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Flavonols and flavones in food plants: a review†

K. HERRMANN*

Summary

In this review the qualitative and quantitative occurrence of flavonols and flavones, particularly in fruit and vegetables, are considered. They occur practically in all plants. Their formation normally depends on light so that they are mainly concentrated in the outer tissues. The concentration of flavonols in free standing leaves exceeds that in other parts of the same plant considerably, except in onions.

Flavonols act as antioxidants and protect the ascorbic acid from autooxidation, for example in fruit juices. On the other hand, flavonols can lead to discolourations. Beneficial effects on the human organism have also been described.

Introduction

Flavonoids (Harborne, Mabry & Mabry, 1975) which have the common skeleton of diphenylpropanes ($C_6C_3C_6$) are of widespread occurrence in plants. They consist mainly of the monomeric flavanols (catechins, leucoanthocyanidins), proanthocyanidins, anthocyanidins, flavones and flavonols, flavanones and chalcones. Isoflavonoids, biflavonoids and neoflavanoids are known also. Together with hydroxycinnamic acid compounds (Herrmann, 1967), which have the basic structure of phenylpropane (C_6C_3) and which are closely related to the flavonoids, flavones and flavonols can be found in almost every plant, particularly in the leaves and petals. In plants flavonols occur more frequently than flavones.

Chemical structure and general occurrence

Flavonols can be regarded as 3-hydroxyflavones and flavones, conversely, as 3-deoxyflavonols. Individual differences arise from the number and distribution of the hydroxyl groups as well as from the nature and extent of alkylation and/or glycosylation of these groups. In common with other flavonoids, the flavones and flavonols most frequently found in plants are those with B-ring hydroxylation in the 3′- and 4′-positions, followed

* Author's address: Technische Universität Hannover, Lehrstuhl für Lebensmittelchemie, Wunstorfer Str. 14, D-3000 Hannover 91.

† Translation by H. Liebmann.

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by those with a hydroxyl group in the 4′-position only. Quercetin and kaempferol are typical flavonols of this type.

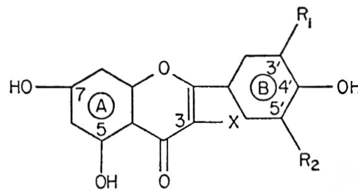

FIG. 1. Flavonols X=OH. Kaempferol: R_1=H, R_2=H; Quercetin: R_1=OH, R_2=H; Myricetin: R_1=OH, R_2=OH; Isorhamnetin: R_1=OCH_3, R_2=H. Flavones X=H. Apigenin: R_1=H, R_2=H; Luteolin: R_1=OH, R_2=H; Tricin: R_1=OCH_3, R_2=OCH_3; Chrysoeriol: R_1=OCH_3, R_2=H.

The anthocyanidins which produce the blue and red colourations of berry fruits, cherries and plums, egg plants, red cabbage and radishes (review: Herrmann, 1972) as well as the mainly weakly coloured yellow flavones and flavonols occur in living cells almost exclusively as glycosides, and mainly as *O*-glycosides.

The preferred bonding site of the sugar radical to the flavonols is the 3-position, much less frequently the 7-position, and only in rare cases the 4′-, 3′-, or 5-positions. In the case of diglycosides, 3-*o*-biosides and 3,7-di-*o*-glycosides occur most frequently. Flavones occur mainly as 7-*o*-glycosides, other glycosides occur only rarely. D-Glucose is the most frequent sugar residue but D-galactose, L-rhamnose, L-arabinose, D-xylose, D-apiose are found also, as well as D-glucuronic acid and, in rare cases, D-galacturonic acid. In general, the sugars of the D-series are bound β-glycosidically while those of the L-series have the alpha configuration.

Acylation occurs with hydroxycinnamic and hydroxybenzoic acids, as well as with benzoic, malonic, acetic and other acids. In these cases the acids are esterified with the sugars and are not attached to phenolic hydroxyl groups. However, flavon(ol) sulphates are known also in which one or more phenolic hydroxyl groups have been esterified with potassium hydrogen sulphate. In general, mono- and diglycosides are prevalent but it seems that triglycosides are also fairly widespread although their structures have been elucidated only in rare cases.

The formation of flavone and flavonol glycosides depends normally on the action of light (see Siegelman, 1964, 1969; Mohr, 1969), so that in general the highest concentrations of these compounds occur in leaves while only traces are found in parts of the plants below the soil surface. The common onion is, however, a well-known exception. The concentrations in leaves can vary considerably.

Flavones and flavonols do not contribute markedly to the colouration of the plant except where they occur in very high concentration, as in the skins of onions, or when they are complexed with metal. Flavonols which make an essential contribution to the yellow colour of a flower differ from the usual hydroxylation pattern by an additional hydroxyl group attached to the nucleus at the 6- or 8-position.

C-Glycosyl compounds which carry a sugar residue attached directly to an aromatic carbon atom are also known. Such compounds occur predominately in the leaves of various cereal crops as derivatives of apigenin and luteolin. In citrus fruits flavanones prevail, particularly rutinosides, which show no flavour, and the very strongly bitter-tasting neohesperidosides of naringenin, isosakuranetin, eriodictyol and hesperetin (Kefford & Chandler, 1970). Some very highly methoxylated flavones have been found also in the oil bearing cells of the peels which so far have not been found in other plants.

Fruits of the Rosaceae contain, besides catechins and their oligomers (proanthocyanidins) mainly glycosides of quercetin. Quercetin glycosides also predominate in vegetables or in the leaves of various vegetables. Frequently glycosides of kaempferol, luteolin and apigenin are also present.

Tea contains quercetin and kaempferol glycosides in concentrations exceeding 1% of its dry matter (Bokuchava & Skobeleva, 1969). Tobacco contains approximately 1% of rutin (quercetin-3-rutinoside) which plays a role in the formation of the brown pigments during the preparation of tobacco leaves (see Stedman, 1968). In hops we found approximately 700 mg/kg of quercetin and approximately 550 mg/kg of kaempferol (Herrmann & Mosel, 1973) which occur in the form of glycosides.

The concentrations of flavones and flavonols, like those of all secondary plant metabolites, vary within certain limits, and are dependent on a number of factors such as growing conditions, degree of ripeness, size of the fruit and variety.

Procedures for the isolation and identification of flavone and flavonol glycosides have been described in the books by Harborne *et al.* (1975) and Mabry, Markham & Thomas (1970). The addition of alkaline or complex forming reagents to flavonoid solutions causes shifts in UV absorption maxima from which it is possible to draw conclusions regarding the position and substitution of hydroxyl groups.

For the determination of flavones and flavonols measurement of absorbance in the UV region can be used. It is usually necessary to purify the plant extract by chromatography. In the method described by Wildanger & Herrmann (1973a,b) polyamide columns are employed, the eluated glycosides are hydrolysed, the aglycones are separated by thin layer chromatography on cellulose plates, and are determined spectrophotometrically. In view of the known resistance of some *o*-glycosides to acid-catalyzed hydrolysis (Harborne, 1965), enzyme-catalyzed hydrolyses were also performed.

Fruit

Fruit varieties which are cultivated or grow wild in Central and Northern Europe contain almost exclusively flavonol glycosides, mainly of quercetin (Table 1). Kaempferol is also found frequently in smaller quantities. Isorhamnetin occurs mainly in pears and sea buckthorn berries. Myricetin is found in blackcurrants, black grapes, cultivated bilberries and cranberries. Flavones have only been detected rarely and in trace quantities.

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TABLE 1. Kaempferol and quercetin glycoside contents of stone and berry fruits, estimated and calculated as mg of aglycon per kg of fresh weight*

Species	Variety	Year of picking	Kaempferol mg/kg fresh weight	Quercetin mg/kg fresh weight
Sweet cherry	Büttners rote Knorpelkirsche	1971	6	6
	Badocsoner Riesen	1974	0	24
	Teickners schwarze Herz	1974	0	9
Sour cherry	Schattenmorelle	1971	17	80
	Schattenmorelle	1974	5	23
Plum	The Czar	1974	2	15
	Wangenheims Frühzwetsche	1971	2	3
	Mirabelle von Nancy	1971	<0·1	<0·1
Peach	Red Haven	1971	0	0
	Mangipane	1971	<0·01	<0·01
	Früher Alexander	1971	2	4
Apricot	—	1971	2	53
Raspberry	Schönemann	1971	<0·1	29
Blackberry	Theodor Reimers	1971	14	33
Black currant	Rosenthals langtraubige Schwarze	1971	<0·1	33
	Rosenthals langtraubige Schwarze	1974	6	33
	Silvergieters Schwarze	1973	10	68
	Silvergieters Schwarze	1974	10	41
Red currant	—	1971	2	27
	Rote Heros	1974	0·1	2
	Heinemanns rote Spätlese	1974	2	11
White currant	—	1971	2	28
	Weiße Versailler	1974	1	7
	Weiße aus Jüterbog	1974	0·2	3
Gooseberry	Weiße Triumph	1971	<0·1	<0·1
	Rote Triumph	1971	0	<0·1
Bilberry, wild	—	1971	0	32
Bilberry, cultivated	Clon 908	1973	6	160
	Heerma I	1974	0	105
	Heerma II	1974	0	159
Elderberry	—	1973	0	237
	—	1974	0	105

* Wildanger & Herrmann, 1973b; Starke & Herrmann, 1976b.

All varieties of fruits of the Rosaceae family investigated by us as well as various currants, gooseberries and grapes contain isoquercitrin (quercetin-3-glucoside). The data presented in Table 2 relate only to compounds whose occurrence has been established unequivocally (see also Herrmann, 1970).

TABLE 2. Flavonol glycosides of common fruits and vegetables

Fruit or vegetable	Flavonol glycosides	Reference
Apple	Quercetin glycosides, e.g. Qu-3-glucoside, Qu-3-galactoside, Qu-3-rhamnoside	
Pear	Qu-3-glucoside, Isorhamnetin-3-glucoside, Isorhamnetin-3-rhamnosylglucoside, Isorhamnetin-3-rhamnosylgalactoside	Nortjé & Koeppen (1965)
	Isorhamnetin-3-(O-malonyl)-glucoside	Nortjé (1966)
	Qu-3-glucoside, Qu-7-xyloside, Isorhamnetin-3-glucoside, Isorhamnetin-3-galactoside, Isorhamnetin-3-rutinoside	Duggan (1969)
Quince	Qu-3-glucoside, Qu-3-galactoside	Gumbaridze (1972)
Sour cherry	K-3-glucoside, K-3-rutinoside	Schaller & von Elbe (1970)
	K-3-rutinoside, Qu-3-glucoside, Qu-3-galactoside, Qu-3-rutinoside, Qu-4′-glucoside, K-3-glucoside (?)	Shrikhande & Francis (1973)
Apricot	Qu-3-glucoside, Qu-3-rutinoside	el-Sayed & Luh (1965)
Plum, yellow	Qu-3-glucoside, Qu-3-rhamnoside, Qu-3-arabinoside	Williams & Wender (1953)
Strawberry	K-3-glucuronide, K-3-glucoside, Qu-3-glucuronide, Qu-3-glucoside, K-7-glucoside (?)	Ryan (1971)
Raspberry, red	K-3-glucuronide, Qu-3-glucuronide, Qu-3-glucoside (?), Qu-3-galactoside (?)	Ryan & Coffin (1971)
Cranberry	Qu-3-galactoside, Qu-3-rhamnoside, Qu-3-arabinoside, Myricetin-3-arabinoside	Cansfield & Francis (1970)
Grape	K-3-glucoside, Qu-3-glucoside, Myricetin-3-glucoside, Qu-3-glucuronide	Ribéreau-Gayon (1964)
Asparagus	Qu-3-rutinoside	Stevenson (1950); Wöldecke & Herrmann (1974a)
Endive	K-3-glucuronide, K-3-glucoside, K-3-(O-malonyl)-glucoside (?)	Wöldecke & Herrmann (1974d)
Leek	K-3-glucoside, K-3-xylosylglucoside	Starke & Herrmann (1976c)
Lettuce	Qu-3-glucuronide, Qu-3-glucoside, Qu-3-(O-malonyl)-glucoside, Luteolin-7-glucuronide	Wöldecke & Herrmann (1974d)
Onion	Qu-4′-glucoside, Qu-3,4′-diglucoside, Qu-4′, 7-diglucoside	Herrmann (1958); Brandwein (1965); Starke & Herrmann (1976a)
Rhubarb	Qu-3-glucoside, Qu-3-rhamnoside, Qu-3-rutinoside	Blundstone (1967)
Spinach	6-Methyl-quercetagetin, 3′, 6-Dimethyl-quercetagetin, Kaempferol glycosides	Zane & Wender (1961)
	3-Methyl-6, 7-methylendioxyquercetagetin + 3 Quercetagetin derivatives	Oettmeier & Heupel (1972)
Tomato	Qu-3-rutinoside	Wöldecke & Herrmann (1974b)

Abbreviations used: K = kaempferol; Qu = quercetin.

Since the formation of flavonol glycosides depends on light, it is not surprising that they are found mainly in the skins of fruits (Workman, 1963; Walker, 1964; Wildanger & Herrmann, 1973b). Their distribution in pome fruits is summarized in Table 3. The concentration of flavonols in pome fruits, however, is considerably lower than that reported for apples (e.g. Workman, 1963; Walker, 1964) and we attribute this to the fact that in our analyses we have been able to eliminate interfering compounds.

TABLE 3. Distribution of kaempferol and quercetin glycosides within pome fruits and some vegetables, estimated and calculated as mg of aglycone per kg of fresh weight

Species	Variety	Outer parts of the tissues (skin, peel)		Remaining tissues	
		Kaempferol (mg/kg)	Quercetin (mg/kg)	Kaempferol (mg/kg)	Quercetin (mg/kg)
Apple	'Weißer Klarapfel'	< 1	98	< 0·01	2
	'Gravensteiner'	2	58	0	< 1
	'Cox Orangenrenette'	7	263	< 0·1	< 1
Pear	'Williams Christ'	12	28	0	< 0·1
Quince	'Portugiesische Quitte'	210	180	< 0·01	< 0·01
Bell pepper (outer parts of the tissues = 28% of fruit)	'Yolo wonder'	0	63	0	< 1
Kohlrabi	'Primavera'	6	7	< 1	< 1
Small radish	'Saxa Treib'	27	0	0·6	0
Horse-radish		76	0	1·5	0
Scorzonera	'Hoffmanns schwarze Pfahl'	< 1	< 1	< 1	< 1
Potato	'Clivia'	c. 0·2	0	c. 0·1	0
	Clivia, total green by chlorophyll	57	47	9	c. 0·2
Asparagus	'Huchels Auslese', white tips	< 0·1	0·3	0	< 0·1
Tomato	Contains nearly the total flavonol content in the outer parts of the tissues, which were 4–5% of the fruit				
Cucumber	Contains traces of flavon(ol) glycosides only in the peel				

Although unripe berries of blackcurrants contain only traces of myricetin (< 1 mg/kg) but 10–20 mg/kg quercetin, the former is the dominant flavonol in ripe blackcurrants. This is in agreement with the hydroxylation pattern of anthocyanidins found in these fruits. Blackcurrants contain mainly delphinidin, corresponding to myricetin, and only small quantities of cyanidin. In 1971 and 1974 we found in 'Rosenthals langtraubige Schwarze' 55 and 43 mg/kg myricetin and in 1973 and 1974 in 'Silvergieters Schwarze' 95 and 41 mg/kg myricetin calculated on the fresh weight of the fruit (Wildanger &

Herrmann, 1973b; Starke & Herrmann, 1976b). These flavonols occur in the form of glycosides. Our analytical results are of the same order of magnitude as the values reported by Morton (1968) and agree also with findings from the north of the U.S.S.R. (Samorodova-Bianki & Kulikova, 1966). Redcurrants and whitecurrants, on the other hand, contain only traces of myricetin glycosides while cultivated bilberries can contain up to 70 mg/kg of myricetin in the form of glycosides (Starke & Herrmann, 1976b). The concentrations of flavonols found by us in apricots grown in Italy are in the same range of 5–90 mg/100 g dry matter, depending upon cultivar, as found by Melkumyan (1969) in Armenia (U.S.S.R.). Grapes have not been analysed by us since they are not usually grown in northern Germany. Analyses by two-dimensional thin layer chromatography on cellulose of Russian wine prepared by must fermentation, revealed the presence of 19–22 mg/l of quercetin monoglycosides (Bokuchova *et al.*, 1971).

When the changes in the concentration of flavonols during the development of the fruit are investigated, it is found that in blackcurrants the amount of glycosides of quercetin, and particularly of myricetin, increases markedly during ripening (darkening) of the berries (Starke & Herrmann, 1976b; Samorodova-Bianki & Kulikova, 1966). In cultivated bilberries the myricetin content in ripe fruit is also relatively high. On the other hand, ripe red- and whitecurrants, sour cherries, plums, cultivated bilberries, elderberries and tomatoes possess lower concentrations of kaempferol and quercetin glycosides than the unripe fruit. Similar changes in concentration have been observed for the catechins and hydroxycinnamic acid derivatives during the development of the fruit (Mosel & Herrmann, 1974a,b; Stöhr & Herrmann, 1975a,b; Stöhr, Mosel & Herrmann, 1975).

Vegetables

In a considerable number of vegetables only fairly small concentrations of flavone and flavonol glycosides have been detected. We found concentrations around 1 mg/kg fresh weight, and lower amounts in carrots, radish, rutabaga, scorzonera, beet, white asparagus without tips or with white tips, cabbage without the outer leaves (white, red, Savoy cabbage), peeled kohlrabi, cauliflower (edible parts), chicory, peas, cucumber, egg plants and potatoes (Eloesser & Herrmann, 1975; Wöldecke & Herrmann, 1974a; Wildanger & Herrmann, 1973c; M. Wöldecke, unpublished). On the other hand, the leaves of root vegetables, potatoes and asparagus frequently contained concentrations in excess of 1000 mg/kg. Data on the occurrence of flavonol glycosides in vegetables are given in Table 2. Findings are only quoted if the compounds in question have been identified beyond doubt (see also Herrmann, 1970).

Leaves

Flavonols in plants are nearly always found in free-standing leaves. Onions only are an exception, but in all other vegetables investigated by us the concentration of flavonols in the leaves was many times higher than that in other tissues of the same plant. Investi-

gations on peas and broad beans have shown (M. Wöldecke, unpublished) that the flavonol content is highest in leaves, lower in pods, and lowest in unripe seeds (Table 4).

Where heads are formed from leaves such as in lettuce, endives (Wöldecke & Herrmann, 1974c) and in Brassica species (Wildanger & Herrmann, 1973c) the flavonol

TABLE 4. Kaempferol and quercetin glycoside contents of leaves and other parts of vegetables, estimated and calculated as mg of aglycon per kg of fresh weight

Species	Variety	Leaves		Other parts of the same plants		
		Kaempferol (mg/kg)	Quercetin (mg/kg)		Kaempferol (mg/kg)	Quercetin (mg/kg)
Brussels sprout	'Unigrade'	75	50	Sprouts	40	25
Cauliflower	'Gordan'	270	30	Curd	2	1
Kohlrabi	'Primavera'					
	open air	80	25	Peel	6	7
				Flesh	< 1	< 1
	glasshouse	5	< 0·1	Flesh	< 0·1	< 0·1
Kale	'Halbhoher grüner extra-krauser'					
	open air	250	50			
	glasshouse	70	35			
Small radish	'Saxa Treib'	825	0	Root	8	0
	'Eiszapfen'	150	30	Root	1·3	< 0·1
	'Neckarperle' (glasshouse)	16	0	Root	1	0
Radish	'Kitzinger Sommer'	130	35	Root	*c.* 0·3	0
Rutabaga	'Seefelder'	400	40	Root	*c.* 0·3	*c.* 0·1
Horse-radish		1600	50	Root	20	0
Scorzonera	'Einjährige Riesen'	25	230	Root	< 1	< 1
Potato	'Hansa'	50	770	Tuber	1	2
	'Grata'	60	1000	Tuber	1	2
Tomato	'Ronald V'					
	open air	20	420	Fruit	0·2	7
	glasshouse	4	155	Fruit	0·2	2·5
Pea	'Juwel'	140	1580	Pod without seeds	3	125
				Seed	< 1	< 1
	'Aldermann'	150	1590	Pod without seeds	5	130
				Seed	< 1	< 1
Broad bean	'Felix'	800	1340	Pod without seeds	28	36
				Pod	5	19

concentration drops markedly from the outer to the inner leaves. Investigations of several varieties of lettuce and endives have shown that, probably due to the action of sunlight, the outer, mainly green leaves contain a much higher level of flavonols than the inner, mainly yellow ones. In the case of lettuce and endives, these levels appear to vary with the chlorophyll content as indicated by the green colour. Savoy cabbage, for example, without the outer leaves contained less than 1 mg/kg of quercetin and kaempferol while whole Savoy cabbages with the outer leaves had a concentration of kaempferol of 29 mg/kg and 5 mg/kg of quercetin. The green outer leaves of leek showed ten times more kaempferol and quercetin glycosides than the edible white parts.

TABLE 5. Flavonol glycoside contents of lettuce and endives, estimated and calculated as mg of aglycon per kg of fresh weight*

Species, variety	Per cent of the total leaves	Quercetin	
		(mg/kg)	Calculated for all the leaves of the head (mg/kg)
Lettuce 'Blanco', open air			
outer leaves	48	60	29
inner leaves	52	3·4	1·9
Lettuce 'Valentine', open air			
outer leaves	59	462	273
inner leaves	41	7·6	3·1
Lettuce 'Valentine', glasshouse			
outer leaves	55	10·8	6·0
inner leaves	45	< 1	< 1
Endives, open air		Kaempferol	
outer leaves	58	258	150
inner leaves	42	5·7	2·4

* Wöldecke & Herrmann, 1974c.

