stored for 3 weeks)

Treatment	Induction period (days)					
	Dehydrating oil			Adherent oil		
	Without additive	BHT	Sesamol	Without additive	BHT	Sesamol
Control*	9·0	9·0	9·0	—	—	—
First dehydration	6·0	11·0	10·5	19·0	29·0	31·0
Fifth dehydration	7·0	12·0	11·0	11·0	29·0	31·0
Tenth dehydration	7·0	10·5	12·5	13·5	19·0	22·5
Fifteenth dehydration	6·5	10·5	14·5	8·0	18·0	22·5

*Control value determined before addition of antioxidants.

TABLE 10. Dehydration of herring: effect of antioxidants on the stability of the dehydrating oil (samples stored for 6 weeks)

Treatment	Induction period (days)					
	Dehydrating oil			Adherent oil		
	Without additive	BHT	Sesamol	Without additive	BHT	Sesamol
Control*	8·0	8·0	8·0	—	—	—
First dehydration	5·5	9·0	8·0	18·5	23·5	34·0
Fifth dehydration	7·0	10·0	10·0	7·5	19·5	30·5
Tenth dehydration	7·0	9·5	12·5	13·5	15·5	19·0
Fifteenth dehydration	6·5	8·0	13·0	7·0	12·5	15·5

*Control value determined before addition of antioxidants.

give values for the induction periods as high as that for unheated arachis oil (Table 12).

The protective action of sesamol was more pronounced after several dehydrations. It was established that, during processing, fat was transferred from the fish to the dehydration vessel, and it would be surprising if the α-tocopherol of the fish oil were not transferred at the same time. Consequently, the tocopherol content of the drying oil would rise continuously. A synergistic action of α-tocopherol and sesamol would, therefore, be the most likely explanation for the behaviour of oil treated with sesamol.

The oil adhering to the product was, in general, more resistant to autoxidation than corresponding samples of the dehydrating oil, and this difference was main-

TABLE 11. Dehydration of herring: effect of antioxidants on the stability of the dehydrating oil (samples stored for 9 weeks)

Treatment	Induction period (days)					
	Dehydrating oil			Adherent oil		
	Without additive	BHT	Sesamol	Without additive	BHT	Sesamol
Control*	7·5	7·5	7·5	—	—	—
First dehydration	6·5	10·5	9·0	17·5	21·5	22·0
Fifth dehydration	6·5	10·5	9·0	7·5	17·5	23·5
Tenth dehydration	6·0	9·5	12·5	12·5	11·5	12·0
Fifteenth dehydration	6·5	8·5	12·5	6·5	10·0	15·0

*Control value determined before addition of antioxidants.

TABLE 12. Dehydration of herring: effect of antioxidants on the stability of the dehydrating oil (samples stored for 11 months)

Treatment	Induction period (days)		
	Without additive	BHT	Sesamol
Control*	2·0	2·0	2·0
First dehydration	2·5	8·0	6·5
Fifth dehydration	3·0	7·0	8·5
Tenth dehydration	3·0	7·5	9·0
Fifteenth dehydration	2·5	6·0	7·5

*Control value determined before addition of antioxidants.

tained throughout storage. The improvement in stability was again attributed to the contribution of natural antioxidants from the fish during prolonged contact with the oil.

The presence of either BHT or sesamol in the drying medium greatly increased the induction period of the samples of adherent oil, sesamol once more proving to be the more effective. It was concluded that these antioxidants acted on the oil by reinforcing the natural antioxidants of the fish.

Measurement of the linoleic acid content of the oil provided additional evidence for the stability of the drying medium and the adherent oil. Although it is not possible

to detect minute changes in the composition of lipids by gas–liquid chromatography, the analyses clearly showed that autoxidation of the oil was neither significantly accelerated in the course of dehydration nor during a lengthy period of storage.

When the moisture content of an oil exceeds a certain level, hydrolysis may occur, resulting in the liberation of free fatty acids. It seemed of interest, therefore, to determine whether the passage of large volumes of water through the oil medium in the course of the dehydration process would promote hydrolysis.

Fresh arachis oil was found to have a very low content of free fatty acids (0·1% as oleic acid) which did not change significantly after 9 months storage. The values for samples of the drying medium, examined after drying fifteen batches of cabbage and meat were 0·1 and 0·2%, respectively, and again no change on storage was noted.