Growing plants in glasshouses also reduces the flavonol content, particularly in the outer leaves, as we have shown for kale, Brussels sprouts, lettuce, kohlrabi and small radishes. Compared with the leaves, all other parts of these plants have very much lower flavonol contents. The same is true for the flavones. Parsley grown in the open was found to contain 238 mg/kg of apigenin and approximately 310 mg/kg of chrysoeriol, while the same variety grown nearby under glass had only 47 mg/kg of apigenin and approximately 50 mg/kg of chrysoeriol (Wildanger & Herrmann, 1973c).

TABLE 6. Flavonol or flavone contents of some vegetables, estimated and calculated as mg of aglycon per kg of fresh weight*

Species	Variety	Kaempferol mg/kg fresh weight	Quercetin mg/kg fresh weight
Cabbage, white	Industrieweiß Marne	<0·1	<0·1
Cabbage, red	Lagerrot Marne	<0·1	6
Savoy cabbage	Hammer		
	with outer leaves	29	5
	without outer leaves	<1	<1
Broccoli		30	6
China cabbage	Kantoner	11	3
Leek	9 varieties	90–200	10–25
Chive		10	300
		Apigenin mg/kg fresh weight	Luteolin mg/kg fresh weight
Carrot	4 varieties	<1	<1
	but leaves	350–800	400–1500
Cellery root	Invictus	75	14
	but leaves	930	500

* Wildanger & Herrmann, 1973c; Starke & Herrmann, 1976c; Eloesser & Herrmann, 1975.

Asparagus

The underground parts of the plants showed very low concentrations of flavones and flavonols. In the asparagus spears with white tips just coming to the surface we found 1·1 mg/kg quercetin and 0·2 mg/kg kaempferol and 90% of these flavonols were concentrated in the tips. When the tips had developed a blue colour after exposure to light, the asparagus spears showed concentrations of 6·7 mg/kg of quercetin and 0·7 mg/kg of kaempferol. The flavonol glycosides were almost entirely concentrated in the tips and in the skin of the spears. Stevenson already found in 1950 with the analytical methods then available that the rutin content in the tips of green asparagus was three to four times higher than that in the remainder of the spears. An increase in growth was accompanied by an increase in rutin content up to 100 mg/100 g fresh weight. When we investigated the leaves formed from the spears we found 3800 mg/kg quercetin and 150 mg/kg kaempferol in plants free from stalks. Thus approximately 1·5% of the dry weight consists of flavonols, expressed as aglycones. Calculated as rutin, the main flavonol glycoside of asparagus, this would represent approximately 2·5% of the dry weight (Wöldecke & Herrmann, 1974a).

Root vegetables and potatoes

Only very small amounts of flavone and flavonol glycosides have been found in root vegetables, mainly below the limits of quantitative analytical methods for aglycones (Eloesser & Herrmann, 1975). Since the roots of different plant families are used as root vegetables, it would appear from our analyses that roots in general contain only traces of these flavonoids. This would account for the scarcity of information in the literature on the occurrence of flavone and flavonol glycosides in roots. In celeriac the flavone concentrations are somewhat higher. This can be attributed to the fact that celeriac tubers are thickened roots including hypocotyl and primary shoot.

When potatoes (variety 'Clivia') were exposed to light for one month so that they were coloured green by chlorophyll, the flavonol level calculated as the aglycones, rose from approximately 0·1 mg/kg kaempferol and zero quercetin to 22 and 13 mg/kg respectively, with the major quantities located in the skin.

Onions

Unlike other vegetables and in contrast to white-skinned varieties, onions (*Allium cepa* L.) with coloured skins are exceptional in their flavonol content. Outer dry skins contain 2·5 to 6·5% quercetin as reported in the literature (Herrmann, 1958; Varshney & Ali, 1971; Kaufmann, el Baya & Meinsen, 1969). Quercetin appears mainly in the free form, i.e. as the aglycone (67–86% of total quercetin in ten samples) and only to a smaller extent as spiraeoside (quercetin-4′-glucoside). The epidermis of onion scales contains quercetin glucosides exclusively, mainly as spiraeoside. This is formed first, the formation of diglucosides follows during storage and increases continuously. There appear to be no flavonols in the mesophyl. The flavonol concentration decreases from the outer to the inner scales with higher levels in the outer than in the inner epidermis (Starke & Herrmann, 1976a).

In the green leaves, the synthesis of the flavonols depends upon the action of light. Glucosides of both kaempferol and quercetin are formed, but no spiraeoside nor the known diglucosides of the scales. During drying in the open an accumulation of flavonols takes place. This is associated with the formation of free quercetin and spiraeoside, but not of free kaempferol (Starke & Herrmann, 1976a).

Onions with white skins (variety 'Weisse Frühlingszwiebel') showed only traces of flavonols, e.g. 10 mg/kg total quercetin in dry skins. This is consistent with previous findings (Herrmann, 1958; Varshney & Ali, 1971). The protocatechuic acid concentration was also very much lower than in coloured skins (Schmidtlein & Herrmann, 1975). Only traces of flavonols were found in garlic.

The scales of onions illustrate well the fact that the flavonols are localized mainly in the outer tissues. This applies also to fruits, tubers and roots, the highest concentrations of flavonols being in the skin and peel (cf. also Tronchet, 1972). The remaining parts of the plants contain mostly less than 1 mg/kg of flavonols calculated on a fresh weight basis (see Table 3).

TABLE 7. Total concentration of quercetin and spiraeoside in the epidermis of the scoler leaves of onions (Stuttgarter Riesen) in mg/kg fresh weight)*

	1st–3rd scale		4th–8th scale	
	Outer epidermis	Inner epidermis	Outer epidermis	Inner epidermis
Total quercetin	24000	540	10600	400
Spiraeoside	20000	640	6900	620
Spiraeoside quercetin as percentage of total quercetin	54	78	42	100

Note: Scale 1 is the outermost non-dried scale.
* Starke & Herrmann, 1976a.

Discolourations

Flavone and flavonol glycosides are not attacked, or are only relatively slowly attacked, by oxidative enzymes such as *o*-phenoloxidases (e.g. Baruah & Swain, 1959). In the presence of transfer substances such as chlorogenic acids (caffeyl-quinic acids) and catechins, they are oxidized more rapidly with the probable formation of dimers as a first step.

Black discolouration occurs as a result of a reaction with iron of flavonols and flavones possessing ortho-dihydroxy groups (quercetin, luteolin). Other colour changes are possible with different salts, e.g. aluminium salts. Trace concentrations of about 1 mg/kg fresh weight are insignificant as far as discolourations are concerned. On the other hand, larger amounts of about 100 mg/kg have marked effects. The relatively high content of rutin in green asparagus leads to greenish-black discolourations of the spears and the liquid in the can, particularly noticeable at the tips which are rich in flavonols. These colour changes occur from a few minutes until up to 3 hr after opening of the can. This phenomenon is of economic importance and has been investigated by a number of authors in the United States (Stevenson, 1950; Davis *et al.*, 1961; Hernandez & Vosti, 1963; Lueck, 1970). The discolouration is caused by complex formation of rutin with ferric ions formed by oxidation and dissolution of iron from the cans. The formation of the water-insoluble rutin-ferric-complex is dependent on pH, and does not occur when sufficient stannic ions are in solution (Hernandez & Vosti, 1963). Canned white asparagus with white tips is not subject to such discolourations (see Table 3) but asparagus with coloured tips can show darkening by reaction with dissolved iron.

In the peels of tomato, chlorogenic acid and quercetin may be found in concentrations up to 500 mg/kg (Wöldecke & Herrmann, 1974b). These compounds are of some importance in the preparation of tomato products. Discolourations of the type occurring in green asparagus can take place by chelation of phenolic compounds with heavy metals. The reaction can be avoided by removal of the skins, as is done in the prepara-

tion of canned tomato purée, since the flavonols occur almost exclusively in the skins (Table 3). Quercetin is believed not to be of importance in the discolouration of fruits since these contain catechins, proanthocyanidins and chlorogenic acids in considerable quantitities (Mosel & Herrmann, 1974a,b; Stöhr & Herrmann, 1975a,b; Stöhr, Mosel & Herrmann, 1975). These phenols are oxidized easily by enzymic action, yielding brown reaction products, and are also easily discoloured by iron. Similarly, in celleriac discolourations can be attributed to the relatively high chlorogenic acid concentrations rather than to the luteolin content. In the discolouration of broad beans quercetin and myricetin, despite their concentrations of 20 mg/kg and 10 mg/kg respectively, are of far lesser importance than the proanthocyanidins.

Significance of flavonols and flavones

Phenolic compounds in plants are significant in several respects. It has been known for a number of years that flavonols and flavones can act as antioxidants and can, for example, protect vitamin C from oxidation (cf. Herrmann, 1973). Heimann *et al.* (1953) and Heimann & Reiff (1953) have shown that quercetin, which occurs most ubiquitously in natural products, is a better inhibitor of the auto-oxidation of ethyl linoleate than the usual antioxidants, ethyl and propyl gallate and the tocopherols. It has been shown also that quercetin in concentrations of 15 mg/100 g is a very active antioxidant for butter fat and lard (Richardson, El-Rafey & Long, 1947; Morris & Riemenschneider, 1949). Other flavonols show similar effects although less actively than quercetin (Kaufmann & el Wahab el Baya, 1967). The position and the number of free hydroxyl groups in flavonols are responsible for their antioxidative activity. It is believed that the 3-hydroxyl group in conjunction with the 2,3-double bond are of decisive significance. If the 3-OH group is bound glycosidically or if it is absent, as in the case of flavones, the antioxidative activity is weakened (Kaufmann & el Wahab el Baya, 1967).

The antioxidative activity which has been observed in a number of vegetables and spices may be due partly to flavonols, probably derivatives of quercetin (Pratt & Watts, 1964; Pratt, 1965). Extracts of the skins of yellow onions which are rich in quercetin inhibit, in a concentration of 0·01%, the auto-oxidation of methyl linoleate which is used frequently as a test substance for the investigation of the oxidation of unsaturated fats (Kaufmann, el Baya & Meinsen, 1969).

A series of investigations has shown that flavonols in particular can stabilize ascorbic acid, especially in the presence of heavy metal ions, such as those of copper, which can destroy ascorbic acid rapidly. As was shown in the case of blackcurrant juice, flavonols in the juices of berry fruits contribute to the protection of vitamin C (Davidek, 1960; Clegg & Morton, 1968; Harper, Morton & Rolfe, 1969). Here the action of quercetin, as well as that of kaempferol, are of considerable importance, while the glycosides are less effective. The addition of 0·01% quercetin to canned apple juice held in cold storage resulted in a 50% reduction in the rate of loss of vitamin C (Waksman & Letan, 1971).

Some flavonoids have characteristic pharmaco-dynamic activities which have been exploited therapeutically for many years. Owing to its easy availability, rutin is best known in this connection, and is contained in a number of medical preparations.

Szent-Györgyi in his classical investigations has attributed vitamin characteristics (vitamin P, P = permeability vitamin) to certain flavonoids, and it is known that certain flavonol glycosides such as rutin have a definite advantageous effect on the stability and permeability of capillary blood vessels. According to Böhm (1967) these flavonoids are especially useful in the treatment of conditions where there is an increased tendency to bleeding as a result of reduced resistance of the capillary blood vessels or increased capillary permeability. This is true in cases of high blood pressure where the danger of bleeding owing to capillary fragility is increased, and diabetes where damage to blood vessels can occur. Anti-spasmolytic, anti-inflammatory and anti-allergic properties of a number of flavonols have also been reported, as well as beneficial effects upon the circulation and heart (Böhm, 1967; Gabor, 1972). Such flavonoids with biological activities are often referred to as bioflavonoids. It is, however, not generally accepted that bioflavonoids possess true vitamin activities, and many of the pharmacological properties reported for these compounds remain to be substantiated more convincingly.

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The use of a rotational fitting technique in the interpretation of sensory scores for different characteristics

J. M. HARRIES AND H. J. H. MACFIE

Summary

A rotational fitting technique, previously described, has been applied to two sets of data from experiments involving sensory perception, to examine mutual relationships between the characteristics scored. In both cases, considerable insight has been gained into the way in which assessors scored the characteristics, which can be used as the basis for economy in designing future experiments of the same type.

Introduction

The eating quality of food has often been defined (e.g. Amerine, Pangborn & Roessler, 1965) as an integration of attributes, but what these attributes are is often a matter for conjecture. An investigator interested in determining what effect some factor in production may have on eating quality usually has to decide what characteristics to assess for his samples and, even when these are obvious, he is faced with the difficulty of examining their interrelationships in interpreting the data. Clearly the more economic an investigator can be in terms of the sensations that the assessors are asked to record, the better, but there is little information available in the literature about the relative importance of the different components of sensation in sensory assessment. Much current research therefore tends to examine each feature of eating quality separately, with little attempt either to amalgamate them into fewer variables, or to study their overall meaning. Statistical techniques to help an investigator in this situation seem to be scarce.

Banfield & Harries (1975) described one such technique and used it to assess the performance of judges in sensory tests by considering their scores to be the coordinates of the samples in multidimensional space, and fitting the separate configurations together. The residual inconsistency was used to examine mutual incompatibilities amongst the judges in a series of experiments concerned with the visual assessment of carcass quality of beef animals. The main concern in that paper was 'with people as judges rather than with beef as such' and the purpose of this paper is to show that the

Authors' address: Meat Research Institute, Agricultural Research Council, Langford, Bristol, Avon BS18 7DY.

technique is equally useful for studying relationships between the attributes themselves.

Correlation coefficients are of little value in this situation, since they are necessarily bivariate. To compare scores for two characteristics one must either average over the judges for each characteristic or calculate a separate coefficient for each judge. In contrast the technique used here produces a single residual inconsistency index between two characteristics which takes into account different scoring strategies of any number of judges.

The technique is applied to two sets of data: the visual scores used by Banfield & Harries (1975) and textural assessments of cooked samples of beef described by Harries, Rhodes & Chrystall (1972). The results throw new light on the way a judge, perhaps unconsciously, appears to use his sensory information to score different characteristics.

Material and methods

Visual assessments

A series of investigations into the composition of sides of beef (Pomeroy *et al.*, 1974) included trials of a method of visual assessment. Full details of the methods with traditional statistical analysis of the results, have been given by Harries, Pomeroy & Williams (1974). The data used here consist of the scores given by seven judges to 180 sides of beef for each of the ten following characteristics, on arbitrary seven-point scales, in which increasing scores reflected increasing amount (1—5) or improvement (6—10) as defined by mutually acceptable criteria.

(1) Subcutaneous fat (as proportion of side weight).
(2) Volume of kidney knob and channel fat.
(3) 'Feathering' (i.e. fat intermingled with lean between the ribs).
(4) Muscle (as proportion of side weight).
(5) Muscle to bone ratio.
(6) Conformation of buttock.
(7) Conformation of rump.
(8) Conformation of loin.
(9) Conformation of forerib.
(10) Overall conformation.

Five of the judges were experienced fatstock officers (referred to in this paper as 'experts'). The other two had specialist butchering experience and will be referred to as 'butchers'.

Textural assessments

For the second set seven textural assessments made by nine assessors (referred to as tasters) on forty-two beef roasts were used. This is a sub set of the data first described by Harries *et al.* (1972).

Each taster scored each sample for the following quantitative characteristics.

(1) Initial resistance.
(2) Initial wetness.
(3) Overall juiciness.
(4) Cohesion.
(5) Hardness.
(6) Overall texture.
(7) Chew count.

The first six characteristics were scored by placing a cross on a 100 mm line, and subsequently measuring the distance from one end. The seventh was a simple count of the number of chews necessary before swallowing.

Rotational fitting

A method of rotational fitting first described by Gower (1971) involves the centralization of each set of data points, followed by dilation and rotation of the sets to minimize the sums of squares of the distances between corresponding samples. A simplified explanation of the process was given by Banfield & Harries (1975) who used the technique to examine relationships between twelve visual assessment judges.

The same method is used here to examine relationships between the characteristics. Thus for the visual assessments data we have two configurations of points for each characteristic; the 180 points lying in five dimensions (one dimension for each expert) and the 180 two-dimensional points scored by the butchers. As there are nine tasters the textural assessments study produces seven configurations of forty-two points lying in nine dimensions.

Treating butchers, experts and tasters separately, we centralize, dilate, and finally rotate each characteristic configuration to every other. Each rotation produces an M^2 value which is the sum of squares of distances between corresponding points after rotation. This is a measure of how similar the internal structures of the two configurations are when differences due to location, scale and orientation are removed. Thus a low M^2 value indicates that the judges' perceptions of the differences between the samples were similar for the two characteristics in question. This process was repeated for each pair of characteristics.

Principal coordinate analysis

For each of the three sets of configurations, experts, butchers and tasters, the end product is a set of M^2 values which represents the overall squared distances between characteristics. Principal coordinate analysis, described by Gower (1966) can be used to produce the coordination of each characteristic relative to the principal axes so as to reproduce exactly the given (M^2) distances. A plot of the coordinates relative to the first two principal axes gives the best two-dimensional representation of their relative positions.

Both the rotations and the principal coordinate analyses were performed using the GENSTAT programming language (Nelder *et al.*, 1971).

Results

Visual assessments

Experts. Figure 1 shows the ten carcass characteristics relative to the first two principal axes of their M^2 values. Three main clusters appear. In the bottom left hand corner

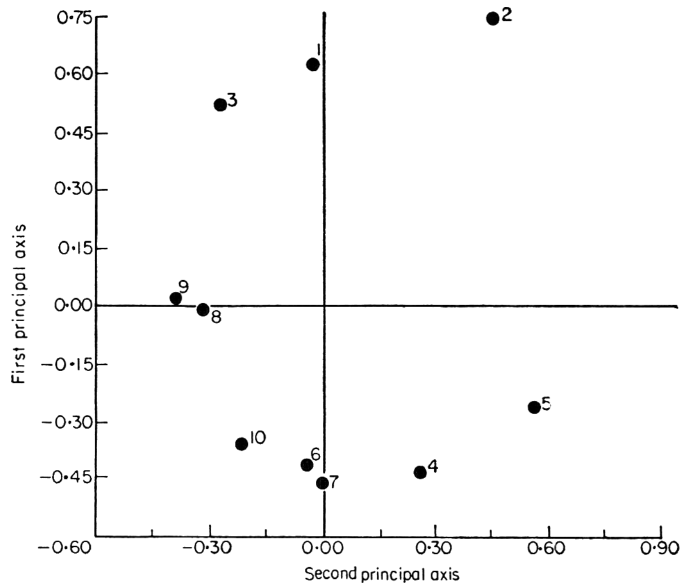

FIG. 1. Plot of ten carcass characteristics scored by the seven experts relative to the first two principal axes.

overall conformation (10) is closest to conformation scores of buttock (6) and rump (7). The proportion of muscle in the side (4) is equidistant from these two and muscle to bone ratio (5). Similarly subcutaneous fat (1) falls in between volume of kidney knob (2) and feathering (3). The third cluster, conformation scores of loin (8) and fore rib (9), lies between the first two.

Butchers. Figure 2 gives the corresponding picture for the butchers. For the present purpose interest centres on the *relative* positions of the characteristics. Again there are three main clusters. The bottom cluster is more compact than that of the experts but does not contain muscle to bone ratio (5). This is now very close to subcutaneous fat (1) in the top right quadrant of Fig. 2, along with volume of kidney knob (2). Conformation of foreribs (9) is now equidistant from that of loin (8) and feathering score (3).

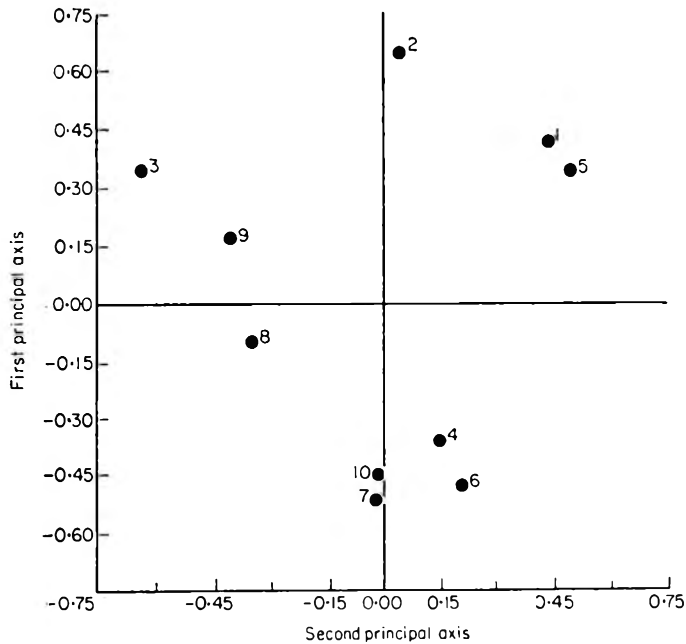

FIG. 2. Plot of ten carcass characteristics scored by the two butchers relative to the first two principal axes.

Textural assessments

Figure 3 shows the positions of the seven textural characteristics relative to the first two principal axes of the M^2 values. There is only one tightly packed cluster, comprising initial resistance (1), cohesion (4), hardness (5) and overall texture (6). The other three characteristics, initial wetness (2), overall juiciness (3) and chew count (7), are almost equidistant from each other and from the main cluster.

Discussion

Interpreting the figures

The three figures are two-dimensional representations of multi-dimensional distances and therefore should be interpreted with care. Nevertheless if two characteristics are close to each other we can infer that the judges' perceptions of the differences between the samples were similar for both the characteristics. Therefore, if we pick one member of each of the clusters in Figs 1–3 we can discard the other members as giving us very little extra information about the differences between samples.

Visual assessments

The overall picture for the experts shows an encouraging discrimination between

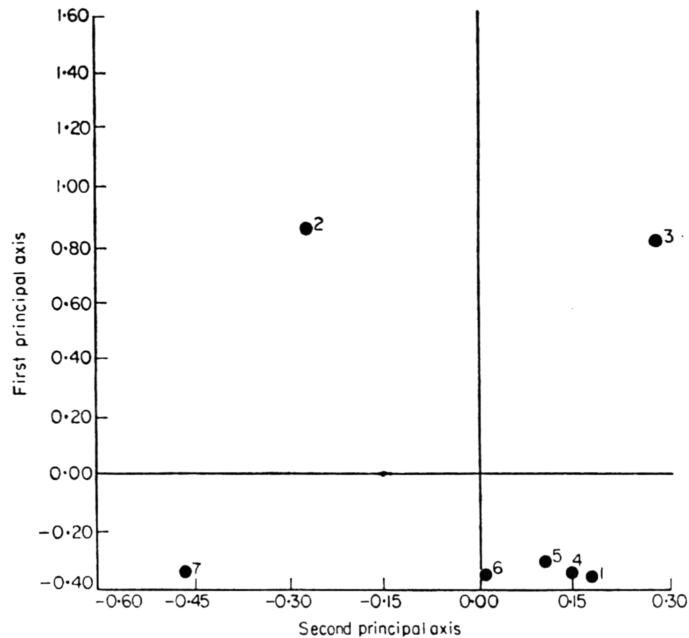

FIG. 3. Plot of the seven textural characteristics scored by nine tasters relative to the first two principal axes.

characteristics, but it appears that one of loin (8) and forerib (9) conformation could be discarded and also the scores for buttock (6) and rump (7) are very close.

The butchers' scores for proportion of muscle in side (4), conformation of buttock (6) and rump (7) give almost the same internal structure as that produced by overall conformation scores (10). On the basis of these data the first three could be eliminated with very little loss of information. In addition one of muscle to bone ratio (5) or subcutaneous fat (1) could be removed. But the butchers appear more discriminating in their scores of loin (8) and forerib (9) conformation and neither of these could be discarded. The two sets of visual judgements reveal some sharp differences in their ability to discriminate between characteristics. It is interesting to attempt to relate these differences to differences between the two types of judge in their attitude to a side of beef. The experts are presumably more objective than the butchers, who are accustomed to making a snap decision on the retail value of a carcass.

Thus the experts show overall conformation lying between the rump, buttock and the loin, forerib clusters. It is a composite score of the two anatomical sections. The butchers clearly have the commercially important rump and buttock in mind when they score overall conformation. Similarly the butchers appear to equate differences in subcutaneous fat (1) with variation in muscle to bone ratio (5), whereas the experts score them quite differently. The retail value of loin and forerib joints are clearly affected by

the 'feathering' level, and this may explain why this characteristic is so much closer to them in the butchers' picture.

It is also interesting to note that in Fig. 1 the fatness characteristics 1, 2 and 3 are clearly differentiated from the others along the first principal axis; this is a different result from that obtained earlier (Harries *et al.*, 1974) when a principal component analysis of the raw data failed to indicate 'the hoped-for result, that the conformation variables would at some point stand out from the fatness variables'.

Textural assessments

In Fig. 3 it is clear that the first principal axis separates the two juiciness characteristics (2 and 3) from the tough/tender characteristics (1, 4, 5, 6 and 7). This is consistent with the findings of Harries *et al.* (1972). Figure 3 also suggests that a substantial reduction in the number of characteristics scored could be made without seriously impairing the discriminating power of the tasters. Initial resistance (1), cohesion (4), and hardness (5), tell us very little more about the differences between the roasts than the simple overall texture scores (6). In contrast, the two moisture characteristics (2 and 3) and the chew count (7) are quite distant from each other and the texture cluster.

The fact that chew count (7) is separated from the other textural characteristics is interesting in the light of its history as a method of evaluating the tenderness of meat. It has obvious attractions because of its simplicity and clear relationship with texture. In use, however, it proved unreliable. Burrill, Deetherdt & Saffle (1962) found the chew count to be the least satisfactory measure of tenderness, whilst Harrington & Pearson (1962) found that both the chew count itself and its repeatability on adjacent pieces of pork varied considerably between tasters but it gave high correlation with a Warner-Bratzler sheer force measurement when the correlations were calculated for each taster separately. Szczesniak & Torgeson (1965) have pointed out some of the difficulties inherent in its use (such as the definition of the end-point, and the fact that tasters may adjust the force of the chewing motion to the texture of the meat rather than its frequency) and confirmed the wide variation between different tasters. In the present context, this variation does not matter, since an adjustment is made for it. The fact that after centralization, dilatation and rotation, it is still separated from characteristics 1, 4, 5 and 6 by the principal coordinate plot means that tasters must be using information of a different kind in evaluating it. This may be because it is the only frequency-type score, the others being force-based.

Thus there is again a difference between the present results and those obtained earlier (Harries *et al.*, 1972) by means of a factor analysis which indicated that two factors, thought to be 'toughness-tenderness' and 'juiciness', explained about 95% of the variation in the data. It is important to distinguish the two statistical methods, especially since in a factor analysis, rotation of the axes to maximize the explained variance is often done, though in the earlier analysis no such rotation was used. The method used here

rotates the original data sets, after centralization and dilation, to minimize the residual variation between them and the findings have added to our knowledge of mutual relationships between the characteristics. The separation of chew-count (7) from the toughness characteristics (1, 4, 5, 6) along the second principal axis can perhaps be explained as above, but no ready explanation can be advanced for the separation of initial wetness (2) from overall juiciness (3) along the same axis.

Conclusions

The use of rotational fitting and principal coordinate analyses of the resulting residual distances can help to improve the practice of sensory assessment in two ways. It can indicate that set of characteristics for which the judges are most discriminating. Secondly it can be used to compare the discrimination hierarchies of different types of judges. This will help the choice of different characteristics and judge types according to the purpose of the investigation. The application of the technique to two sets of data cannot be expected to result in firm conclusions about the particular characteristics examined, but the tentative examination of those given above is enough to indicate the potential of the technique in future work.

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Effect of calcium ions and low-methoxyl pectin on the drained weight of grapefruit segments

I. J. KOPELMAN, S. MIZRAHI AND R. SCHAB

Summary

The admixture of low-methoxyl pectin and calcium ions, and its effect on the final drained weight (FDW) in a well-controlled system of grapefruit segments, were studied.

The increased FDW in the presence of the above admixtures is mainly attributable to formation of a gel layer which settles on and between the segments and increase the FDW both directly and through reduction of water outflow.

Introduction

Grapefruit segments are an important product in the citrus industry. Factors effecting the quality of grapefruit packs are mainly taste, firmness, texture, appearance and drained weight. The drained weight, being an important economic factor (and one of the hardest to control during process and storage) has been the subject of various investigations. The general parameters relevant to its behaviour were investigated, inter alia, by Berezovsky (1970), Levi *et al.* (1969) and Mannheim & Bakal (1968). It was also shown recently (Kopelman, Mizrahi & Kochba, 1975) that the drained weight at equilibrium—the final drained weight (FDW) of grapefruit segments is flow-rate dependent, because of interference of the faster flow of the water with the slower migration of the solutes. It was also found to be sensitive to commonly used additives such as calcium salts (Kopelman *et al.*, 1975; Mannheim & Bakal, 1968; Olsen *et al.*, 1966; Sidewell & Cain, 1955) and low-methoxyl pectin or LMP (Berezovsky, 1970; Board *et al.*, 1966; Mannheim & Koteck, 1967).

The present study deals with the admixture of these additives and its effect on the final drained weight in a well-controlled grapefruit segment system.

Materials and methods

Materials

Grapefruit segments (lye-peeled) were drawn from a commercial production line prior to canning.

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

Low-methoxyl pectin, Purple Ribbon grade, Obi-Pektin AG, Bischofszell, Switzerland; purified by three successive extractions with ethanol-hydrochloric acid solution (2 volumes ethanol + 1 volume 0·15 N hydrochloric acid), followed by washing with ethanol and vacuum-drying at 70°C for 12 hr.

Sugar. The 46°Bx syrup, deionized (in order to remove all traces of calcium) by passing through a cationic (IR 120, Koch Light Labs, Colnbrook, Bucks, England) and an anionic bed (IRA-410, Rohm & Hass Co, Philadelphia) down to 600 KΩ electric resistance (determined by bridge type conductometer, type E 382, Metrohm, AG, Herisau, Switzerland).

SO_2 (preservative). Five per cent aqueous solution, passed twice through the cationic bed for the same purpose.

Isoionic stock solutions. Fifteen per cent w/v $CaCl_2$ and 11·86% w/v NaCl were combined to yield any desired concentration of Ca^{++} at constant total ion concentration and a uniform dilution effect. (Equal volume of each of the solutions contains equal amount of ions.)

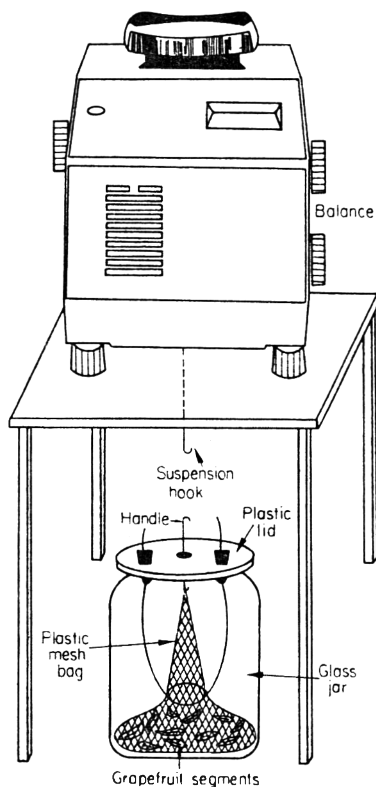

FIG. 1. Experimental set-up (schematic).

Experimental

The equipment used is shown in Fig. 1. A 150 g sample of grapefruit segments was placed in a plastic mesh bag (mesh size about 5 mm) inside an 800 ml glass jar with a plastic lid. The mouth of the bag was tied with a nylon string and the bag suspended (from a hooked handle driven through the lid) and immobilized by an adjustable stainless steel ring assembly. An equal amount of 46°Bx sucrose syrup was poured into the jar, with the specified amount of $CaCl_2$ stock solution and the balancing NaCl. The pectin (0·1%, based on total weight) was solubilized in the syrup under vigorous mixing. All samples, preserved with SO_2 (1000 ppm, based on total weight), were pasteurized in a 90°C water bath for 25 min and water-cooled immediately.

A model system (5 mm glass spheres) was also tested in 46°Bx syrup, this time with 1800 ppm Ca^{++}, under the same regime.

Drained-weight determination. The drained-weight pattern was observed by lifting the mesh bag periodically out of the syrup, suspending it (by the hook of the lid handle) from a hook provided at the bottom of a balance (semi-analytical, Sartorius model 2257, accuracy $\pm$0·005 g), and weighing after a 30 sec delay allowed for draining. The drained weight data in the diagram are averages of eight replicates, with standard error of about 0·5% final drained weight.

Results and discussion

The drained weight pattern v. time is given in Figs 2 and 3 for isoionic concentration systems with and without LMP, respectively, with the Ca^{++} level as parameter. The curves are seen to lack the 'turning point' typical of many canned fruits (Sterling, 1969; Berezovsky, 1970), suggesting system permeable in practice to all solutes.

A cross linked system has reduced ability to swell thus admixture of Ca^{++} can be expected to reduce the FDW, mainly by increasing the degree of cross-linking through formation of intermolecular calcium pectate bonds within the segments. By contrast, admixture of LMP in the presence of Ca^{++} may lead to formation of a gel which would settle on and between the segments and increase the FDW both directly and through reduction of the water outflow (the FDW is known to be flow-rate dependent—Kopelman *et al.*, 1975). Accordingly (Fig. 4), under an isoionic regime, and at a given LMP level, the FDW increases with the Ca^{++} level up to a certain 'saturation' point, beyond which the trend is reversed. Obviously, the lower the given LMP level, the lower also the optimal Ca^{++} level; this is seen when no LMP is added and the process involves only the pectin naturally present in the system, in which case the curve peak is shifted to the left (Fig. 4).

The effect of gel formation is also demonstrated by the glass sphere model (Table 1). The syrup/LMP/Ca^{++} system showed an increase of 45% over the original weight, while all other four variants showed approximately the same—and much smaller—

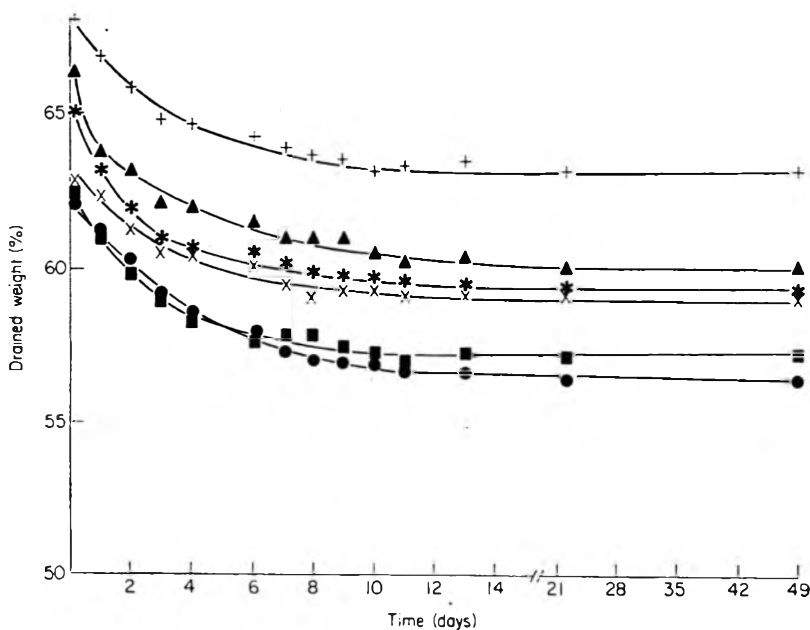

FIG. 2. Effect of Ca^{++} ion on drained-weight pattern at isoionic salt concentration. Ca^{++}: * 0 ppm; ▲ 180 ppm; × 360 ppm; ■ 720 ppm; ● 1080 ppm; + no salt.

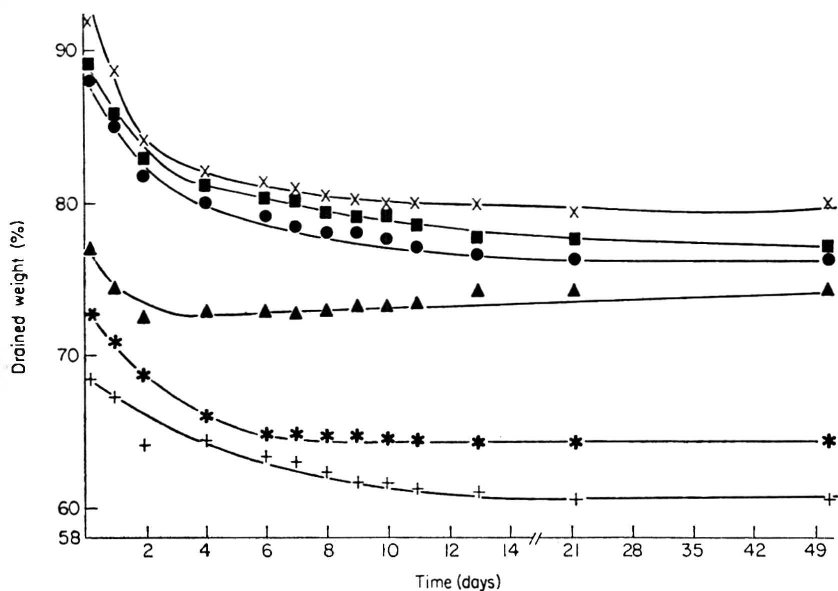

FIG. 3. Effect of Ca^{++} on drained-weight pattern in the presence of LMP, at isoionic salt concentration. Ca^{++}: * 0 ppm; ▲ 180 ppm; × 360 ppm; ■ 720 ppm; ● 1080 ppm; + no salt.

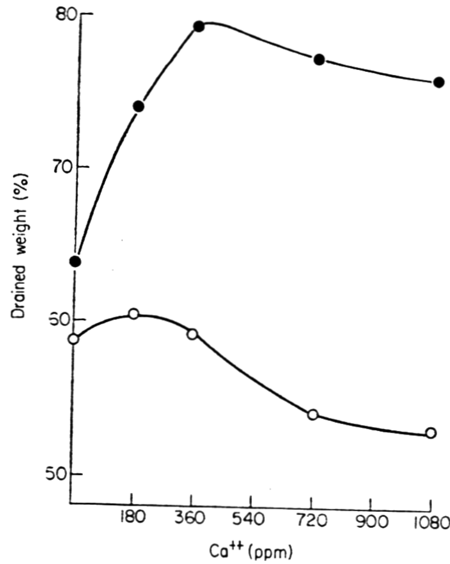

FIG. 4. Effect of Ca^{++} ion on FDW at isoionic salt concentration. ● With added LMP; ○ without added LMP.

TABLE 1. Drained weight in glass-sphere model

	Increase in weight (%)
Water	10
Syrup	10
Syrup + Ca^{++}	11
Syrup + LMP	15
Syrup + Ca^{++} + LMP	45

increase. The effect was also observable visually: the three component mixture became turbid with a noticeable increase in viscosity, while in the other variants the mixture remained clear.

Figure 5 shows that, in view of the high correlation coefficient, the long-term FDW of a given system can be predicted from the initial change rate of the drained weight determined on the basis of less than 24 hr data. The above could be useful in monitering the process by providing an early indication of the FDW normally equilibrated at a much later period.

In conclusion, the increase FDW in the presence of the above admixtures is mainly

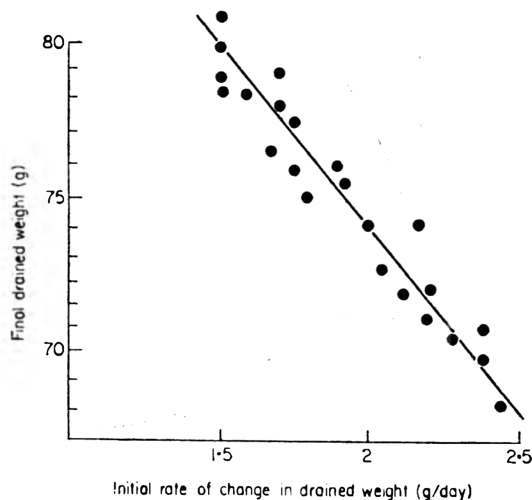

FIG. 5. Effect of initial change rate of drained-weight on FDW. FDW $= -11{\cdot}2$ rate g/day$+96$ ($r=0{\cdot}945$).

attributable to formation of a gel, which acts both as (a) added 'ballast' and (b) further barrier for water outflow, which in turn has an indirect effect upon establishing a higher FDW at equilibrium.

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Studies of mango processing

I. The foam-mat drying of mango (Alphonso cultivar) puree

R. D. COOKE, G. R. BREAG, C. E. M. FERBER, P. R. BEST AND J. JONES

Summary

An experimental thin layer drier is described for evaluating foam-mat drying characteristics. Polyglyceryl stearate was found to be the most effective foaming agent for Alphonso mango puree. A stable mango foam could be prepared using this surfactant without prior concentration of the puree or the use of viscosity increasing additives. The dependence of the drying rate on temperature, foaming agent concentration and air flow rate is reported. A readily re-hydrateable powder of 2% moisture content could be produced by drying at 70°C for 20 min. This drying rate compares favourably with earlier studies of the foam-mat drying of citrus concentrate. The vitamin C content of the puree was decreased by 80% during foam-mat drying, and this powder was less acceptable than a product obtained by spray-drying.

Introduction

The mango (*Mangifera indica* L.) is grown in large quantities in many parts of the tropics especially India, the cultivar 'Alphonso' being one of the most popular. The puree is used in nectars, drink bases and flavouring agents (Bhatnagar & Subramanyam, 1973). Mango utilization, whether in the producer country or for export to premium markets is limited by the perishable nature of the fruit and the short harvest season. The production of a dried puree could assist in overcoming this problem and reduce transport and storage costs.

Hertzendorf & Moshy (1973) have reviewed the application of foam-mat drying to many heat sensitive materials, including fruit juices. Foam-mat drying depends on the generation of a heat-stable foam using a suitable surfactant-stabilizer (Morgan & Ginnette, 1960). Drying rates at mild temperatures are enhanced because of the increased gas-liquid interface, furthermore the open structure of the product ensures rapid re-constitution. Vacuum techniques and high temperatures are not employed with consequent cost reduction and minimization of heat damage. The surface area of the

Authors' address: Ministry of Overseas Development, Tropical Products Institute, 56–62 Gray's Inn Road, London WC1.

foam may be increased by 'cratering' with a controlled air blast (Morgan *et al.*, 1961), though some foams cannot be cratered effectively presumably because of surface tension or viscosity considerations.