Biological value of the protein of dried herring

The drying of herring by the vacuum-oil process results in a fall in the fat content of the fish. A comparison is made with freeze-dried herring in Table 13. In preparing

TABLE 13. Fat, nitrogen and protein content and NPU of dehydrated fish samples

Sample	Fish tissue: fat content (%)	Diet for assay		NPU
		N content (%)	Protein (%) (N% × 6·25)	
Freeze dried	52·30	2·29	14·32	70
Vacuum-oil dried	45·90	2·23	13·92	73

the diets for biological assay, a small amount of arachis oil was, therefore, incorporated with the oil-dried herring in order to give two diets of similar composition with respect to the percentage of calories derived from protein. The results of the assay are summarized in Table 13. It will be seen that the heating of the fish during dehydration in oil had no detrimental effect on the quality of its proteins. On the contrary, the dried product was, if anything, superior to freeze-dried herring.

In 1958, Pellett determined the nutritive value of meat processed under similar conditions, and came to the same conclusion. The NPU of beef steak dried in oil *in vacuo* did not differ significantly from that of an acetone-dried product.

Conclusion

In evaluating a method of food preservaton by dehydration, the most important criteria are that the process should be inexpensive, the nutritive value of the material

should not be altered during dehydration, and the product should retain its palatability and nutritive value over a reasonable period of storage.

The drying of foodstuffs in edible oil *in vacuo* has been shown to satisfy the first and second of these criteria. In the experiments described above, factors which might influence the shelf-life of the product were investigated. Foodstuffs which are particularly susceptible to spoilage, such as green leafy vegetables, meat and fish containing a large amount of highly unsaturated fat were used in the tests.

The choice of arachis oil as the drying medium proved a fortunate one, since the oil displayed a natural resistance to autoxidation under the conditions of the experiment. Furthermore, all three foodstuffs dried, contrary to expectations, had the effect of improving the stability of the oil.

It was originally thought that the development of rancidity in the oil which adhered to the dried product might seriously limit its shelf-life. The beneficial effect, resulting from the interaction of product and drying medium was, however, even more striking in samples of the adherent oil, and was attributed to the transfer of natural antioxidants from the foodstuffs into the oil.

It is concluded that, even without recourse to the use of synthetic antioxidants, foodstuffs dried in oil *in vacuo* would resist autoxidation for a considerable time. The addition to the oil of an antioxidant such as BHT would, however, extend the shelf-life of the dried product.

Since sesamol was found to be as effective as BHT, sesame oil might be added to a dehydrating oil not itself endowed with natural antioxidants, or it might be used as the dehydrating medium.

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Some aspects of the statistics of small numbers of triangular taste tests

V. D. LONG

Summary. The binomial distribution of random selections of odd samples in small numbers of triangular taste tests has been applied to estimate: (a) scores establishing a difference between samples, (b) the extent of true discrimination shown by any significant score, (c) the fixed-panel size needed to establish difference or similarity, and (d) decisive scores in a sequential test scheme. It is shown that conclusions may be reached with fewer tests than the published literature suggests, thereby affording a saving of tasting effort.

Introduction

It is well known that interpreting the results of tasting tests involves risks of concluding that samples are: (a) different when they are indistinguishable, and (b) similar when they are in fact different. The risk first may be reduced to an acceptable size by requiring disproof of similarity at a high level of statistical significance. Tables of significant scores for seven or more tests were calculated for this purpose by Roessler, Warren & Guymon (1948). The second risk arises by inferring from the absence of statistical disproof of similarity, that the samples are similar. To control this risk it is necessary to carry out a sufficient number of tests, the number depending on the criterion of 'similarity' to be applied. Steiner (1966) has considered the number of tests required to reduce both risks to 5% with three standards of 'similarity' and has pointed out that sequential methods are more efficient for a given level of risk than those involving a fixed number of tests. His main results were presented as tables of decisive scores for sequential procedures involving two standards of 'similarity': for routine use, less than 50% true discrimination; and for more selective occasions, less than 25% true discrimination.