Several types of foam stabilizers have been tested with the aim of producing foams that withstand spreading, cratering and drying (Hart *et al.*, 1963). Foam collapse increases the drying time, reduces product quality and hinders de-traying. Previous studies (Morgan *et al.*, 1961) indicated that foaming of various citrus juices required high soluble solids contents (pre-concentration of the juice being necessary); alternatively the use of 'stiffening' agents such as carboxy-methyl celluloses, guar gum or pectins in addition to a suitable surfactant was recommended (Brown *et al.*, 1973). Citrus concentrate foams (2·2 mm thick) can be dried to 2% moisture content in 15–20 min at 70°C (Morgan *et al.*, 1961). Bates (1964) has reported oven stability tests of foamed tropical fruit products, including mango puree (unstated cultivar). A foam of limited stability was produced using 1% glycerol monostearate, but no drying characteristics or product analyses were described for this or the other products mentioned. Jayaraman *et al.* (1974) recently reported the foam-mat drying of mango and other fruit purees using 2% of a distilled monoglyceride as a foam stabilizer. The product was dried at 80°C for 30 min followed by 65–70°C for 30–90 min, and $CaCl_2$ desiccant was required to further reduce the moisture content to below 3%. Vitamin retention or drying characteristic data were not presented. Information about the drying characteristics of fruit juice foams and about the resultant product quality is superficial (Hertzendorf & Moshy, 1973).

The present paper reports a drier system for the evaluation of foam-mat drying characteristics: the selection of a suitable stabilizer for the production of Alphonso mango foam: the drying characteristics of the cratered foam: and analyses of the dried products. The foam-mat dried powder is compared with a product obtained by spray-drying, and was found to be of inferior quality.

Materials and methods

Production of mango foams

The difficulty of obtaining air-freighted mangoes in the U.K. limited these investigations to the popular cultivar 'Alphonso', imported from India. Green, mature mangoes (60 kg) were ripened at 30°C. The ripe fruit was pulped in a paddle-pulper (Brierly, Collier & Hartley Ltd, Rochdale) through a 0·124 mm screen to remove peel and stones. The puree obtained (corresponding to about 70% of the fresh weight of the whole fruit) was frozen and stored at −10°C prior to use. The pH of the puree was 4·1 and the total soluble solids content was 21·5° Brix.

Foams were produced in 300 g lots using a Kenwood domestic mixer operated at maximum speed for 10 min. Various fatty acid esters, proteins, gums, celluloses and pectins were evaluated as possible foam stabilizers (Table 1). These additives were

generally prepared as stock suspensions by homogenizing (MSE laboratory homogenizer) for 2 min in hot (70–80°C) distilled water. The cooled suspensions (usually 20% w/v or more) were added to the puree to the required levels, in the range 0·5–2·5% of the total solids present in the puree (i.e. dsb). Foam stability was assessed by measurements of density and by the foams' capacity to remain supported on the drying trays.

Experimental dryer

Two thin layer driers (described below) with automatic weighing facilities were modified for this study by the construction of perforated aluminium trays to support the foam. The sixteen gauge aluminium trays had 3·17 mm diameter holes on 4·76 mm centres as described by Morgan *et al.* (1961). The data obtained from the drying trials were processed by a Digico Micro 16 V computer (Digico Ltd, Stevenage, Herts). Each drier (Fig. 1) employs a centrifugal fan to blow air over a bank of thermostatically

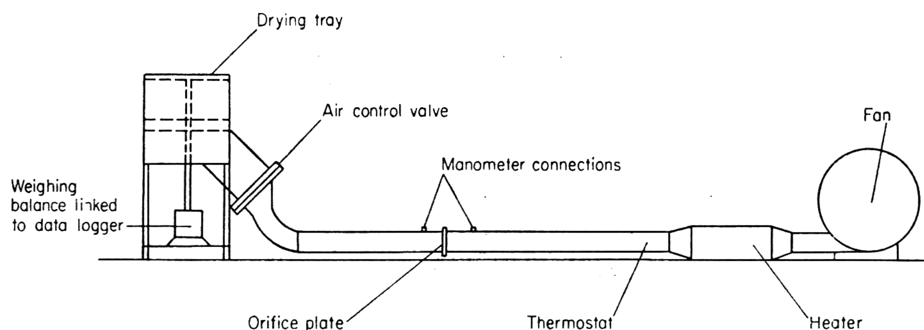

FIG. 1. Diagram of thin layer dryer system.

controlled electric heaters which heat the drying air to the required temperature. The heated air then passes through a 75 mm diameter stainless steel pipe, fitted with an orifice plate (designed to British Standard 1042, 1964, part 1), into a plenum chamber before exhausting to atmosphere via the drying tray. The air flow rate is regulated by an iris valve located prior to the plenum chamber, and measured as the pressure drop across the orifice plate using an inclined manometer. Inside the plenum chamber a wire mesh and a 100 mm deep honeycomb structure is used to reduce turbulence and to ensure the even passage of drying air through the drying tray. Air drying temperatures over a range of 30–85°C and air flow rates of 0·5–3·5 mm/sec per sq. mm of drying area are available. The drying tray is supported by a central rod which is linked to a specially adapted weighing system.

At steady state conditions, the material to be dried is placed on the drying tray and the loaded tray is tared. As drying progresses, the time for every 0·5 g of moisture evaporated is recorded automatically through a Harwell 6000 series data logger on to a teletype unit (Atomic Energy Research Establishment, Harwell, Oxon.).

Evaluation of drying characteristics

The foam was spread on to the tray with a scraper to give a layer not exceeding 2·2 mm thick and a tray loading of 1·22 kg/m^2. The mat of puree was then cratered by moving a controlled air blast (about 45 m/sec) along the underside of the tray.

Drying was carried out with air flow rates of 2·3–3·0 mm/sec per sq. mm of free area of drying tray at temperatures between 50–80°C. The trays have about 40% free area for the passage of drying air, resulting in an air speed in contact with the foam varying between 580–750 mm/sec. The final moisture content of the dried material is determined in the laboratory so that drying rates at various stages of the drying process can be computed.

Spray-dried product

A sample of the mango puree was spray-dried for comparison purposes in the quality assessment of the foam-mat dried product. A cocurrent Kestner laboratory spray-drier (APV Ltd, Crawley, Sussex) fitted with a centrifugal atomizer operated at 14 000 rpm was used. The puree viscosity was reduced by a treatment with 0·1% w/v Ultrazyme (Ciba-Geigy Ltd, Basle, Switzerland) for 2 hr at 30°C. This feedstock atomized uniformly, but difficulties were encountered owing to the slow flow rate through the gravity feed system of this drier (causing fluctuations in the outlet temperature). A 4 kg sample of the puree was dried under stable drying conditions by diluting this feedstock with distilled water (2 : 1 v/v respectively). The feed rate was 250 ml/min and the inlet and outlet temperatures were 154°C and 88°C respectively.

Product analyses

Product moisture contents were determined by drying to constant weight at 70°C under vacuum. Ascorbic acid and dehydroascorbic acid were determined fluorimetrically by the o-phenylenediamine method of Deutsch & Weeks (1965), as modified by Blundstone (1974). Total and reducing sugars were determined spectrophotometrically using potassium ferricyanide, as described by Gaines (1973). Carotenoids content was estimated by extracting the puree (or the reconstituted powder) with hexane-acetone (3 : 2 v/v) containing 0·2% (w/v) $MgCO_3$, and measuring the absorbance of the extract at 436 nm (A.O.A.C., 1970). β-carotene type III (Sigma Chemical Co Ltd, Kingston upon Thames) was used as standard. All assays were performed in duplicate, and the data derived from the dried powders were related to fresh puree by allowing for moisture and foam stabilizer contents.

Results and discussion

Drying characteristics—foam-mat drying

The different types of surfactants, emulsifiers and thickening agents tested as foam stabilizers are shown in Table 1. Polyglyceryl stearate (as Crester KZ, Croda Foods Ltd, Widnes) and glycerol monostearate (GMS, British Drug Houses, Poole) were

TABLE 1. Types of additive tested as mango foam stabilizers

Fatty acid esters	Glycerol monostearate, polyglyceryl stearate, calcium stearyl lactylate
Proteins	Egg albumen, soyflours, soyflour hydrolysates
Gums	Guar gum, locust bean gum, calcium or potassium carragheenan, sodium alginate
Celluloses	Carboxymethyl cellulose, methyl cellulose, ethyl methyl cellulose.
Pectins	Citrus pectin and low-methoxyl pectin

found to be the most effective additives. The GMS foams were marginally heat stable at the 1·5% dsb level; polyglyceryl stearate produced heat stable foams at the 1·5% level, of density between 0·33–0·40. Calcium stearyl lactylate at the 2% level produced high density foams (0·85) that collapsed quickly at room temperature. The effectiveness of polyglyceryl stearate as a mango foam stabilizer indicated that mango puree could be foam-mat dried without the need for additional 'stiffening' agents or increasing the soluble solids content. The celluloses, pectins, gums and proteins tested in the present study were ineffective as stabilizers (i.e. negligible reduction in puree density after the 10 min mixing procedure) and also had a negligible effect in combination with GMS. The failure of these additives to enhance the foam forming ability is presumably related to the high consistency and viscosity of the puree. Similarly, dissolving cane sugar in the puree to increase the solids to 40° Brix did not improve foaming ability using GMS as stabilizer. Consequently, the drying studies reported below were undertaken using polyglyceryl stearate as the stabilizer.

Approximately fifty drying trials were done to establish the effects of temperature, flow rate and stabilizer concentration on the drying rate. A foaming agent concentration of 1·5% dsb was selected for the majority of the drying trials giving a tray loading of 1·22 kg/m^2. Increasing the additive concentration resulted in an enhanced drying rate, but reduced the foam density and gave a lower loading of foam per surface area of tray. A comparison of the drying characteristics of two foams of differing stabilizer contents is shown in Fig. 2. The moisture content versus time curves (Fig. 3) show the increase in drying rate with temperature. The mango foam could be dried to less than 3% moisture within 20 min, which compares favourably with the results of earlier studies using orange juice concentrate (Bissett *et al.*, 1963).

The drying rate profiles (Fig. 4) show that there are two falling rate periods and that the rate of change in the drying rate increases as the foam dries. This is in contrast to the usual behaviour of agricultural drying processes (Hall, 1957). However, foam-mat drying profiles are product dependent: Komanowsky, Sinnamon & Aceto (1964) reported only one falling rate period on foam-mat drying glue, whereas Chandak & Chivate (1972) found a progression of constant rate phases when foam-mat drying calcium carbonate. They ascribed this to periodic bursting of successive layers of foam bubbles, effectively exposing new surfaces as drying proceeds.

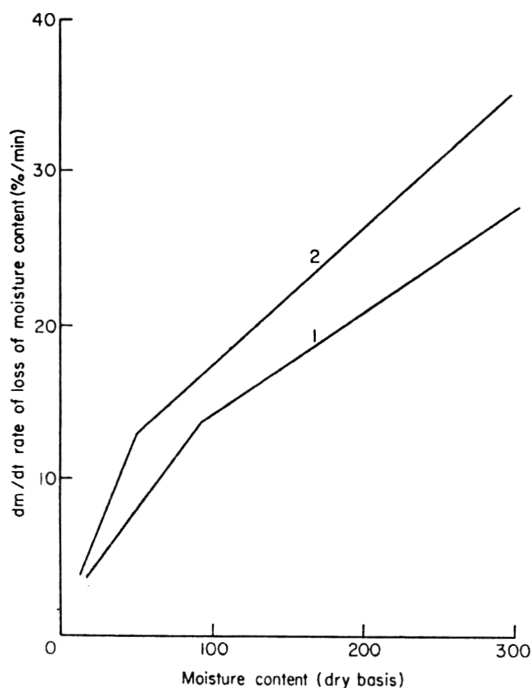

FIG. 2. The dependence of drying rate on the stabilizer content of the foam. The two foams were dried at 72°C with an air flow rate of 580 mm/sec as described in 'Materials and methods'. (1) 1·0%; (2) 1·5%.

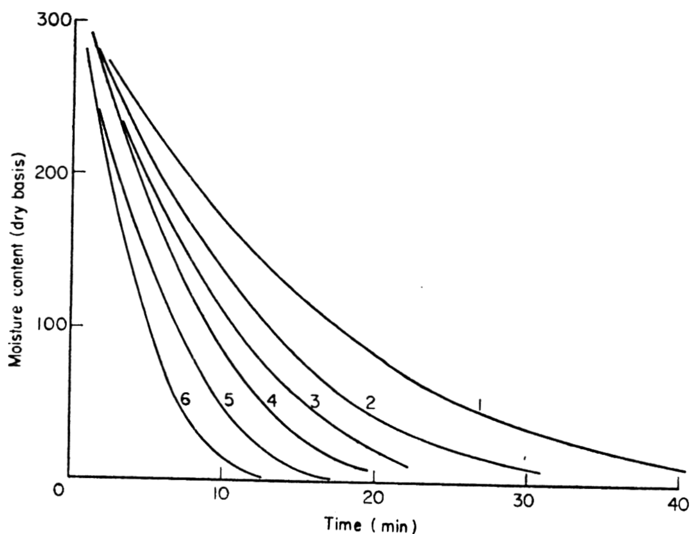

FIG. 3. Characteristic drying curves at various temperatures at fixed stabilizer content (1·5%) and air flow rate (580 mm/sec). (1) 51°C; (2) 56°C; (3) 60°C; (4) 66°C; (5) 74·5°C; (6) 80°C.

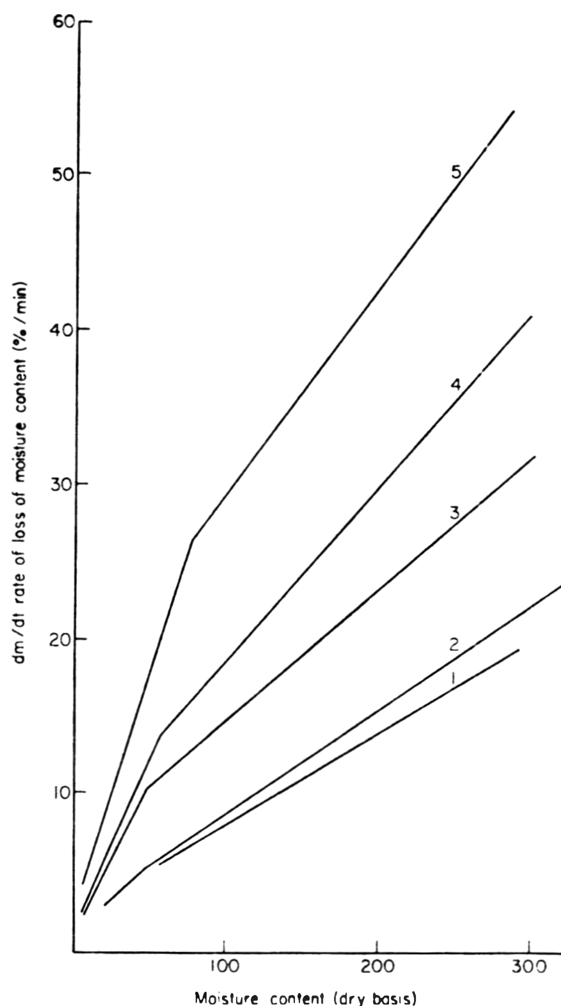

FIG. 4. The change in rate of loss of moisture versus moisture content at various temperatures (1·5% stabilizer content and 580 mm/sec air flow). (1) 51°C; (2) 56°C; (3) 66°C; (4) 74°C; (5) 80°C.

Increasing the air flow rate from 580 to 750 mm/sec within the temperature range 50–70°C increased the drying rate in the early stages of drying, but the attainment of a moisture content of less than 5% was not achieved more rapidly (Fig. 5). The effect of a higher air flow rate became less marked as the air temperature increased and at 70°C was negligible.

Drying characteristics—spray drying

Spray-drying of viscous heat-sensitive materials containing a high proportion of hygroscopic substances (e.g. sugars) is often associated with problems of non-uniform

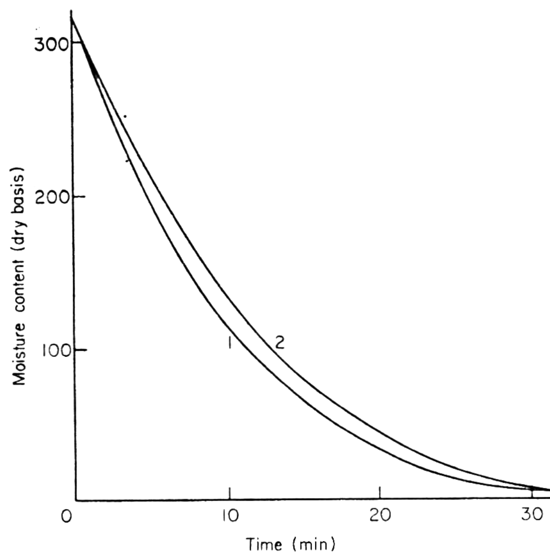

FIG. 5. Characteristic drying curves showing the dependence on air flow rate (1·5% stabilizer content and 56°C). (1) 75 cm/sec airflow; (2) 58 cm/sec airflow.

atomization and wall deposition (Ponting, Stanley & Copley, 1973). Brennan, Herrera & Jowitt (1971) have described the wall deposition problem enountered in the spray-drying of orange juice concentrate, and indicated that wall deposition is dependent on the thermoplasticity and hygroscopicity of the powder. Wall deposition was not a serious problem with mango puree; the product was a fine, free-flowing powder of bright colour. However, most of the product remained in the lower part of the chamber instead of reaching the cyclone collector. This powder could readily be dislodged by knocking the chamber walls, suggesting that the air-broom or vibrating hammer devices available on larger spray-driers would overcome this problem.

Product analyses

The drying conditions for various samples of product, are given in Table 2 and the analyses in Table 3. The total and reducing sugar contents of the products are very similar to the fresh puree. Extensive losses of vitamin C occur during foam-mat drying; about half of this loss can be accounted for as an increase in dehydroascorbate. This vitamin C loss is not simply caused by excessive drying, since an 80% loss was found in samples analysed after only 16–18 min of drying at 66–70°C (i.e. at 2·0–2·5% moisture content). The foaming operation itself was not accompanied by detectable reduction in vitamin C content ($<5\%$), while holding the foam at room temperature (22 ± 2°C) for 45 min resulted in a loss of about 25% of the vitamin C, most of which could be accounted for by the increase in dehydroascorbate. This suggests that a combination of heat and air is required for the vitamin C and the dehydroascorbate losses reported in Table 3.

TABLE 2. Drying treatments of samples analysed in Table 3

Sample	Foaming agent (% dsb)	Foam density (g/ml)	Drying temp. (°C)	Air-speed (cm/sec)	Drying time (min)
A	1·5	0·40	76	75	26
B	1·5	0·39	72	75	30
C	1·5	0·39	60	75	35
D	1·0	0·56	71	75	30

TABLE 3. Chemical analyses of foam-mat and spray-dried mango powders

Sample	Moisture content (% wet basis)	Ascorbic acid (mg/100 g puree)	Dehydro-ascorbic acid (mg/100 g)	Total sugars (g/100 g)	Reducing sugars (g/100 g)	Total carotenoids (mg/100 g)
Mango puree	78·2	52·0	28·1	17·1	3·2	13·4
A	2·2	10·8	51·3	17·2	3·0	12·1
B	2·2	1·2	47·3	17·5	2·7	10·2
C	3·2	12·5	47·7	17·2	2·7	10·8
D	4·7	5·3	44·3	17·0	3·0	9·5
Spray-dried (removed from chamber)	1·0	30·4	8·2	16·7	3·1	7·8
Spray-dried (removed from cyclone collector)	5·1	42·3	25·0	17·0	3·1	10·7

This behaviour is in marked contrast to that observed with orange juice concentrate (Bissett *et al.*, 1963) and grapefruit juice concentrate (Berry *et al.*, 1965) which were foam-mat dried at 71°C for 26 min with no detectable loss of vitamin C (determined by 2,6-dichlorophenol-indophenol titrations). Berry *et al.* (1965) observed that orange juice concentrate is more sensitive to heat damage than grapefruit juice concentrate (judged by sensory evaluation), i.e. orange powder dried at 82°C for 14 min was detectably different from orange juice control, whereas changes were only detectable after 26 min at this temperature with grapefruit samples.

The spray-dried sample had a much better vitamin C retention (Table 3), the collector material retained about 80%, whereas that removed from the chamber at the end of the run retained about 55% of the vitamin C. The latter sample was exposed to temperatures between those of the inlet and outlet of the drier for an average time of 12 min which presumably accounts for the greater loss. The carotenoid contents of

samples dried by either foam-mat and spray drying were slightly reduced (Table 3); the spray-dried sample from the chamber exhibiting most damage.

The spray-dried and foam-mat dried products were free flowing, finely divided powders, which were readily rehydratable in cold water; nectars (equivalent to 1 + 1 + 0·3 parts (w/w) of puree + water + sugar) were prepared for sensory evaluation. The spray-dried product was a bright and attractive colour in contrast to the darker foam-mat dried product. The latter was rated poorly in simple taste panel assessments (seven judges familiar with mango taste) possessing a distinct off-flavour. The spray-dried sample was slightly lacking in flavour compared with control nectar, but was preferred to the foam-mat dried material. This assessment reflects the heat damage indicated by the chemical analyses; consequently the poor quality of the product does not recommend this application of foam-mat drying. Foaming and drying in an inert atmosphere might improve the quality of the product (Ponting *et al.*, 1973) but this is not usually economically feasible.

Acknowledgments

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Studies of mango processing

II. Deep freezing of mango slices

R. D. COOKE, C. J. ALLISON, J. BALDRY, J. C. CAYGILL, C. E. M. FERBER, L. KANAGASABAPATHY AND M. J. VEALE

Summary

Alphonso mango slices in syrup were frozen under thirteen different conditions, stored for three months at −28°C and the quality of the products assessed chemically, microbiologically and organoletically. The quality was insensitive to the freezing rate (cryogenic freezing, blast freezing or domestic deep freezing) and to the addition of 0·1% vitamin C to the cover syrup. Presoaking in $CaCl_2$ prior to pasteurization caused a marked firming of the slices. Soaking the slices in acidic syrup followed by mild pasteurization greatly decreased the microbial counts but had an adverse effect on flavour. Fruit frozen in 40° Brix syrup had a better flavour than that frozen in 20° Brix syrup. Acceptable slices with good nutrient retention were produced by freezing in 40° Brix syrup using a domestic deep freezer.

Introduction

The mango (*Mangifera indica* L.) is grown in large quantities throughout the tropics, and Alphonso is a popular dessert cultivar. The perishable nature of the fruit and its short harvest season severely limit its utilization (Krishnamurthy & Subramanyam, 1973). The deterioration of fresh fruits can be prevented by freezing, while the deleterious reactions which accompany other preservation methods (e.g. canning, dehydration) usually occur at negligible rates in the frozen material (Finkle, 1971). An alternative to canning is often sought in developing countries because of high local tin plate costs (Mehta, 1975).

Excessive softness is a common fault of deep frozen fruit and vegetables; softening has been attributed to cell rupture and separation caused by ice crystal formation (Weier & Stocking, 1949). Improved texture has been associated with faster freezing rates (Fennema & Powrie, 1964) which may be related to the smaller size of the ice crystals formed. Three freezing methods of different rapidities (cryogenic, blast and domestic deep freezing) were employed in the present work. Textural improvements

Authors' address: Ministry of Overseas Development, Tropical Products Institute, 56–62 Grays Inn Road, London WC1.

related to calcium-pectate formation in the cell wall (Guadagni, 1949) were also investigated by presoaking fruit in $CaCl_2$ prior to freezing.

Fruit slices are usually frozen in cover syrup to exclude air, enhance flavour and inhibit enzymic browning (Ponting, Feinberg & Boyle, 1968). Textural improvements, perhaps due to the cryoprotective effect of the syrup, have also been claimed (Joslyn & Hohl, 1948). Addition of vitamin C has been reported to improve the colour of lightly coloured frozen fruits such as apples, peaches, apricots and pears (Guadagni, 1969), and vitamin C addition to different cover syrups was investigated in these trials. Allah & Zaki (1974) reported that the vitamin C retention of deep frozen mangoes was improved by a brief pasteurization treatment; a similar treatment was also investigated in this work. The quality of the products was assessed by chemical and microbiological analysis and by sensory evaluation after a period of storage under deep-frozen conditions.

Materials and methods

Preparation of mango slices and description of pretreatments

Green, mature Indian mangoes (50 kg of Alphonso cultivar) were ripened at 30°C. The firm, ripe fruits were rinsed in tap water, drained, and peeled. The cheeks of each fruit were cut off and sliced (approximate slice dimensions: 90 × 20 × 15 mm). The residual flesh was rejected because of the difficulty of obtaining uniform slices. Slices were produced in 3 kg lots, mixed, weighed and transferred to the appropriate syrups within 1 hr of peeling.

The mango slices were frozen under thirteen different conditions as shown in Fig. 1. The number of variables studied necessitated this fractional factorial design, the data from which was studied using analysis of variance techniques. Three pretreatment alternatives were used; P_1: no pretreatment; P_2: soaking the slices in 20° Brix sucrose syrup containing 2% (w/v) citric acid (syrup pH 1·98) for 15 hr at 4°C. The slices were enclosed in perforated stainless steel baskets to ensure total immersion in the syrup (the syrup : slice ratio was 1 : 1 w/w). The slices were then pasteurized by immersion in 20° Brix syrup containing 2% citric acid for 1 min at 70°C, as described by Dougherty & Koburger (1972). The slices were drained for 5 min at room temperature (18°C) and weighed into the trays containing the appropriate cover syrups ready for freezing. P_3: this varied from P_2 in that the presoak syrup contained 2% (w/v) $CaCl_2$.

The cover syrups used were 20° or 40° Brix sucrose syrups both containing 0·1% (w/v) citric acid (S_1, S_2 respectively) and with or without 0·1% (w/v) vitamin C (A_1, A_2 respectively). The vitamin C (L-ascorbic acid) was purchased from Koch-Light Ltd, Colnbrook. The pHs of these syrups were 2·75, 2·72, 2·64 and 2·61 for S_1A_1, S_1A_2, S_2A_1 and S_2A_2, respectively.

Freezing methods

The slices were frozen in 178 × 83 × 44 mm aluminium trays (Alcan Foil Ltd,

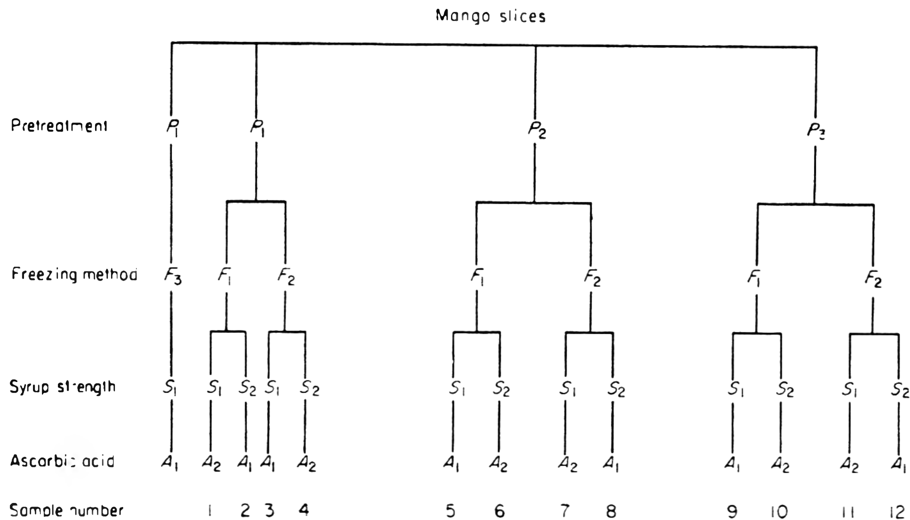

FIG. 1. Experimental design. P_1: no pretreatment; P_2: soaked in 20° Brix syrup containing 2% citric acid, followed by pasteurization; P_3: soaked in 20° Brix syrup containing 2% citric acid and 2% $CaCl_2$, followed by pasteurization. F_1: domestic deep freezer; F_2: blast freezer; F_3: liquid nitrogen freezer. S_1: 20° Brix cover syrup; S_2: 40° Brix cover syrup. A_1: no ascorbic acid added to the cover syrups; A_2: 0·1% ascorbic acid added to the cover syrups.

Chesham, Bucks) containing 310 g ± 10 g fruit (about fourteen slices), and 250 g of the cover syrup. Three freezing methods were employed; F_1: domestic deep-freezing, F_2: air-blast freezing, F_3: cryogenic freezing. The domestic deep-freezer and the blast freezer were operated at −28°C, and the air speed in the blast freezer was 5·7 m/sec. The trays were frozen with their lids in place and the centres of the fruit slices took 5 hr and 2·2 hr to attain −10°C for F_1 and F_2, respectively. These rates were measured with a miniature temperature recorder and HS (125 × 4·8 mm) thermistor probes (Grant Instruments Ltd, Cambridge). The trays were left in the freezers until the slices had reached −28°C, i.e. about 4 hr in the blast-freezer and 15 hr in the domestic deep-freezer. The trays for F_3 were frozen without their lids in place and spent 33 min in a liquid nitrogen freezing tunnel of belt size 0·61 × 3·66 m (at British Oxygen Ltd, South Wimbledon, London SW19). The lids were then fitted and the trays transported (1 hr) to this institute in contact with solid CO_2 in insulated boxes. The trays frozen by these various methods were then stored in a cold room at −28°C ± 2°C for three months prior to the start of the analyses. Guadagni (1969) reported that no measurable changes in quality occurred on storage of many frozen fruits for five years at this storage temperature. This indicates that the results of this study should be representative of much longer storage periods. Two trays of each sample were prepared, one for chemical and one for taste panel analysis.

Product analyses—chemical and microbiological analyses

Samples were thawed under controlled conditions (placed in a refrigerator at 4°C for 17 hr prior to immediate analyses) and two slices and a syrup sample from each tray were removed for microbiological analyses, i.e. total viable counts using plate count agar at 37°C and 27°C for two and three days respectively. The possible presence of coliforms, *Staphylococcus aureus*, salmonellae and pathogenic anaerobes was also investigated by standard techniques. Six slices were removed for textural analysis using an Instron tensile testing machine TM–SM–L (Instron Ltd, High Wycombe, Bucks). An 8-mm probe was used to traverse each slice (15 mm); the first yield point and the maximum force encountered were measured at least twice per slice. These two measurements gave similar results and only the first yield points are recorded here.

The remaining six or seven slices in each tray were drained for 2 min on a nylon mesh support, dipped in 800 ml distilled water for 15 sec at room temperature and re-drained for 2 min. The slices were then homogenized for 3 min (MSE laboratory homogenizer) and two samples of each puree were analysed in duplicate for moisture content, ascorbic acid, dehydroascorbic acid, total and reducing sugars and total carotenoids as described earlier (Cooke *et al.*, 1976). Total titratable acidity was determined in a centrifuged, diluted puree sample after boiling for 1 min and cooling under vacuum. This was titrated to pH 8·0 using a TTT2 module linked to an ABU 11 autoburette (Radiometer, Copenhagen, Denmark), and the titres expressed as mg citric acid/100 g mango slice.

Sensory evaluation

The taste panel consisted of seven non-European judges who were familiar with mangoes and seven European judges who were initially unfamiliar with the fruit. They were asked to score the sweetness, firmness and flavour of the two samples, to comment on the appearance, fibre content and presence of off-flavour and to rank the samples.

Twelve assessments were organized at each of which the cryogenically frozen sample ($P_1F_3S_1A_1$) was presented to the judges with one of the other twelve samples. The scores of the cryogenically frozen sample were used as a control to compensate for changes in response of the judges. The samples were thawed as described above and warmed to room temperature immediately before presentation to the panel by two 30 sec exposures to microwave irradiation in a Merrychef 85 oven (Merrychef Ltd, Reading).

Results

The total bacterial counts of the frozen pasteurized samples was nil or trace but those of the unpasteurized samples (P_1) were much higher ($5 \times 10^3 - 2 \times 10^4$ at 37°C). However, no pathogens were isolated and all the samples were considered safe for human consumption.

The chemical analyses of fresh mangoes are shown in Table 1. The large standard

TABLE 1. The chemical composition of fresh, ripe alphonso mangoes

	Number of mangoes analysed	Mean	Standard deviation
Ascorbic acid (mg/100 g)	12	63·8	4·8
Dehydroascorbic acid (mg/100 g)	12	9·8	3·1
Total carotenoids (mg/100 g)	8	11·4	1·2
Total titratable acidity (mg/100 g)	8	525	197
Total sugars (g/100 g)	8	14·8	1·4
Reducing sugars (g/100 g)	8	3·0	0·6
First yield point (g)	6	129	56

deviation of the total titratable acidity is a reflection of the marked change in fruit acidity during ripening (Krishnamurthy & Subramanyan, 1973). The texture of the fruit slices, as measured by first yield point is also very variable. Tables 2 and 3 show the compositions of the various frozen samples, and indicate that little loss of nutrients occurred during the freezing treatments. The vitamin C contents of those samples not fortified with ascorbic acid decreased by about 15% and the dehydroascorbic contents by 30–50%. Reducing sugars also decreased by 30–50%. Carotenoids decreased by 15–30%. Total titratable acidity was increased by the acidic pretreatments P_2 and P_3;

TABLE 2. The vitamin retention of the deep-frozen slices; the fresh mango data is derived from Table 1 and the control data is the average of four sets of determinations (Materials and methods)

Sample number	Moisture content % wet basis	Ascorbic acid mg/100 g	Dehydroascorbate mg/100 g	Total carotenoids mg/100 g
Fresh mango	78·2	63·78	9·75	11·36
Control	78·8	54·26	6·15	9·80
1	79·2	82·62	8·16	9·54
2	74·9	55·93	6·58	10·39
3	78·1	52·40	4·92	9·21
4	70·8	81·56	10·36	9·72
5	77·9	54·23	4·66	9·52
6	71·4	77·92	5·07	10·23
7	78·4	73·81	5·73	9·15
8	72·9	56·84	4·52	9·74
9	76·1	55·98	4·11	7·74
10	73·2	87·41	6·70	9·22
11	77·1	77·62	4·93	9·28
12	72·1	53·37	4·31	8·92

TABLE 3. The acidity and sugars contents and first yield points of the deep-frozen slices

Sample number	Total sugars (g/100 g)	Reducing sugars (g/100 g)	Acidity (mg/100 g)	First yield point (g)
Fresh mango	14·83	3·03	524·6	129
Control	15·25	1·66	426·3	89
1	12·57	1·61	599·3	102
2	19·78	1·73	444·9	82
3	15·62	1·76	440·2	102
4	19·73	1·79	446·3	128
5	15·35	1·59	642·0	114
6	24·70	1·44	510·9	78
7	14·72	1·67	598·5	91
8	20·58	1·50	506·9	82
9	18·51	2·08	596·5	256
10	19·46	1·71	632·8	208
11	19·18	2·06	634·9	196
12	21·97	2·05	585·2	214

and total sugars were, naturally, increased by the 40° Brix syrup. The yield points of the slices were lower than those of fresh material in all treatments except $CaCl_2$ (P_3), in which an increase of at least 50% occurred.

These data were examined by analysis of variance to determine which of the treatment parameters caused significant differences in product composition (Table 4). Texture is

TABLE 4. The significance of the effect of the freezing parameters (P, F, S, A) on the chemical composition of the product

Freezing parameter	Moisture content	Ascorbic acid	Dehydro-ascorbate	Carotenoids	Reducing sugar	Total sugar	Acidity	Yieldpoint
Pretreatment (P)	—	—	**	—	**	*	**	**
Freezing method (F)	—	—	—	—	—	—	—	—
Syrup strength (S)	**	—	—	**	*	**	**	—
Ascorbic acid addition (A)	—	**	**	—	—	—	—	—

*, ** Differences significant at $P=0\cdot05$, $0\cdot01$ respectively.

an important attribute of a frozen fruit product (Ponting *et al.*, 1968), and all treatments except $CaCl_2$, caused softening which latter treatment firmed the slices rather too much. Further experiments are required to devise conditions producing a slice texture similar to the fresh product. The higher syrup strength caused osmotic dehydration, the

moisture content means for S_1 and S_2 being 77·8% and 72·6%. This was accompanied by a corresponding increase in total sugars (16·0 and 21·0 g/100 g respectively) and a decrease in reducing sugars (1·8 and 1·7 g/100 g) and acidity (585 and 521 mg/100 g). The small increase in total carotenoids at the higher syrup concentration (9·0 and 9·7 mg/100 g) may have a similar cause.

The pretreatments increased the slice acidity (means of P_1, P_2, P_3 482, 565, 612 mg/%) and slightly increased the total and less certainly the reducing sugar contents (16·9, 18·8, 19·8 and 1·7, 1·6, 2·0 respectively). The acidity of the frozen slices was less variable than that of the fresh slices presumably because of equilibration with the syrups (standard deviation reduced from 197 to about 50). The dehydroascorbate contents were reduced by the pretreatments (7·5, 5·0, 5·0 respectively), perhaps the result of inactivation of ascorbate oxidase. The freezing rates (F_1, F_2, F_3) caused no significant effects. The ascorbic acid fortification affected only the ascorbic acid and dehydroascorbate contents of the slices (means of 54·8, 80·2 and 4·9 and 6·8 respectively for A_1, A_2).

TABLE 5. The significance of the effect of the freezing parameters on the sensory evaluation of the products

	Sweetness	Firmness	Flavour	Appearance	Fibre	Off-flavour	Preference relative to control
Pretreatment	*	**	**	—	—	*	*
Freezing method	—	—	—	—	—	—	—
Syrup strength	**	—	**	—	—	—	**
Ascorbic acid	—	—	—	—	—	—	—

Table 5 shows the significant results of the analysis of variance of the taste panel scores. The firming effect of the $CaCl_2$ treatment is evident (scored as slightly too firm and perceived as more fibrous); but the flavours of both P_2 and P_3 were less acceptable than P_1 (lower flavour scores, less sweet, increased incidence of off-flavours and decreased preference relative to control). The judges familiar with mangoes were significantly more sensitive to off-flavours and sweetness than the naive judges. The samples frozen in 40° Brix syrup were judged sweeter, the flavour considered more acceptable and were preferred relative to the control. The addition of ascorbic acid and the different freezing procedures had no organoleptic effects. The cryogenically frozen control had a slightly different appearance (not significant) owing to some cracking of the slices, and this hindered measurement of the slice yield point.

Thawing conditions (17 hr at 4°C) were carefully controlled since preliminary experiments had indicated that vitamin C and reducing sugar contents are sensitive to thawing rate. Thawing the cryogenically frozen control by maintaining it at 20°C for

17 hr prior to analyses decreased the vitamin C content by 25% and the reducing sugar by 30% relative to the standard thawing condition. Rapid thawing of the control (placing it in a water bath at 40°C for 1 hr) yielded a similar sugar content but a 15% increase in vitamin C relative to standard thawing.

Discussion

The beneficial effect of rapid freezing on frozen fruit texture is controversial (Ponting *et al.*, 1968). The quality of frozen soft fruits such as strawberries and sliced tomatoes is improved by liquid nitrogen freezing (Wolford, Ingelsbie & Boyle, 1967), whereas fruits such as peaches appear less sensitive (Luyet, 1968). The texture and appearance of Kensington and Common mangoes were improved relative to blast-freezing by freezing within 10 min by immersion in 60° Brix syrup precooled to −21°C (Leverington, 1957). The present study does not indicate that the three freezing methods used affect the quality differently. Faster freezing rates would be obtained by cryogenic freezing without cover syrup, and this might improve the texture retention. However, flavour and colour problems may be associated with fruit frozen in the absence of cover syrup (discussed in Introduction).

The firmness of the product was increased by the $CaCl_2$ pretreatment. Both the yield point data and the sensory evaluation indicate that the slices were firmed too much and further experiments are required to optimize the texture of the slices. Netto, Bleinroth & Lazzarini (1971) presented taste panel data suggesting that 0·07% $CaCl_2$ included in the cover syrup slightly improved the colour and texture of Haden mangoes stored at −20°C for four months. Ranganna, Sastra & Siddappa (1961) reported that $CaCl_2$ (0·025–0·05%) slightly increased the firmness of canned Bangalora mangoes; higher concentration made the slices rather tough and adversely affected the flavour. This treatment did not improve the texture of canned Alphonso or Raspuri mangoes, but adversely affected the flavour. Both pretreatments employed (P_2 and P_3) involved soaking in citric acid followed by a mild pasteurization. The taste-panel and chemical analysis suggest that their similar low flavour scores may be related to the heat treatment and enhanced acidity; no flavour changes can be attributed to the $CaCl_2$ alone based on the present work. The pretreatments caused a marked decrease in the microbial counts of the products. A similar treatment (Dougherty & Koburger, 1972) produced an acceptable Keitt mango product after three months storage at 2°C. The present data indicate that this treatment adversely affects the flavour and is unnecessary in terms of the microbial load of the frozen products. However, this pasteurization pretreatment may be necessary for commercial deep-freezing operations under tropical conditions. Allah & Zaki (1974) reported that the vitamin C retention of frozen Balady mango juice was improved by pasteurization (85°C for 2 min). No significant improvement was observed with the milder heat treatment of the slices used here.

The vitamin C retention of many fruits is a good quality index because of its depen-

dence on the oxidative enzyme activity of the fruit and related effects on colour and flavour (Guadagni, 1969). Aliaga & Luh (1971) and Netto *et al.* (1971) have both reported browning problems associated with the freezing of Haden mangoes and the beneficial effects of added vitamin C. Similarly, Saleh, El-Said Amer & Shehata (1960) reported improved colour retention on freezing three Egyptian mango cultivars in the presence of vitamin C. The bitterness of deep-frozen Langra mango puree can be diminished by de-aeration (Mukerjee, 1969). The present studies indicate that the only significant effect of vitamin C incorporation was enhanced vitamin C and dehydroascorbate concentrations in the slices. The vitamin C retentions of 85–90% suggest that there is little oxidative activity occurring and indeed Alphonso mangoes do not brown on exposure to air for several hours. Orr & Miller (1955) reported that no browning occurred with the twenty-one Hawaiian cultivars tested, though improved colour retentions of frozen slices were reported on twelve months storage at −18°C. Further experiments using Alphonso mangoes frozen for longer periods are required to check whether such slow changes are occurring. Earlier work by Guadagni (1969) indicates that this is unlikely at the storage temperature used (−23°C). Adsule & Roy (1974) reported poor vitamin C retentions on deep freezing several Indian mango cultivars in a cover syrup containing 0·5% vitamin C. This may be related to the storage temperature (−12·2°C) used over the nine month interval.

The 40° Brix syrup treatment produced a sweeter product preferable to that produced with the 20° Brix syrup. This preference may arise from the acidity caused by some of the treatments. The osmotic dehydration effect of the stronger syrup caused minor changes in chemical composition but did not alter the texture. Syrup concentrations higher than 30° Brix have been reported to cause toughening, shrivelling and colour loss in some deep frozen fruits (Ponting *et al.*, 1968).