Both the treatments of Roessler *et al.* and Steiner approximated the chance frequency of selection of the odd sample to a Gaussian distribution. By contrast, the present treatment considers risks (a) and (b) for triangular taste tests in relation to the binomial distribution which actually obtains. It gives statistically significant scores for

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disproving similarity with fewer than seven tests, and proposes an alternative sequential test scheme which converges more rapidly than that of Steiner.

Probability of chance selection

The foundation of all statistical treatments of triangular tests is the probability of random selection of m odd members from n sets of three, each set comprising one odd and two similar samples. By considering the possible outcome of increasing numbers of tests it is easily seen that the probability (p) is given by:

$$p(m) = \frac{n!\ 2^{n-m}}{(n-m)!\ m!}\left(\frac{1}{3}\right)^n. \tag{1}$$

Values of this probability may be found by interpolation of tables published by the National Bureau of Standards (1950), or can readily be evaluated from:

$$p(o) = (\tfrac{2}{3})^n,$$

$$p(m) = \frac{1+n-m}{2m}p(m-1).$$

In the present work the frequency distribution defined by equation (1) will be used first to estimate the scores of successes (i.e. the number of correct selections of odd samples from a given number of triangles) necessary to disprove two null hypotheses: (i) no true discrimination between samples, and (ii) partial discrimination with fractional success rate, f. The former corresponds to the work of Roessler *et al.*, and the latter, with a suitable choice of f, to that of Steiner on risk (a). Secondly, the frequency distribution will be used to estimate the number of tests required to reduce risk (b) to an acceptable level. The application of this result to a sequential test scheme will then be considered.

Null hypothesis

No discrimination

This null hypothesis will be disproved by a score of m successes in n tests, where the sum of probabilities of gaining m or more successes by chance is not greater than the acceptable fractional risk (R_a) of error of the first kind. Algebraically this condition for establishing significant differentiation is:

$$\sum_{i=m}^{n} p(i) \leqslant R_a. \tag{2}$$

Evaluation of the decisive score (m) for risk (R_a) equal to 0·050, 0·010 and 0·001 gives results in substantial agreement with Roessler *et al.* for values of n from 7 upwards. For example, comparing all results for numbers of tests less than 50 shows that the

earlier figures over-estimated by one the decisive score for 16, 23, 30, 35, 42, 47 and 48 tests at the 5% level; and for 24 and 33 tests at the 1% level. Scores for the 0·1% level were under-estimated by one for 11, 13, 31, 33, 35 and 44 tests.

Values of the decisive score for fewer than seven tests are given in Table 1 (the 0·1% risk level is excluded because this is not reached until seven tests are made).

TABLE 1. Scores establishing a difference between samples with risks of error of 5% and 1%

No. of tests	Minimum number of correct selections proving significant differentiation	
	$R_a = 0{\cdot}050$	$R_a = 0{\cdot}010$
1	I	I
2	I	I
3	3	I
4	4	I
5	4	5
6	5	6

I=Impossible.

Partial discrimination

To test the hypothesis that the fraction of tests in which true discrimination occurred was f, a score of m successes out of n trials may be regarded as equivalent to a test of a null hypothesis of no detectable difference for a score of $(m-fn)$ out of $n(1-f)$. That is, after allowing for the supposed genuine distinction, the residue is tested for randomness. Thus a score of m successes out of n tests is evidence of true discrimination at a fractional level at or above $m-n^*-1$ where n^* is the minimum successful score required to disprove randomness of selection in $n(1-f)$ tests. Adopting the 5% level of risk used by Steiner,this limiting level of the true success rate which must be attained to get a given score reduces to the value

$$\frac{2m-(n+2)}{n}$$

for values of n less than 16.

From the above reasoning the score necessary to establish true discrimination at or above a given fractional level may be calculated. In particular, values for Steiner's 'routine' and 'selective' levels of 0·50 and 0·25 are given in Table 2.