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A comparison of methods for measuring the volatile components of apple fruits

MICHAEL KNEE AND STEPHEN G. S. HATFIELD

Summary

Volatile compounds produced by apple fruits (*Malus domestica*, Borkh.) were separated and estimated by gas chromatography. Procedures were devised for estimation of compounds in air surrounding whole apples and discs of apple tissue, and in apple juice. Concentrations of volatiles in air passing over apples were influenced by tissue permeability, concentrations in peel or cortex, and the extent of enzymic hydrolysis of esters passing through the peel. The complexity of these effects prevents assessment of the importance of compounds in apple flavour based on their concentrations around whole fruit. The ratios of concentrations established in air around cortical discs to concentrations in juice were similar to air/water partition coefficients observed for standard compounds. Measurement of concentrations above cortical discs is simple and sensitive and can be related to flavour, through the olfactory threshold concentrations of the compounds detected.

Introduction

Volatile compounds, some of which are responsible for the flavour of apples, have usually been analysed by collecting them from air passing over whole fruit ('headspace' technique), or by extraction of disintegrated tissue (Nursten, 1970). Neither method measures the concentrations of compounds experienced by the consumer, and these are probably similar to those established in the vapour phase when pieces of apple tissue are enclosed in suitable vessels. The present work includes analyses of volatiles collected in this way, and attempts to compare these with whole apple headspace and tissue concentrations. The results are compared with measured air/water partition coefficients for standard compounds, some of which were determined previously (Buttery, Ling & Guadagni, 1969). The measured headspace concentrations of individual compounds are also compared with estimates of threshold concentrations for olfactory detection (Flath *et al.*, 1967).

Authors' address: East Malling Research Station, East Malling, Maidstone, Kent ME19 6BJ.

Materials and methods

Apples

For most of the experiments apple fruits (*Malus domestica*, Borkh, cv. Cox's Orange Pippin) were harvested on 2 October 1975 from fourteen-year-old trees on MM 104 rootstocks and stored until required at 3·3°C in atmospheres of air, 2% O_2: 98% N_2 or 1% O_2: 99% N_2.

Gas liquid chromatography

Routine analyses were done using a 1·7 × 4 mm (i.d.) column packed with 15% SE 30 on Chromosorb W (60–80 mesh) at 100°C, with water-saturated N_2 as carrier gas at 40 ml min^{-1}, and a flame ionization detector. Analytical standards were prepared by injecting known volumes of appropriate compounds into a glass flask closed with a glass stopcock equipped with a septum.

More detailed separations of headspace volatiles were carried out on a 150 × 0·5 mm i.d. open tubular column coated with Carbowax 1500, temperature programmed from 40°C to 130°C at 1·25°C min^{-1} and held at 130°C for 60 min, with N_2 as carrier gas at 2·5 ml min^{-1}, and a flame ionization detector. Volatiles emanating from whole apples were trapped on short columns of Chromosorb 101, from which they were displaced in a stream of nitrogen at 150° into a 1 mm (i.d.) 'U' tube cooled in liquid N The 'U' tube was then heated to 150° to displace the volatiles on to the capillary column.

Air/water partition coefficients

Air/water partition coefficients were measured by injecting into the chromatograph samples of the vapour above dilute aqueous solutions of volatile compounds, and by indirect injection of the solution itself. The results were broadly consistent with earlier findings (Buttery *et al.*, 1969), and showed the expected linear relationship between log partition coefficient and carbon number.

Volatiles from whole apples

Single apples were enclosed in glass vessels, supplied with a constant flow of air through a length of standard bore capillary, to which was applied air at a constant pressure. Samples (1 ml) of air were taken at the exit of the vessels in a glass syringe and quickly injected into the chromatograph.

Volatiles from apple slices

Plugs 1·5 cm diameter were cut transversely from the peel to the core. A razor blade was used to cut peel discs from plugs with the minimum of cortical tissue adhering; the average weight of such discs was 0·15 g. Cortical discs were cut 0·2 cm thick and 1 cm in from the peel.

Preliminary work showed that esters and alcohols were rapidly absorbed by vaccine

caps which are a convenient closure for vessels containing discs. Single discs were, therefore, enclosed in 10 ml glass syringes and incubated at 20° for 1 hr. Samples were taken with a 1 ml glass syringe through a minimal length of P.T.F.E. tubing joining the needles of the two syringes.

Volatiles in apple juice

A rapid method was required for measurement of concentrations of volatile compounds in solution in apple tissue. Direct injection of apple juice proved impossible because thermal decomposition of other tissue components gave rise to peaks which masked those due to volatiles. Juice, obtained by squeezing tangential slices cut from whole apples into a small vial cooled in ice, was injected indirectly; 1 μl juice was injected into a 21 gauge hypodermic needle which was then attached to a glass syringe containing 1 ml of air, and this was used to displace volatile compounds on to the GLC column. This is referred to below as 'indirect injection'.

Results

Volatiles from whole apples

When 'headspace' samples from vessels containing single ripe Cox's Orange Pippin apples were injected on to the packed column, the major peaks had retention times similar to those of butanol, butyl acetate, butyl butyrate and hexyl acetate. Smaller peaks corresponding to 2-methyl propyl acetate, 2 or 3-methyl butyl acetate, butyl propionate, ethyl butyrate, hexyl butyrate and hexanol were also found. When concentrated headspace volatiles were applied to the capillary column the presence of the same compounds was confirmed and no other major components were separated. Additional minor components had retention times similar to ethyl pentanoate, ethyl hexanoate, ethyl octanoate, propyl acetate, propyl butyrate, and pentyl acetate; each of these amounted to less than 50% of the quantity of 2 or 3-methyl butyl acetate detected.

Volatiles from apple discs

When a disc of apple fruit tissue was enclosed in a syringe the concentrations of esters and alcohols in the air rose rapidly over the first few minutes and then remained nearly constant (Table 1). Table 2 shows the uniformity of concentrations of individual compounds present in syringes containing replicate discs from a single apple.

Volatiles in apple juice

Chromatographic responses per unit mass of standard compounds were compared for direct liquid, indirect liquid and vapour injections. The results showed that, in the indirect method, only a proportion of a volatile compound present in solution was eluted on to the GLC column by the air used to flush the needle containing the sample.

TABLE 1. Time course of change of concentration (ng ml^{-1}) of volatile compounds in syringe containing a single cortical disc from a Cox's Orange Pippin apple

Time after enclosure (min)	1	7	13	19	25	31	260
Butanol	4·0	6·4	15·4	10·8	8·6	8·0	10·0
Butyl acetate	80	177	188	188	194	194	196
2 or 3-methyl butyl acetate	1·5	5	4·2	3·8	3·4	3·4	4·2
Hexyl acetate	19	52	60	70	68	71	83
Butyl butyrate	0·6	3·0	2·6	3·0	3·4	3·4	0·3

The apple had been stored in air at 3·5° for eight weeks and 0·5 ml samples were withdrawn at each time for injection into the chromatograph.

TABLE 2. Reproducibility of measurement of concentration of volatile compounds in syringes containing cortical discs from a single Cox's Orange Pippin apple

Compound	Mean concentration (ng ml^{-1})	Standard error
Butanol	2·73	0·09
2-methyl propyl acetate	6·36	0·23
Butyl acetate	168	5·8
2 or 3-methyl butyl acetate	3·95	0·21
Hexyl acetate	96	5·5
Butyl propionate	2·09	0·07

Eight discs were cut, from an apple obtained from a commercial store in January 1976, and incubated individually in syringes.

This proportion varied from around 35% for alcohols to 80% for esters, and decreased as carbon number increased in a homologous series. Results obtained by direct liquid injection and vapour injection agreed, except for higher alcohols, where vapour injection gave lower results (e.g. 30% less for hexanol). This could be due to absorption by glass, a known disadvantage of syringe injection (Buttery *et al.*, 1969). The results obtained by any one method were consistent (standard error of about 5% of the mean) for a given compound; vapour standards were injected at the same time as samples of apple volatiles, and factors relating indirect liquid injection to vapour injection for each compound were used in calculating their concentrations in apple juice.

The consistency of the indirect injection technique applied to apple juice is shown

in Table 3. Juice samples were routinely taken from tangential slices because they were more easily obtained in this way than by pressing cortical discs.

TABLE 3. Reproducibility of estimation of concentrations of volatile compounds in juice from Cox's Orange Pippin apples

Compound	Concentration $\mu g\ ml^{-1}$ in juice from	
	Tangential peel slices	Cortex slices
Butanol	47·4 (2·2)	32·1 (2·3)
Hexanol	8·2 (0·27)	4·4 (0·69)
Butyl acetate	14·0 (1·3)	16·4 (2·74)
2 or 3-methyl butyl acetate	0·2 (0·04)	0·3 (0·05)
Hexyl acetate	5·5 (0·27)	4·4 (0·69)
Ethyl butyrate	0·4 (0·05)	0·5 (0·09)
Butyl butyrate	2·3 (0·24)	4·3 (0·35)

The peel and cortex slices were cut from two different apples which had been stored for eleven weeks at 3·5°C in air.
Standard errors are shown in parentheses ($n=5$).

Relationships between methods of measuring apple volatiles

Several apples were enclosed in individual vessels, each with an air flow at 3·75 ml min^{-1}. The apples were selected from a range of storage conditions, so that they would have a wide range of volatile contents. Volatiles from whole apples, peel discs, cortex discs and juice were measured, and the relationships between these measurements for selected compounds are shown in Table 4, in terms of linear regressions.

The regressions of cortex headspace on juice contents for butanol, butyl acetate and hexyl acetate approximated to their observed air/water partition coefficients. Coefficients of whole apple headspace to juice contents approximated to the air/water partition for butanol but were considerably less for the esters. Consequently the coefficients for whole apple headspace to cortex and peel disc headspace approximated to unity for butanol and a fraction for the esters. The quantities of butyl butyrate detected in cortex headspace and juice injections were small and variable; substantial quantities were found in peel headspace and whole apple headspace, and the regression coefficient with its standard error shows that these were correlated. No similar relationship was found for other compounds, mainly because of the high standard errors of estimations of the small amounts present in whole apple headspace and juice samples.

Cortex headspace and olfactory thresholds

The concentrations of volatiles present in cortex disc headspace samples for apples

TABLE 4. Air/water partition coefficients and the relation between various methods of measurement of selected volatile compounds from apples

Compound	Mean concentration ($ng\ ml^{-1}$) in				Regression coefficients				Air/water partition coefficient
	Juice (j)	Whole apple headspace (w)	Peel disc headspace (p)	Cortical disc headspace (c)	b_{wj}	b_{cj}	b_{wp}	b_{wc}	
Butanol	20200	6·80	5·63	4·52	$2·46 \times 10^{-4}$ ($0·30 \times 10^{-4}$)	$2·25 \times 10^{-1}$ ($0·21 \times 10^{-4}$)	0·660 (0·170)	1·06 (0·120)	$2·49 \times 10^{-4}$ ($0·23 \times 10^{-4}$)
Butyl acetate	10500	30·5	59·5	94·2	$2·02 \times 10^{-3}$ ($0·60 \times 10^{-3}$)	$6·71 \times 10^{-3}$ ($2·2 \times 10^{-3}$)	0·165 (0·084)	0·213 (0·050)	$8·42 \times 10^{-3}$ ($0·06 \times 10^{-3}$)
Hexyl acetate	1870	15·3	37·8	56·7	$7·33 \times 10^{-3}$ ($1·64 \times 10^{-3}$)	$19·0 \times 10^{-3}$ ($5·2 \times 10^{-3}$)	0·463 (0·116)	0·140 (0·086)	$14·1 \times 10^{-3}$ ($0·48 \times 10^{-3}$)
Butyl butyrate	152	6·2	13·5	1·02	—	—	0·339 (0·060)	—	$16·8 \times 10^{-3}$ ($0·23 \times 10^{-3}$)

Measurements were carried out on fifteen apples, stored in various atmospheres for twelve weeks at 3·5°C and transferred to air at 15°C for fourteen days. Air/water partition coefficients are means of four determinations. Standard errors are shown in parentheses.

from different storage conditions (Table 5) can be compared with their published olfactory thresholds (Flath *et al.*, 1967). The concentrations of one compound, hexyl acetate, were consistently greater than its threshold (2 ng ml^{-1}). Butyl acetate varied above and below its threshold value (66 ng ml^{-1}), and 2 or 3-methyl butyl acetate was

TABLE 5. Concentrations (ng ml^{-1}) of volatile compounds detached in headspace of cortical discs of Cox's Orange Pippin apples which had been stored in various atmospheres

Compound	Storage atmosphere			
	1% O_2:99% N_2	2% O_2:98% N_2	2% O_2:98% N_2 + air	Air
Butanol	0·90 (0·24)	2·5 (0·63)	3·4 (0·45)	9·4 (0·80)
Hexanol	0·31 (0·06)	0·62 (0·49)	0·51 (0·11)	0·93 (0·15)
2-methyl propyl acetate	1·43 (0·27)	2·22 (0·33)	2·33 (0·61)	2·22 (0·36)
Butyl acetate	26 (7·2)	59 (16)	104 (9·3)	165 (16)
2 or 3-methyl butyl acetate	1·41 (0·23)	1·85 (0·33)	1·78 (0·29)	2·19 (0·14)
Hexyl acetate	28 (3·3)	61 (13)	39 (9·3)	71 (3·6)
Butyl propionate	1·08 (0·07)	1·73 (0·16)	2·19 (0·16)	2·8 (0·51)
Butyl butyrate	0·18 (0·18)	0·56 (0·25)	0·95 (0·18)	2·02 (0·48)
Number of observations	3	4	3	4

The apples had been stored for twelve weeks at 3·5°C in the atmosphere indicated, (six weeks in 2% O_2:98% N_2 followed by six weeks in air for 2% O_2:98% N_2+air) and transferred to air at 15°C for fourteen days.

Standard errors are shown in parentheses ($n=4$).

close to the threshold of 2-methyl butyl acetate (5 ng ml^{-1}), but the other compounds identified as peaks from the packed column were well below their thresholds. The additional esters detected with the capillary column would be unlikely to exceed their thresholds, assuming the relation between the whole apple and cortex headspace to be similar for these compounds to those for the major esters.

Discussion

Most of the compounds tentatively assigned to peaks eluting after injection of volatile compounds from Cox's Orange Pippin apples into the gas chromatograph were also found by Grevers & Doesburg (1962), and, with the remainder, have all been recorded from a number of other apple varieties (Nursten, 1970).

Analysis of whole apple headspace volatiles establishes the spectrum of compounds produced by an apple variety, but a number of factors prevent evaluation of importance of compounds in fruit flavour from such data.

An apple in a sealed container should establish partial pressures of volatile com-

pounds in equilibrium with the concentrations dissolved in its cell contents, in accordance with Henry's law. If air were passed over the apple the external concentrations should fall to reflect the rates of production by the tissue, and the internal concentrations should reach an equilibrium with the external, determined by rates of synthesis and diffusion. Diffusion should be in accordance with Fick's law, that is proportional to the difference between internal and external concentrations. Other gaseous apple metabolites, carbon dioxide (Fidler & North, 1971) and ethylene (Burg & Burg, 1965), behave in a way which is consistent with Fick's law. If the concentration of a volatile compound established above a cortical disc is taken as a measure of the concentration in the intercellular spaces of an intact fruit (C_{in}) then a Fick's law constant (K) can be calculated using this figure, the concentration above the whole apple (C_{out}), and the flow rate of the air stream (F).

$$\text{Rate of diffusion} = F.C_{out} = \mathrm{K}(C_{in} - C_{out})$$

The primary data summarized in Table 4 were analysed to obtain regressions of $F.C_{out}$ on $(C_{in} - C_{out})$ of 0·84 (S.E. = 0·30) for butyl acetate and 0·82 (S.E. = 0·37) for hexyl acetate. These are comparable to constants for CO_2 (0·90) and ethylene (0·61) obtained by measuring internal and external concentrations of these gases for similar apples, and suggest that esters follow the same diffusion paths as CO_2 and ethylene.

The air/water partition coefficient for CO_2 is close to unity and for ethylene approximately four. Diffusion of these gases from the intercellular spaces out of the apple can quickly lead to equilibrium between solution concentration and the rates of synthesis. However, esters have air/water partition coefficients around 0·01 so that equilibration between rate of synthesis, solution concentration and diffusion is slow. Low flow rates of air passing over apples would cause an accumulation of esters over several days, while fast flow rates would cause a similarly slow decline. It is erroneous, therefore, to calculate rates of production from concentrations of esters found in air streams passing over apples.

These observations apply to esters present throughout the apple. Butyrates, particularly butyl butyrate, appear to be mainly concentrated in the peel of Cox's Orange Pippin apples and contribute to the whole apple headspace out of proportion to their average concentration in the apple.

Alcohols, particularly butanol, were found to occur at higher concentrations outside than inside the apple. This cannot be explained in physical terms, but other experiments have shown that esterase activity is present, particularly in the fruit peel (M. Knee, S. G. S. Hatfield & P. Henderson, unpublished). Alcohols are probably produced from esters passing from cortex through the peel and out of the apple.

The complexity of factors influencing the composition and quantities of volatile compounds released by whole apples precludes general conclusions about their relation to internal concentrations, and the evaluation of their role in apple flavour. The numerical relationships established between whole apple headspace and tissue con-

centrations in the above work would apply to that particular fruit under the experimental conditions, and not to other fruit or other conditions.

The concentrations of esters detected in juice from air stored Cox's Orange Pippin apples were comparable to those reported for similar material by Drawert *et al.* (1969), who extracted esters from apple tissue by partitioning into a non-polar solvent. The alcohol concentrations in the present work were much higher, possibly due to low recoveries in solvent partitioning. However, the indirect injection technique used in the present work suffers from lack of sensitivity and consequently it is reliable only for the major volatile components.

Measurement of volatiles in the headspace of fruit tissue discs is simple and sensitive, though it has not been widely used. It has the advantage that flavour assessment and analysis can be carried out with single apples. A possible disadvantage is that compounds might be metabolized during equilibration, although the rapid establishment and stability of concentrations over several hours suggest that this is not so. The similarity of the regression coefficients for cortex disc headspace on juice concentrations, for an alcohol and two esters, to their air/water partition coefficients confirmed this impression, and showed that the analyses of cortex headspace can be interpreted in terms of a simple physical system. These analyses are a guide to concentrations experienced by a consumer. Taking account of olfactory threshold concentrations (Flath *et al.*, 1967) hexyl acetate, butyl acetate and 2 or 3-methyl butyl acetate are expected to contribute to flavour. However, a number of compounds occurring at concentrations below their individual thresholds may complement each other in mixtures so as to give a perceptible flavour (Guadagni *et al.*, 1963). Compounds present very low concentrations, but with olfactory thresholds of the order of 0·0001 ng ml^{-1}, for example ethyl 2-methyl butyrate (Flath *et al.*, 1967), could have escaped detection. Furthermore, aldehydes formed on crushing apple tissue, and found in apple juice (Flath *et al.*, 1967), were not detected by the techniques employed in this work, although they may be important in flavour.

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Meat quality and protein quality

A. E. BENDER AND M. ZIA

Summary

Samples of meat of good and poor eating quality were assayed biologically for protein quality and analysed for connective tissue content. The low quality meat had NPU 69 and 23·6% connective tissue; the high quality meat had NPU 82 and 2·5% collagen.

Introduction

The price and eating quality of meat are usually determined by the content of connective tissue (Laakkonen, 1973) yet it is generally stated that all cuts of meat have the same nutritive value irrespective of eating quality. For example, 'the cut of meat has no influence on the nutritive value. In other words the nutrients found in beef chuck are as adequate as those found in porterhouse steak' (Fleck & Munves, 1962). 'Regardless of the species the amino acid composition of the portions of flesh foods are relatively constant' (Robinson, 1967). 'The costliness of meat can be considerably diminished by selecting cheaper cuts, which are equal in nutritive value to the dearer kinds though inferior in tenderness and flavour' (Hutchinson, 1969). Price & Schweigert (1971) concluded that the essential and non-essential amino acid composition of meat protein is quite constant regardless of the species of the cut or the organ from which the meat is obtained but excepted meats containing 'large' amounts of connective tissue because this is relatively poor in tryptophan and tyrosine.

Many authors refer back these conclusions to the work of Mitchell & Carman (1926) who, on the contrary, suggested that different cuts of meat may vary in their biological value (BV). They stated that 'the ordinary assumption that the protein in cheap cuts of meat is equal in nutritive value to the protein in expensive cuts of meat can only have reference to digestibility. Any assumption that they are equal with respect to the biological value of their nitrogen is quite unfounded.' These authors went on to say 'it is a matter of common observation that different cuts of meat differ in texture and particularly in tenderness. These differences presumably depend upon differences in the proportion of connective tissue to muscle tissue. In all probability the nitrogen of connective tissue would possess a lower biological value than the nitrogen of muscle

Authors' address: Department of Nutrition, Queen Elizabeth College, Campden Hill, London W8 7AH.

tissue.' Mitchell & Carman did not make any measurements of biological value but based their conclusions on the chemical differences in the nitrogen compounds found in the different cuts. Later, Mitchell, Beadles & Kruger (1927) reported that a cut of veal, 'evidently very fibrous' had BV of only 62 compared with beef (unspecified) at 69 and pork at 74. They also refer to an unpublished experiment in which the BV of a particularly fibrous piece of meat was as low as 56. The same authors found that the BV of pork cracklings (consisting essentially of connective tissue) was only 25 compared with that of 79 for pork tenderloin. The former was later erroneously referred to as gelatin which has, in fact, BV zero (Bender, Miller & Tunnah, 1953).

Mitchell *et al.* (1927) suggested that the increased amount of connective tissue will decrease the value of the nitrogen for maintenance and growth but admitted that they had no quantitative data of the amount of connective tissue present.

No biological measurements on different cuts of meat appear to have been made subsequent to those of Mitchell and co-workers. Collagen is very low in sulphur-amino acid content; methionine 0·80 g/16 nitrogen (compared with 2·56 g in muscle) and cystine 0·08 g (compared with 1·28 g in muscle) (Orr & Watts, 1957). Dvorak & Vognarova (1969) calculated chemical scores from amino acid analyses and obtained low values for cuts rich in connective tissue. They found that beef shank contained only 1·45 g methionine per 16 g nitrogen compared with beef fillet at 2·73 g and showed a relationship between chemical score calculated from S-amino acids and hydroxyproline content but did not verify their findings by biological assay.

In the present work the net protein utilization and hydroxyproline content (as a measure of connective tissue) were measured on two cuts of beef, one at each end of the extremes of quality and price.

Methods

Samples of meat

Two samples of meat were purchased: fillet beef steak at £1.40 per lb and shin at £0.48 per lb. Visible fat was trimmed off and the meat was minced and dried and partially defatted by stirring with acetone—2·5 l per 500 g meat—and drying in air. Samples were not cooked.

Protein quality

Net protein utilization (NPU) was determined by the carcass method of Bender & Miller (1953). Hooded rats purchased from Animal Supplies Ltd at twenty-one days of age were kept on stock diet for one week, before being fed the experimental diets for ten days. Each kilogram of diet contained 400 g meat (providing 10% protein in the diet) 490 g starch, 50 g arachis oil, 20 g vitamin mixture in starch base and 40 g mineral salt mixture.

Assays were also carried out on the meat protein diets supplemented with 1·4 g DL-methionine per kilogram diet.

Chemical determination

Nitrogen was determined by the Kjeldahl method.

Hydroxyproline was determined in triplicate on acid-hydrolysed samples by the method of Switzer & Summer (1971). Collagen content was calculated on the basis that it contains 14% hydroxyproline.

Results

Table 1 shows that the NPU of the lower quality beef, shin, was 69 compared with 82 for fillet. Both samples were limited by the S-amino acids since their NPUs were

TABLE 1. Quality of meat protein and collagen content

Sample	NPU duplicates		Mean	Hydroxyproline g/100 g protein	Collagen g/100 g protein
Shin	68	70	69	3·31	23·6
+ methionine	88	90	89		
Fillet	81	83	82	0·36	2·5
+ methionine	99	97	98		
Shoulder (cooked)	76	80	78	1·9	13·5*

* From Bender & Husaini (1976).

increased to 89 and 98 respectively by supplementation with methionine. The intermediate values of shoulder meat found by Bender & Husaini (1976) are included for the purposes of comparison.

The difference between the values for the methionine-supplemented samples indicate a relative deficiency of a second amino acid in the lower quality meat—according to Dvorak & Vognarova (1969) this could be phenylalanine.

Table 1 also shows that the protein of shin contains 23·6% collagen compared with 2·5% for the fillet.

Conclusions

Dvorak & Vognarova (1969) showed that the content of essential amino acids of beef proteins decreased with increasing content of hydroxyproline; beef shank contained less methionine than fillet (1·45 compared with 2·73 g/16 g nitrogen) which reflects the

low content of sulphur amino acids in connective tissue, namely, 1% compared with 3·7% in muscle. The present work appears to be the first in which biological assay has been related to chemical determination of connective tissue on the same material and confirms the earlier, although apparently forgotten, conclusions of Mitchell & Carman (1926) that cheaper cuts of meat which are richer in connective tissue have protein of lower biological value.

Meat is generally stated, for example by FAO (1968), to have NPU 74; the publication gives a range from 62 to 78 but it is not stated whether this range is due to variability of assay or to different samples of meat. It is clear that a product termed 'meat' may have NPU considerably lower than the accepted value of 74 for a number of reasons; the meat may contain more connective tissue than the samples examined biologically, many different types of products are loosely termed 'meat' and, finally, there can be processing damage.

It is unlikely that differences between high and low quality meats such as those reported here, namely NPU 82 and 69 will be of any practical importance in the diet as a whole and it is noteworthy that the price differential is very much greater than the difference in nutritional value.

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Nutritive value of proteins in a canned meat product

A. E. BENDER AND HUSAINI

Summary

Nine samples of canned meat-with-onions-and-gravy were assayed biologically for net protein utilization and analysed for hydroxyproline as a measure of the collagen content.

NPUs ranged from 40 to 59, compared with 78 for moderate quality meat; two samples contained 20% collagen compared with 14% for the moderate quality meat.

The NPU of meat autoclaved in the laboratory in the presence of wheat flour and glucose was 70 compared with a value of 78 for the same meat autoclaved alone.

It is concluded that the low NPU value of the canned meat products is due both to the high content of connective tissue and to the loss of available methionine on canning in the presence of the other foodstuffs.

Introduction

There is abundant literature reporting that the protein quality of meat is little affected by processing (reviewed by Bender, 1972). For example, in a range of products—bacon, pork, ham and chicken—Thomas & Calloway (1961) showed no loss of any of the essential amino acids when the meat was subjected to processes like freeze drying, cooking and canning. Mayfield & Hedrick (1949) found no fall in protein quality when meat was roasted in an open pan at 163°C with an internal temperature of 80°C—Biological Value (BV) was the same as that of raw meat, namely 79. Even meat that had been browned in the oven for 30 min and heated in the can for 85 min at 121°C still had BV 74.

Many authors fail to state the internal temperature, which is obviously more important than the cooking or sterilizing temperature. Wheeler & Morgan (1958) found no damage when pork was heated at 120°C for 1 hr but after 2 hr, when presumably the heat had penetrated, there was a fall in PER from 2·1 to 1·7. Further evidence comes from the work of Poling, Shultz & Robinson (1944) who found no damage when pork was heated 'to' 128°C for 3 hr with an internal temperature of 107°C.

Authors' address: Department of Nutrition, Queen Elizabeth College, Campden Hill, London W8 7AH.

Lysine is sensitive to heat damage, thus Dvorak & Vognarova (1965) showed 10% loss of available lysine in beef heated 3 hr at 70°C and 20% loss at 121°C.

Since the quality of meat protein is limited by the sulphur amino acids (methionine plus cystine) loss of available lysine will have no effect on bioassay results unless it is excessive; only destruction or reduced availability of the S-amino acids will reduce the quality of the protein.

There is some evidence that when meat is heated in the presence of other foodstuffs there is a greater fall in nutritive value than when it is heated alone. Hellendoorn *et al.* (1971) examined the protein quality (net protein utilization) of six meat-and-vegetable canned meals; protein quality fell after canning in three of the six and all showed a fall after three years' storage. Products containing pork, beef and bacon suffered falls of 12%, 18% and 30% respectively on canning, with total falls of 20%, 31% and 40% after five years' storage. The authors stated at that time that nothing was known about the nutritive value of processed whole meals as distinct from meat processed alone.

It was decided to examine a number of canned preparations of meat-with-onions-and-gravy in which the meat is heated in the presence of the other foodstuffs and compare them with samples prepared in the laboratory.

Methods

Four different samples of one brand of canned minced beef-with-onions-and-gravy and three samples of stewed steak-with-onions-and-gravy were purchased at different times in an attempt to obtain different batches. A single sample of each of the two types of a similar product of another brand was also examined. The ingredients shown on the label were beef, onions, wheat flour, salt, caramel, beef extract, hydrolysed protein and flavouring.

Raw shoulder beef was purchased from the butcher and minced to pass a 9-mm sieve. One portion was heated with one-tenth part of water for 1 hr at 115°C in an autoclave. A second portion was mixed with 6% of its weight of wheat flour, 3% glucose and one tenth part of water (to simulate a gravy) and similarly autoclaved.

All samples were dehydrated and defatted by mixing with two volumes of acetone, repeated twice and drying in air.

Biological and chemical determinations were as described by Bender & Zia (1976).

Results and discussion

Table 1 shows that the four samples of brand A minced meat preparation varied considerably in protein quality, ranging from NPU 40 to 56. There was a much smaller range from 50 to 56 between the three samples of brand A stewed steak. The single sample of brand B minced preparation fell in the same range as brand A; the brand B stewed steak with NPU 59 was slightly above the range of brand A.

TABLE 1. Protein quality of processed meat products (individual results in parentheses)

Canned minced meat with onions and gravy			NPU	Hydroxyproline g/16 g N	Collagen g/16 g N
Sample	1	Brand A sample (a)	40 (42, 40, 38)		
	2	sample (b)	47 (44, 45)		
	3	sample (c)	52 (53, 50)	2·7 (2·5, 2·9)	19·3
	4	sample (d)	56 (53, 59)	3·0 (2·95, 3·1)	21·4
	5	Sample 4 + methionine (1·4%)	70 (67, 72)		
	6	Brand B	55 (54, 55)		
Canned stewed steak with onions and gravy					
Sample	7	Brand A sample (a)	50 (54, 48)		
	8	sample (b)	55 (54, 56)		
	9	sample (c)	56 (59, 53)		
	10	Brand B	59 (57, 61)		
Laboratory preparations					
	11	Autoclaved alone*	78 (76, 80)	1·9 (1·9, 1·9)	13·5
	12	Autoclaved with 'gravy'†	70 (67, 72)	1·9 (1·8, 2·0)	13·5
	13	Sample 12 + DL-methionine (2%)	80 (78, 82)		

* One hour at 115°C.
† 'Gravy' = 6% wheat flour + 3% glucose.

These values may be compared with those for fresh uncooked beef of high eating quality, namely fillet steak, NPU 82, 2·5% collagen and one of low eating quality, namely shin, NPU 69, 23·6% collagen (Bender & Zia, 1976). Shoulder meat (sample 11, Table 1) was intermediate in collagen content between these two examples of the highest and lowest quality meats namely 13·5% but had a high NPU of 78.

Despite its higher collagen content (23·6%) the shin meat referred to had a higher NPU at 69 than samples 3 and 4 (NPU 52 and 56, collagen 19% and 21%). These figures suggest that the low NPUs of these canned meat samples were partly due to processing damage. This was verified by the sample autoclaved in the laboratory with (sample 12) and without 'gravy' (sample 11). In the presence of wheat flour and glucose the NPU was reduced from 78 to 70. The conditions in the laboratory probably differ from those used for the commercial samples and there is no information of the length of time of storage but the measurements indicate that when meat is processed in the presence of other foods there can be a reduction in protein quality in contrast to its stability to autoclaving in the absence of other materials.

Amino acid damage

Sample 5 showed that methionine supplementation of the minced canned product

restored NPU only to 70, whereas the laboratory sample (No. 13) was restored to 80, and other work (Bender & Zia, 1976) has shown methionine-supplemented values of 89 for low quality and 98 for high quality meats. This indicates that damage was done to amino acids other than methionine both in the laboratory processing in the presence of starch and glucose, and to a greater extent in the commercial product. Dvorak & Vognarova (1969) showed chemically that phenylalanine was likely to be the second limiting amino acid in meat rich in connective tissue; at the same time heat damage in the presence of reducing substances results in a fall in available lysine through the Maillard reaction.

Conclusions

It is rare for manufacturers to exercise quality control over the nutritional value of the proteins of their products and there is very little evidence available of the protein quality of processed foods and their variation from batch to batch.

In the canned meats reported on here the protein quality was considerably less than that of good quality meat and varied considerably between batches. There appear to be two reasons for this; the use of meat rich in connective tissue and damage on heating in the presence of other footstuffs such as onions and gravy.

Measurements of protein quality included both the meats and the added ingredients but the latter would make little difference to the combined NPU, since they are small in quantity and especially if wheat flour is a major ingredient since this is limited by lysine which would complement the meat proteins.

The relatively low value of such meat products may have little or no significance in the whole diet since people consuming canned meat products regularly are most likely to obtain far more protein than their minimum needs so that protein quality is of no importance whatever. Furthermore the NPUs observed over a vast variety of diets range only between 80 (in diets where the main protein sources are meat, milk and wheat) to 70 (where the protein is mainly supplied by cereals) (WHO, 1973).

Instances where protein quality may be of importance occur when foods like textured vegetable proteins are being compared with meat, then the quality of the meat may need to be taken into account.

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Determination of freezing and thawing times in the centre of blocks of meat by measurement of surface temperature

STEPHEN J. JAMES, C. BAILEY AND S. ONO

Summary

A mathematical model based on a theoretical analysis of the pattern of heat flow was used to produce a set of freezing and thawing curves at various depths through an infinite slab of lean meat. Analysis of these curves indicated that the end of the freezing or thawing plateau at the block centre should be reflected in a change in the temperature-time gradient ($\Delta T_s/\Delta t$) at or near the surface.

This change was subsequently demonstrated experimentally, and a method has been devised which enables the freezing or thawing time at the centre of a block of meat to be determined by means of a probe inserted just under the surface of the block.

Introduction

There are a number of commercial reasons for accurately determining the thawing or freezing times at the centre of a block of meat. If freezing times are under-estimated blocks may be transferred from the blast freezer to the cold store with the inside of the block still unfrozen which, at worst, may create microbiological problems in the subsequently thawed material, or at best, will impose a product load on the cold store that it was not designed to take. On the other hand, if blocks are left in the blast freezer beyond the required freezing time the whole process becomes inefficient. In thawing it is important from appearance, bacteriological and weight loss considerations that thawing times should be minimal, but hand trimming of the meat cannot be effectively carried out until the centre temperature has reached at least 0°C.

Currently, the progress of freezing or thawing at the thermal centre of large blocks of meat can be followed only by thermocouples placed in the appropriate position. Such a procedure is not very practical, particularly in commercial operations, as thermocouples inserted prior to freezing cannot then be removed from the frozen meat, whilst thermocouples cannot be inserted in frozen blocks prior to thawing without drilling holes. In either case there is no guarantee that the thermocouple will be located at the thermal centre.

Authors' address: Meat Research Institute, Langford, Bristol BS18 7DY.

This paper describes a method of determining the end of the latent heat plateau at the thermal centre of blocks up to 20 cm thick in air freezing and thawing systems without the need for interior thermocouples.

Theoretical considerations

Consider a block of frozen lean meat at a uniform temperature well below its initial freezing point (−1·0°C) placed in an air stream at a temperature T_a (above −1°C); the whole block begins to rise in temperature, the rate of rise being greatest at the surface and decreasing with increasing depth (Fig. 1, Time 1). Once the surface reaches −1°C (Fig. 1, Time 2) a layer of thawed meat is formed which has lower thermal conductivity and a lower specific heat than the adjacent frozen material (Fig. 1, Time 3).

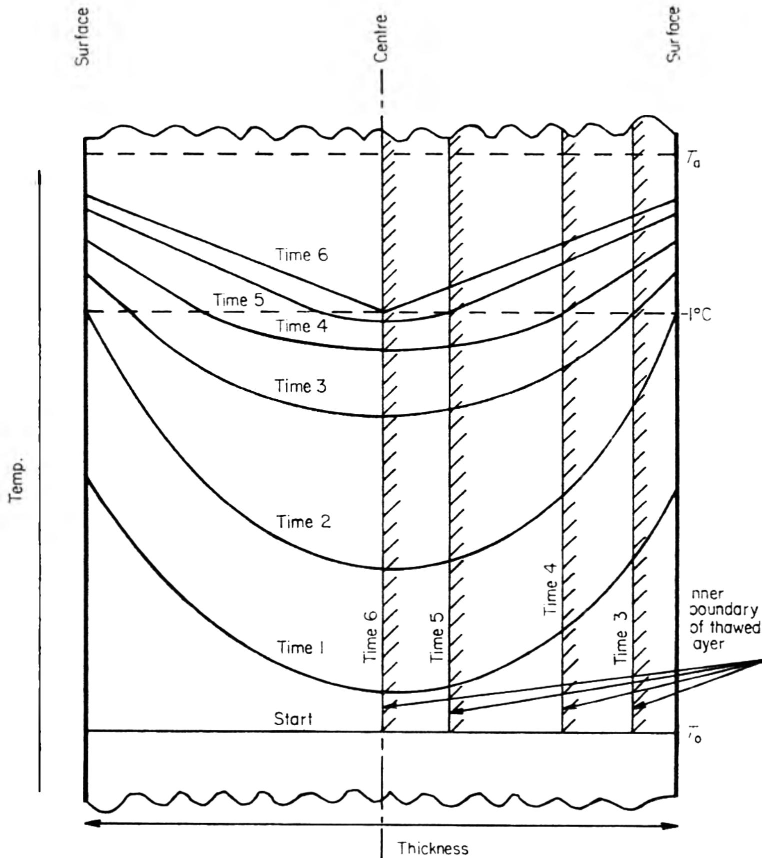

Fig. 1. Change in temperature profile through block with time.

As the thawing proceeds (Fig. 1, Times 4 and 5) the surface temperature of the block continues to rise, consequently reducing the temperature difference between the air and the surface. The rate of rise of the surface temperature is dependent upon this temperature difference and decreases as the difference becomes smaller. When the centre of the block reaches $-1°C$ thawing is completed (Fig. 1, Time 6). Up to this time heat is being supplied to and passes through the slab at a rate which slowly raises the mean temperature of the block, whilst also providing the latent heat required for the phase change. Once the thawing process is complete the phase change demand reduces to zero and the total heat flux is available to rapidly raise the temperature at the centre of the block. This temperature rise reduces the temperature gradient in the block and causes a rapid fall in the rate at which heat flows into the slab. Thus the surface temperature (T_S) shows a marked increase once the centre has thawed. Similar considerations can be used in the case of freezing to show that a marked decrease in (T_S) would be expected when the material is being frozen.

This theory was tested by constructing a mathematical model, based on the numerical method of Dusinberre (1954) for one-dimensional transient conduction, to analyse the pattern of heat flow during the thawing or freezing of blocks of boneless meat (another application of this model has been described in detail by Bailey *et al.* (1974)).

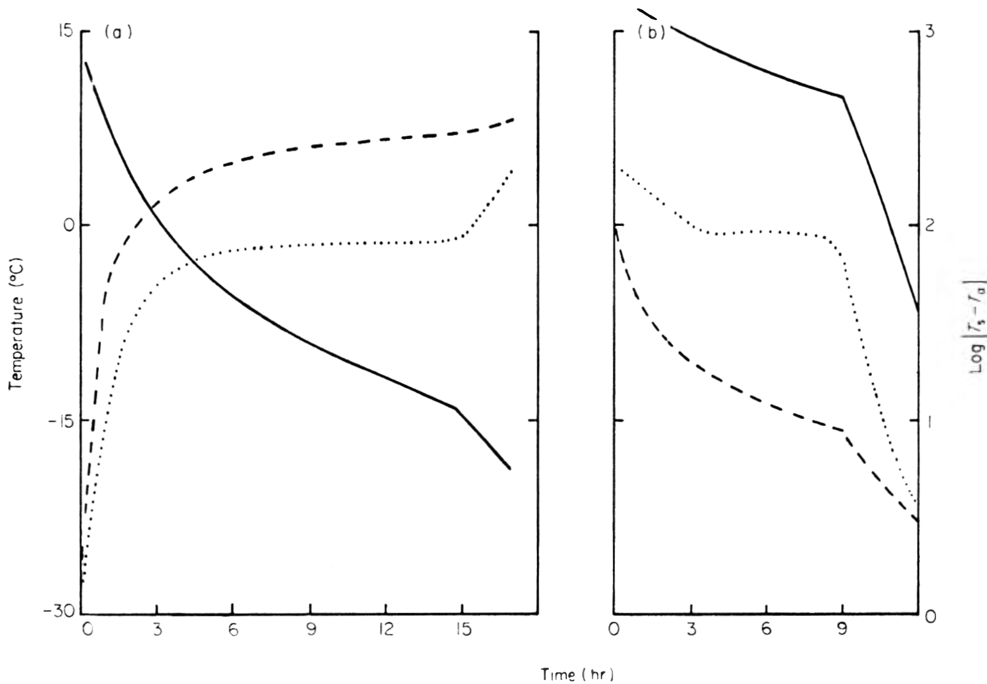

FIG. 2. Theoretical temperature-time curves during (a) thawing and (b) freezing a 10 cm thick meat block with a heat transfer coefficient (H) of 30 W/m² °C. (a) $T_a = 10°C$; (b) $T_a = 30°C$. .... 5 cm deep (T_c); – – – surface (T_s); —— $\log|T_s - T_a|$.

Typical time/temperature curves predicted by this method (Fig. 2) show the change in surface temperature to be expected, an effect which can be emphasized by considering the log of the modulus of ambient and surface temperature difference (log $|T_a - T_s|$). The change in slope of this provides a clear indication of the end of the latent heat plateau, and thus can be used to determine the duration of the freezing or thawing process (Fig. 3).