Number of tests required

The maximum number of tests necessary to show discrimination by a taster at a given level of attainment will be that number which will give significant scores even if he has the worst possible luck in his guesses. At the previously accepted level of risk of

TABLE 2. Scores establishing true discrimination at or above levels of 25 and 50% (risk of error 5%)

No. of tests	Minimum score to establish true fractional discrimination	
	⩾25%	⩾50%
3	3	I
4	4	4
5	5	5
6	5	6
7	6	7
8	6	7
9	7	8
10	8	9
11	8	10
12	9	10
13	10	11
14	10	12
15	11	13
16	11	13

I = Impossible.

not more than 5%, from equation (1) this will involve allowing for at least eight guesses, since this is the smallest number for which $p(o)$ is less than 0·05. It follows therefore that to be sure of detecting a difference at this level of risk where there is a genuine fractional success rate f, it is necessary to obtain statistical evidence for discrimination with a score $(fn+m_r+1)$ out of n, where m_r is the number of correct guesses exceeded in 95% of random selections from $n(1-f)$ tests. From equation (1), m_r will have values 0, 1 and 2 for values of $n(1-f)$ equal to 8–12, 13–16 and 17–20, respectively. For higher values, m_r is satisfactorily estimated by applying Yates's correction to a normal distribution and rounding to the next lowest integer if fractional.

The above general argument will now be applied to a situation where a taster has truly distinguished the odd samples in half the tests, but has randomly selected from the rest (this level of discrimination is of interest since it was proposed by Steiner as being the minimum suitable for establishing a difference between samples in routine testing). For such a taster with a 50% true success rate, the number of tests, n, required to give a 95% chance of statistical proof of discrimination is such that a score of

$$\left(\frac{n}{2}+m_r+1\right) \text{ out of } n$$

is statistically significant. By evaluation of equation (2) for the 5% probability level, the minimum number of tests satisfying this requirement is 16 (two tests fewer than indicated by the table of Roessler *et al.*). The statistically significant score is 9,

which comprises 8 by discrimination and 1 by luck. Thus a score of 8 or less successes out of 16 tests indicates 50% or less true discrimination with a risk of error of frequency less than 5%.

The corresponding calculations for the case of a taster with a 25% true success rate indicates that about 100 tests are required with a significant score of 43, comprising 25 by discrimination and 18 by luck. This number of tests, however, is beyond the scope of the present paper and also the patience of many investigators.

Since 16 tests are required to establish a 'routine' (i.e. 50%) difference with the worst possible luck in sampling, with better luck fewer tests will suffice, the smallest number being 4 as indicated by Table 2. Hence, where a detectable difference of this size occurs, a conclusive result will generally be obtained with less than the maximum number of tests and considerable economy of effort will result from analysing the scores sequentially to decide at each stage of testing whether: (a) a difference between samples, or (b) the similarity of samples, has been proved. If one of these conclusions obtains, the tests may be stopped, and if neither obtains, the tests continue.

As proof of a difference between samples, the results given in Table 1 and by Roessler *et al.* may be used. These are reproduced in the second column of Table 3. Proof of similarity will depend on the degree of difference accepted as similarity and also the allowable risk of error.

TABLE 3. Decisive scores for a sequential test scheme based on a criterion of similarity of less than 50% true discrimination and risks of error of 5%

No. of tests	Difference proved with score greater than:	Similarity proved with score less than:
1	I	I
2	I	I
3	2	I
4	3	I
5	3	I
6	4	I
7	4	1
8	5	2
9	5	3
10	6	4
11	6	4
12	7	5
13	7	6
14	8	7
15	8	8
16	8	9

I = Impossible to decide.

In general, if M is the lowest significant score for N tests, and N is sufficiently large for the risk of missing a real difference to be acceptably small, then clearly a score of less than M successes out of N selections indicates similarity. Hence, if the tests had been stopped at $N-A$ selections, a score of less than $M-A$ would also have been conclusive, since further A tests could only result in a combined score of less than M. However, for high values of A the taster is unlikely to have complete success, and the maximum score indicating similarity will be greater than predicted above.

For the case where a fraction f of choices is truly discriminated, the score for establishing similarity will be less than $(M-fA-B)$, where $(B+1)$ is the minimum score of successes required to disprove random selection from $(1-f)A$ tests. Values calculated in this way for 5% levels of risk and the 'routine' 50% level of true discrimination are also given in Table 3. Needless to say, if the selection from A tests were purely random, the tabulated values would err on the safe side for numbers of tests less than 13. Also, on the other hand, if it were considered desirable to allow always for the case where all A selections were completely successful, it would make little difference to the table, the score for similarity then being less than $9-A$.

Discussion

The results presented in this paper suggest that difference or similarity between samples may be established with fewer tests than the work of Steiner indicates. In particular, the maximum number of tests required to reach a decision at 5% levels of risk, where half the samples were truly discriminated, was shown to be 16 as opposed to Steiner's estimate of 22. This result was then made the basis of the proof-of-similarity criterion of a sequential test scheme in which the scores after n tests were compounded with the maximum likely scores of a further $16-n$ tests and the total score compared with the significant score for 16 tests. The difference and similarity scores of this scheme converge at 16 in contrast to the 35 tests required by Steiner's 'routine' scheme. The economy of effort afforded by a possible reduction in the number of tests required to establish a firm conclusion is clearly desirable, and it remains to point out why the results from the two sources differ.

Dealing with the non-sequential part, Steiner assumed a model distribution which differed from the binomial in both form and variance. He considered results with partial discrimination to be distributed normally about a mean $p_1 n$ with a variance $p_1(1-p_1)n$. Thus the skewness and discontinuous nature of the binomial distribution, which are particularly significant for small values of n, were not taken into account in considering risks of the second kind (b). [Although following Roessler *et al.*, Yates's correction for continuity was applied in assessing risks of the first kind (a).] However, in the present instance difference in form is not a source of discrepancy, since for fewer than 17 tests, predictions at the 5% level of significance for the low-score end of an uncorrected normal distribution agree well with predictions based on the binomial

distribution, *provided the binomial variance is used.* (Generally for 1% and 0·1% levels of significance and for larger numbers of tests at the 5% level, Yates's correction gives a much better approximation.) Thus the difference from Steiner's result must arise from the choice of variance. Using his nomenclature, the variance of the score results from random selection from $1{\cdot}5\ (1-p_1)n$ tests. With a one-third probability of correct selection in any one test, the binomial distribution variance for this number of tests should be $(1-p_1)n/3$. Now since p_1 is always greater than one-third if there is true partial discrimination, Steiner's variance $p_1(1-p_1)n$ is always too high, and in consequence will over-estimate the number of tests required to get a statistically significant score at a specified level of risk of the second kind (b). His variance also has the surprising property of passing through a maximum when $p_1 = \frac{1}{2}$.

The sequential schemes differ in the standards accepted as proof of difference and similarity. The present scheme takes as evidence of difference any score disproving a null hypothesis of no difference with less than 5% risk of error of the first kind (a). Steiner's standard is rather more stringent in that it requires proof of detection of difference at or above a prescribed frequency and is therefore analogous to (but not identical with) disproof of a null hypothesis of the next lowest available level of partial discrimination. This naturally requires a higher success rate, which might result in unnecessary testing. The difference in approach may possibly be best illustrated by an example, say, relating to 16 tests. If half the tests were to result in successful selections by true discrimination, the risk of not getting a score of 9 or more would be less than 5%. Now a score of 9 or more successes establishes the samples are different with a risk of error of the first kind (a) of less than 5%. Therefore a score of 9 would prove a difference exists between samples and have more than a 95% chance of showing this when the difference arises from 50% true discrimination. However, on the other hand, to prove that a difference which is found to exist is due to 50% true discrimination would require according to Steiner 11 successes, and from Table 2, 13 successes. Where proof of difference only is required, clearly the lowest score (i.e. Table 1 and Roessler *et al.*) is adequate. Where proof of a given degree of partial discrimination is required, the highest score (i.e. Table 2) will be safe. The latter is based on straightforward application of the binomial distribution and should, therefore, be free from hidden errors and approximations. It is not clear why the results of Table 2 differ from Steiner's at the 50% level, yet show reasonable agreeement at the 25% level, but it seems likely that the underlying cause may be the application of a continuous variate test to a discrete variate problem. With increasing proportion of the results attributable to chance, as in going from 50 to 25% discrimination, this divergence would be reduced.

The standard of similarity adopted in the sequential procedure proposed here is failure to disprove a null hypothesis of no difference when the number of tests is sufficient for such disproof in 95% of the cases involving 50% true discrimination. For fewer tests, the score indicating similarity is taken as that score which when

compounded with the maximum likely score of such additional tests as are necessary to attain the number specified above, would still be insufficient to disprove the null hypothesis. Thus the results of a small number of tests are taken as evidence of failure to disprove the null hypothesis only when they indicate a similar outcome for a larger number of tests, the size being sufficient to ensure negligible risk of missing a defined 'real' difference. This approach is quite different from Steiner's and leads to higher decisive scores for similarity. His decisive score at 50% partial discrimination for n tests is $(n-5)/2$ rounded to the next lowest integer if fractional. This arises by considering the ratio of the probabilities of a score m out of n arising from: (i) 50% partial discrimination, and (ii) random selection to be less than

$$\frac{\text{risk (b)}}{1-\text{risk (a)}}, \text{ i.e. } \frac{1}{19}.$$

This decisive level of probability ratio is itself defined by an inequality and is, therefore, conservative. Thus it is quite possible that higher scores could be statistically significant, and there seems no reason to doubt that the present method is a valid alternative which has the advantage of requiring fewer tests to reach a conclusive result.

Conclusions

(1) Significant differentiation between samples may be obtained with smaller numbers of tests than included in previously published tables.

(2) Minor revisions of the conclusive scores given in the above mentioned tables may be desirable.

(3) For fewer than 16 tests, a score of m successes out of n tests shows, with 5% risk of error, true discrimination at a fractional level $(2m-n-2)/n$ or above.

(4) The minimum fixed-panel size required to establish either difference with a 5% risk of error, or similarity with a 5% risk of missing cases involving 50% true discrimination, is 16 (a reduction of 27% compared with a previously published estimate).

(5) By regarding fewer than 16 tests as a sample from the fixed-panel size, it is possible to construct a sequential test scheme which converges more than twice as rapidly as the alternative scheme based on a probability ratio test. This rapid convergence should lead to economy of effort in tasting programmes.

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Salt as a preservative

M. INGRAM AND A. G. KITCHELL

Referring to our article, in this journal, on salt as a preservative for foods (Ingram & Kitchell, 1967), Mr T. McLachlan (1967) rightly corrects our statement that it is not used in preserving fruits. We were aware of such practices as he describes (cf. Mossel & Ingram, 1955; Ingram, 1957). However, in the majority of such applications, the preservative action is due as much or more to acidity, either generated by fermentation or naturally present, as it is to salt, as becomes evident if the acidity is diminished (e.g. by yeasts), when the product spoils although the salt remains (cf. Mossel & Ingram, 1955); and, further, it is well established that the action of the salt is directly supplementary to that of the acid and that exactly similar relations obtain between acid and sugar (e.g. Vas, 1957). Hence, we do not regard salt as having a spcial preservative action in such cases.

We cannot follow Mr McLachlan's objections to our use of the word putrefy. It is customarily applied, broadly speaking, only to those types of rotting where volatile free amino-compounds are liberated; and, even within this context, its significance is debatable (see, for example, Ingram, 1963). In foods containing plenty of carbohydrate, fermentation largely inhibits proteolysis and the production of amino-compounds (the well-known 'protein-sparing' action of carbohydrate, cf. Mossel & Ingram, 1955). Also, if any amino-compounds are produced, they are likely not to be free and volatile at low pH levels, but fixed by the acids present. It follows that vegetable foods subject to acid fermentation do not putrefy, in the usual sense of that term.

We are grateful for the historical background which Mr McLachlan has provided, and wish to comment only on one point. He claims that the Public Health (Preservatives, etc., in Food) Regulations were amended in 1940 as a result of public letters in which he pointed out that nitrite is added to hams, etc., as a colouring matter and not as a preservative. Such a view was perhaps defensible at that time, but could now be held only by those ignorant of the facts. Subsequent research (Tarr, 1941; Castellani & Niven, 1955; Eddy & Ingram, 1956; Eddy, 1958) has shown that nitrite possesses a definite preservative action, which may indeed be critical to the safety and stability of non-sterile semi-preserved cured meats (Roberts & Ingram, 1966; Perigo, Whiting & Bashford, 1967; Perigo & Roberts, 1968).

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Book Reviews

Biochemistry of Some Foodborne Microbial Toxins. Ed. by RICHARD I. MATELS and GERALD N. WOGAN.

London: M.I.T. Press, 1967. Pp. 171. 60s.

This book presents a collection of papers delivered at the *Symposium on Microbial Toxins* held during a meeting of the American Chemical Society on 12 September 1966.

This unfortunately is not a readable book, insufficient attention having been paid to presenting material in a form suitable for written, rather than verbal, communication. The method used for the presentation of literature references, which are extensive in some of the papers, interferes with the communication of information from the written page to the mind of the reader.

There are ten papers in all, three concerned with bacterial toxins, one with algal toxins, the remainder with toxins of fungal origin. The papers on staphylococcal enterotoxins and the toxin produced by *Clostridium botulinum* give an insight into the techniques now available for investigating large molecules. The toxic proteins produced by these organisms are responsible for different forms of what we normally refer to as food poisoning although a useful function of this book is to show how diverse the range of micro-organisms responsible for poisoning food materials really is. Although staphylococcal food poisoning can be described as of world-wide occurrence the Bonkrek toxins associated with the contamination of fermented coconut press cake by *Pseudomonas cocovenenans* is an example of a toxicosis with a localized geographical niche, arising in this instance when a village industry in parts of Indonesia gets out of control.

The review of algal toxins covers representatives of the Dinoflagellates, the blue-green and the yellow-brown algae. Some of the dinoflagellates are involved in the aetiology of several shell fish poisons. These compounds are among the most toxic low molecular weight compounds known to be produced in the biosphere.

The section on fungal toxins includes an extensive review of the sporidesmins and related sulphur containing metabolites. The sporidesmins, metabolites of *Pithomyces chartarum*, are implicated in a disorder known as facial eczema which has dogged New Zealand sheep farmers for a very long time.

The phototoxic furocoumarins are in the main associated with a variety of higher plants but are included with the mycotoxins because they are also produced by the mould *Sclerotinia sclerotiorum* growing on celery. The metabolite of *Fusarium graminearum* responsible for an oestrogenic condition in pigs and the toxic metabolites produced by strains of *Aspergillus ochraceus* are also reviewed. The fluorescence of toxins such as aflatoxin when irradiated with ultraviolet light is a property of great value in the detection and assay of these compounds, especially when combined with thin-layer

chromatography. However a paper on the production of fluorescent compounds other than aflatoxins by moulds underlines the care needed in interpreting such results.

For those working on naturally occurring toxins or with interests in this ever-widening field this book will serve the useful purpose of bringing together under one cover material which is disseminated through a wide range of journals.

M. O. Moss

Food in the Future. Ed. J. V. McLouoghlin.

An Foras Talúntais (The Agricultural Institute), 1967. Pp. 136. 30s.

Our friends in Eire have produced an admirably compact symposium on a subject which is, as we write, as much of topical as of prophetic interest. It is a record of the proceedings at a conference held in November 1966, and although impeccably produced, one has to deplore so much delay in the appearance of work of such immediate interest and importance. The contributors, nine in number, comprise: N. W. Pirie and J. Yudkin, on subjects so well associated with their names as to require no enumeration; M. Zinkin on choosing food; R. W. Stephenson on selling it; M. Ingram on preserving it; J. W. S. Selby on packaging it; J. Matthews on engineering it; and G. F. Stewart on product development and research. The introduction, by T. Walsh, the discussion of the different sessions and the summary deal rather more particularly with the problems of Ireland, but many points are raised and dealt with which makes them a well-worth-while part of the book's contents. For instance, Pirie was taken up on the question of the ways and means of introducing unfamiliar foods into the diets of undernourished communities (a question discussed in more general terms by Zilkin) and questions of £ s. d. (or $ and c.) were thoroughly ventilated. The feet, in fact, were firmly on the ground.

Of all the contributions, perhaps that by Zilkin makes the greatest impression in regard to the subject actually under discussion. Having briefly considered nutrition, income, religion and tradition, family and social factors, he emphasizes the extent to which modern developments are undermining attachment to traditional diets by altering fundamentally every one of the factors he discusses.

A reflection, on reaching the end, was how quickly the present overtakes the future. For answers to be ready when they are needed, the necessary work has to be in hand *here and now*.

E. C. Bate-Smith

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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.

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Contents

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