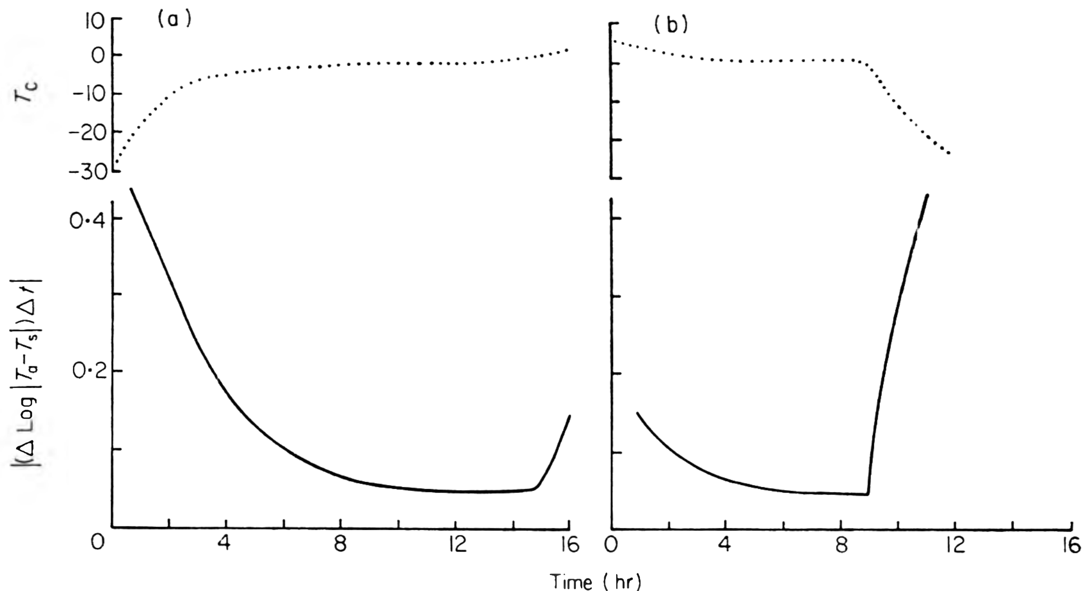

Fig. 3. Theoretical temperature-time and temperature-time gradient curves for 10 cm thick meat blocks during (a) thawing and (b) freezing thawing with an initial air/meat difference of 40°C and a heat transfer coefficient (H) of 30 W/m² °C. (a) $T_a = 10$°C; (b) $T_a = -30$°C. .... (T_c); —— $|(\Delta \log|T_a - T_s|)/\Delta t|$; $\Delta t = 0{\cdot}5$ (hr).

The influence of block thickness, heat transfer coefficient and the different responses shown by freezing and thawing processes can also be predicted using $\Delta \log |T_a - T_s| / \Delta t$. Thick slabs show smaller changes in slope than thin slabs subjected to the same environmental condition whilst, for a given slab, the change in slope becomes more pronounced as surface heat transfer coefficient increases. Freezing processes produce sharper changes in slope than thawing processes for the same heat transfer coefficient and block thickness. The analysis suggests that the method should provide an accurate measurement of the end point of the latent heat plateau for blocks up to 20 cm thick, with heat transfer coefficients between 10 W/m² °C and 90 W/m² °C, and temperatures between 10°C and 30°C for thawing and − 10°C and − 30°C for freezing. This range of conditions adequately covers the majority of air thawing and freezing systems in commercial operations.

Experimental

Material

Blocks were manufactured from chilled vacuum packed beef topsides. From each whole topside a 20 × 20 × 10 cm or 20 × 20 × 5 cm rectangular parallelepiped was removed such that the fibre structure ran parallel to the 20 × 20 cm face.

Container. The meat was inserted into an open Perspex box containing a fixed vertical multi-junction temperature probe mounted through the centre of its base (Fig. 4). This

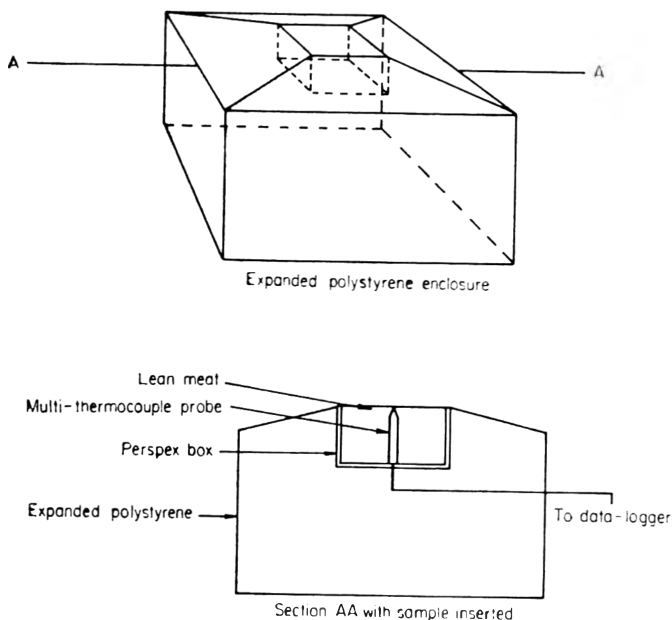

Fig. 4. Simulated 'infinite' block of lean meat.

box was then placed in an expanded polystyrene enclosure with the exposed meat surface flush with the face of the enclosure. The upper surface of the enclosure was inclined slightly towards the box in order to ensure an undisturbed flow of air over the surface of the meat. The complete assembly produced a simulated 'infinite' block with an effective thickness twice that of the inserted sample.

Air thawing. The 'infinite' block assembly was placed in a controlled air-thawing tunnel previously described by Bailey *et al.* (1974). Air velocity was set at 2 to 3 m/sec or 0·5 to 1 m/sec. The tunnel was placed in a room equipped with an evaporator and a bank of heaters, and a sensor in the working section operated a proportional controller to maintain an air temperature of 10° ± 0·5°C. A humidifier controlled by a lithium chloride sensor maintained the relative humidity at 85%.

Air freezing. Freezing was carried out in a small room equipped with a wall-mounted evaporator and two axial flow fans. The 'infinite' block container was positioned to

give air velocities over the surface of the meat of either 2 to 3 m/sec or 0·5 to 1 m/sec. A sensor in the room operated an on-off controller to maintain a room air temperature of $-30° \pm 5°C$. The room had a six hourly defrost cycle.

Measurements. Meat temperatures were measured at 1 cm depths through the block using a copper/constantan thermocouple probe (Bailey *et al.*, 1974). Two further thermocouples cemented to the inner bottom surface of the Perspex box recorded the equivalent 'centre' temperature of the 'infinite' slab. Air temperature was measured using a copper-constantan thermocouple positioned 2·5 cm above the exposed surface of the meat. All thermocouples were connected to a 'Solartron' data logging system measuring to $\pm 0·5°C$ with quick release copper-constantan junction plugs. Air velocities were measured using a 'Wallac' hot wire anemometer located in the air stream immediately above the surface of the meat.

Results and discussion

Practical measurements of $|(\Delta \log|T_a - T_s|)/\Delta t|$ for all experiments using 10 cm and 20 cm thick blocks confirmed the marked slope changes predicted by the model once

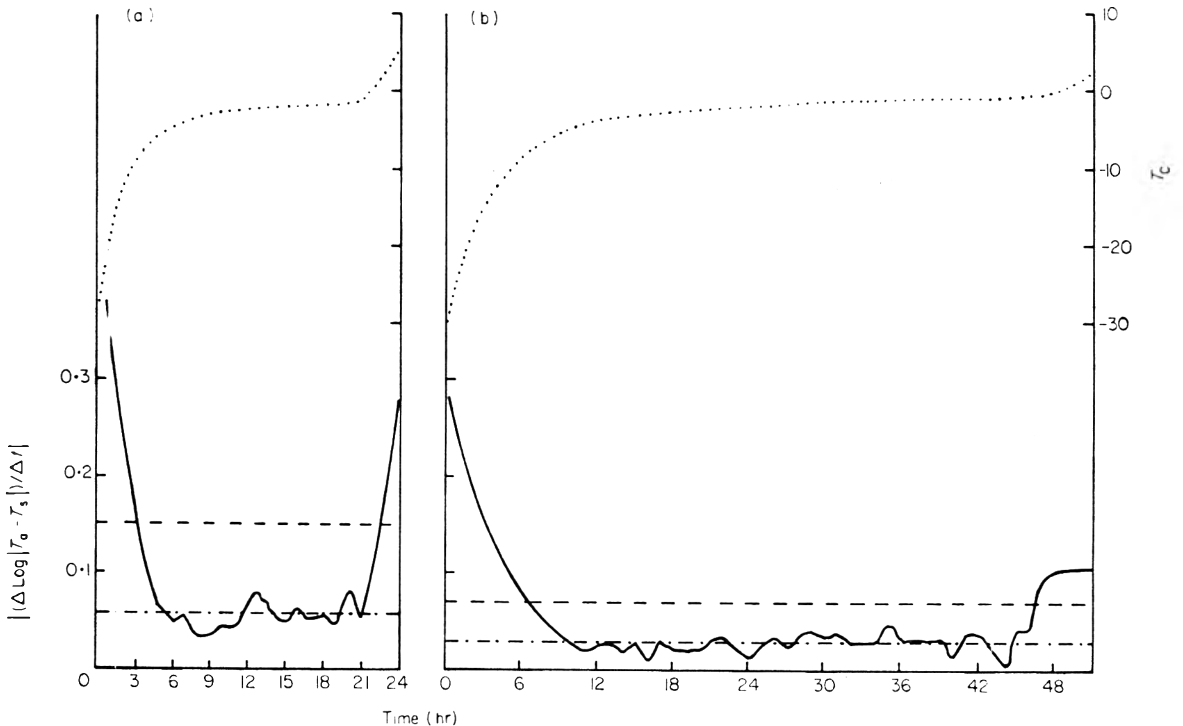

FIG. 5. Typical experimental curves obtained when thawing 'infinite' blocks in air at 10°C, with an air velocity of 0·5 to 1·0 m/sec. (a) 10 cm slab; (b) 20 cm slab. .... T_c; —— $|(\Delta \log|T_a - T_s|)/\Delta t|$; — · — constant; - - - 2·5 x constant; $\Delta t = 0·5$ (hr).

the block centre had reached the end of the latent heat plateau (Fig. 5). In thawing, for example, the curve of $|(\Delta \log|T_a - T_s|)/\Delta t|$ against time was made up of three distinct sections. The initial decrease in slope was followed by a period of relatively constant slope and then a rapid increase. This rapid increase occurred at the completion of the thawing process. Although the mathematical model has shown that the actual value of the 'constant' slope is a function of slab thickness, heat transfer coefficient and ambient air temperature, this value can be readily determined for any application; the end point can then be defined as the time at which the slope exceeds this value. Allowance must be made, in the experimental situation, for fluctuations in composition and conditions which produce random rises in the 'constant' value before the end of the plateau has been reached, by setting a limit above which the slope value must rise to indicate a significant end point. The experimental data indicated that the effects of such process fluctuations are satisfactorily eliminated by setting the limit at 2·5 times the defined 'constant' value.

Table 1 shows the difference between the time to the end of the latent heat plateau determined by a thermocouple in the centre of the block, and that estimated using a factor of 2·5 times the measured 'constant' value of $|(\Delta \log|T_a - T_s|)/\Delta t|$. The error incurred with 10 cm thick blocks was no greater than 1 hr in either freezing or thawing rising to a maximum of 1·5 hr for 20 cm thick blocks an accuracy well within that required for most industrial purposes, and is probably better than the results that would be obtained from probes which, more often than not fail to locate the thermal centre of the block. These errors are in part due to the 0·5 hr intervals used in the temperature determinations at both the surface and centre of the block, and might be further reduced by taking more frequent measurements.

The whole basis of the method is the determination of a change in the meat surface temperature once the centre has passed through the latent heat plateau, and there must therefore be a significant difference between the temperature of the surface during thawing (or freezing) and that of the thawing (or freezing) medium. Such a difference can only be maintained if the surface heat transfer coefficient is low, and the method is consequently viable for air systems but not for processes using water, vacuum steam heat or contact plate heat exchangers. The method has been shown to be successful for uniformly shaped materials such as boned-out blocks of meat where the probe is placed either in the geometric centre of the upper surface or above the thickest part of the block if the surface is irregular. The method could also be employed with irregular shaped materials provided that the surface probe was located at a point above the thermal centre. The accuracy of the method is a function of the choice of the time interval (Δt) and the sensitivity of the surface thermocouple which for optimal results should be capable of detecting changes of $\leqslant 0{\cdot}1$°C. The method has only been demonstrated for lean meat, but could also be applied to any material which shows an abrupt change in thermal diffusivity at its change of state temperature.

TABLE 1. Comparison between time to the end of the latent heat plateau measured with a probe at the block centre and the time to 2·5 × the 'constant' slope, $|(\Delta \log |T_a - T_s|)/\Delta t|$, determined at the surface

Experiment number	Initial meat temperature (°C)	Mean air temperature (°C)	Measured 'constant' slope $|(\Delta \log|T_a - T_s|)/\Delta t|$ $\Delta t = 0 \cdot 5$ (hr)	Time to 2·5 × constant slop (hr)	Measured times to −5° in centre (freezing) 0° in centre (thawing) (hr)	Difference in times
Freezing 20 cm thick slabs, air velocity 2–3 m/sec						
1	8	−31	−0·0440	18·5	18·0	0·5
5	7	−29	−0·0315	24·0	24·0	0·0
7	0	−28	−0·0490	18·0	16·5	1·5
9	6	−27	−0·0429	19·0	20·0	1·0
11	4	−26	−0·0306	22·5	22·0	0·5
Freezing 10 cm thick slabs, air velocity 0·5–1 m/sec						
13	2	−27	−0·0342	10·0	10·0	0·0
15	3	−27	−0·0298	10·5	11·0	0·5
17	2	−27	−0·0456	10·0	10·0	0·0
19	3	−27	−0·0269	11·0	12·0	1·0
21	3	−27	−0·0293	11·5	12·0	0·5
23	4	−27	−0·0330	12·5	12·5	0·0
Thawing 20 cm thick slabs, air velocity 2–3 m/sec						
4	−29	10	−0·0296	48·0	49·0	1·0
6	−29	10	−0·0276	47·0	48·0	1·0
8	−28	10	−0·0310	47·0	46·0	1·0
10	−24	10	−0·0367	42·5	43·0	0·5
Thawing 10 cm thick slabs, air velocity 0·5–1 m/sec						
14	−26	10	−0·0399	21·5	22·0	0·5
16	−28	10	−0·0603	21·5	22·0	0·5
18	−28	10	−0·0592	22·0	22·0	0·0
20	−30	10	−0·0549	23·0	23·0	0·0
22	−30	10	−0·0497	25·5	24·5	1·0
24	−30	10	−0·0557	24·5	24·5	0·0
Accuracy	±0·5°C	±0·5°C	±0·0005	±0·25 h	±0·25 h	±0·5 h

Notation

T = air temperature;
T_c = temperature at centre of slab;
T_s = temperature at surface of slab;
t = time;
Δt = time interval;
ΔT = change in temperature;
$\Delta T_s/\Delta t$ = temperature time gradient at surface;
$|T_a - T_s|$ = modulus of temperature difference between air and surface;
$|(\Delta \log|T_a - T_s|)/\Delta t|$ = modulus of change in natural logarithmic value of $|T_a - T_s|$ with time;
H = surface or film heat transfer coefficient;
x = thickness of slab;
x_1 = thickness of finite slice.

Acknowledgment

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The application of amino acid analysis to the determination of the geographical origin of honey

A. M. C. DAVIES

Summary

Honeys from four geographical areas were characterized by a selection of sixty amino acid ratios, calculated from amino acid analysis data. Tests were carried out on sixteen samples of honey which came from these characterized areas using the selected amino acid ratios and on a reduced set of ratios in which the number of correlated ratios had been reduced. Fifteen samples were correctly identified by the full ratio set and fourteen samples by the reduced set. No sample failed both tests. Forty-two honeys which did not come from these areas were also tested and only one sample was incorrectly identified as originating from a characterized area.

These results support the proposal that amino acid analysis could be used as an instrumental method of determining the geographical source of a honey, in place of the highly subjective method of pollen analysis. However, further work is required before the method could be recommended for official use.

The possibility of applying the same techniques to other problems of food source identity is noted.

Introduction

Man's sweet tooth for honey was developed in primitive times, as is evident from rock paintings in Spain and Africa depicting honey gathering (Crane, 1975). Although honey has been traded in the world for many centuries the present level of trade dates only from the end of World War II as a result of the establishment of modern bee-keeping methods in Mexico, Argentina and Australia. Honey prices vary considerably but one of the factors which determines price is the geographical origin of the honey. At the present time pollen analysis is the generally recognized method of determining the geographical source of a honey (Sawyer, 1975). Pollen analysis is very dependent on expert ability and judgement (Howells, 1969), and chemical methods of characterizing honeys have been sought for many years. Amino acid analysis is one of the methods to attract attention and this was reviewed in an earlier paper (Davies, 1975a). The same

Author's address: Agricultural Research Council's Food Research Institute, Colney Lane, Norwich NR4 7UA.

paper also presented results for the amino acid analysis of ninety-eight honeys with samples from most of the commercially important areas.

A method of utilizing chromatographic data for commodity source identification has been described (Davies, 1975b) and this paper describes the application of the method to the identification of honeys using their amino acid composition.

Methods

Characterization of honey

A honey area was characterized by means of a computer program which determined the sixty most consistent ratios of amino acids using analyses of honeys known to originate from that area. The program allowed for occasional missing amino acids by insertion of a small value representing the limit of detection and reciprocal ratios were excluded by numbering the amino acids and only using those ratios in which the numerator amino acid number was less than the denominator amino acid number. In the tests described twenty-eight amino acids were entered giving a total of 378 ratios, and the program then selected the sixty most consistent ratios, as measured by their coefficients of variation.

Consistency testing

A second computer program was used to test if an unknown sample of honey was consistent with origination from a previously characterized area. The program calculated the specified ratios and used a statistical test (Davies, 1975b), with the null hypothesis that the honey originated in the area under test. The result of this test was the significance level at which this hypothesis could be rejected. Differences which were significant below the 5% level of probability were taken as demonstrating that the honey did not come from the area under consideration. Differences which were not significant even at the 10% level of probability were taken as indicating that the sample was consistent with originating from the test area. Results between the 5 and 10% levels of probability indicated a 'don't know' verdict.

Amino acid analysis data

Except for Table 6, which uses data presented by Bergner & Hahn (1972), the amino acid analyses are from Davies (1975a).

Results

Characterization of honeys

There were sufficient data to characterize honeys from four areas: Australian (Eucalyptus/Clover), Canadian (Clover), Mexican (Yucatan) and United States (Clover). Ten samples of each honey were randomly selected from the available data and sixty ratios were selected from each area. Table 1 shows the ratios which were

Table 1. Selection of amino acid ratios for Australia (Eucalyptus/Clover) honey

Numerator amino acid		Denominator amino acid																	
Name	Number	27	26	25	24	23	20	19	18	17	16	15	14	13	11	10	8	7	5
Glucosaminic	1		*	*		*					*	*	*		*		*	*	*
Amides	5	*	*			*	*			*	*	*		*	*		*	*	
Glutamic	7	*	*			*	*			*	*	*		*	*		*		
Proline	8	*				*	*	*		*		*	*		*				
Glycine	10								*		*								
Alanine	11					*	*			*		*							
Valine	13												*						
Methionine	14	*	*																
Isoleucine	15	*				*	*			*	*								
Leucine	16								*	*									
Tyrosine	17																		
Phenylalanine	18																		
β-Alanine	19					*													
γ-Aminobutyric acid	20					*													
Unknown E†	23	*																	
Lysine	24	*																	
Ornithine	25																		
Histidine	26	*																	
Tryptophan	27																		

* Selected ratio.

† See Davies (1975a).

Note. Only amino acids which were utilized by the selection program are included in the Table.

selected for the Australian area. Most of the selected ratios were formed by common amino acids rather than the unknowns present in the honeys. The selection of ratios with glucosaminic acid and with tryptophan were caused by the absence of these peaks from all samples used for the characterization of the Australian area. Because a constant value was used for missing peaks this provided an unintentional method of selecting the actual amino acid results if they were sufficiently constant. The ratios selected for the other three areas were similar in the amino acids used but differed in the ratios selected.

The method of testing involved the calculation of standardized ratio differences, that is the difference between a sample ratio and the mean value of that ratio, divided by the standard deviation of the mean, i.e. $(x-\bar{x})/s_{\bar{x}}$. It is important that the distribution of standardized ratios is not skew and also that the ratios are independent (i.e. not correlated) variables. Table 2 gives the results of testing the selected ratios. The results

TABLE 2. Tests on ratio selections

Honey area	Skewness	Number of correlated ratios* ($P=0\cdot001$)	Amino acid/peak associated with largest number of correlations	Number of correlations after removal of AA	Number of ratios in reduced set†
Australia	0·05	19	Amides	8	48
Canada	0·02	38	Amides	20	50
Mexico	0·01	45	Alanine	17	48
United States	0·10	53	Aspartic acid	29	48

* Out of the 1770 combinations of sixty ratios.

† The initial sets all contained sixty ratios.

for the skew determinations are all satisfactory as tested by the method of Snedecor (1956), and indicate almost symmetrical distributions of the standardized ratios. In all four areas there were a number of very significantly correlated ratios but these could be considerably reduced by removing all the ratios involving the amino acid associated with the largest number of correlations.

Testing of honeys from the characterized areas

Table 3 gives the results of testing honeys which came from the characterized areas but had not been used in the characterization. The tests were carried out on both the full set of sixty ratios and also on the reduced set after the removal of ratios involving the amino acid which gave rise to the majority of the very significant correlations.

Testing of honeys from non-characterized areas

Forty-two honeys from non-characterized areas were tested against the characterized

TABLE 3. Identity testing of honeys from characterized areas

Source of sample	Number of ratios used	Number of samples	Number of tests which were significant at the given probability level									
			Tested against actual source $P=$					Tested against the other three sources $P=$				
			20	10	5·0	1·0	0·1%	20	10	5·0	1·0	0·1%
Australia	60	3	2	1	0	0	0	0	0	0	0	9
Australia	48	3	2	0	1	0	0	0	0	0	0	9
Canada	60	6	5	1	0	0	0	0	0	0	0	18
Canada	50	6	5	1	0	0	0	0	0	0	0	18
Mexico	60	4	4	0	0	0	0	0	0	0	0	12
Mexico	48	4	2	2	0	0	0	0	0	0	0	12
United States	60	3	1	1	0	1	0	0	0	0	0	9
United States	48	3	1	1	1	0	0	0	0	0	0	9
Total	60	16	12	3	0	1	0	0	0	0	0	48
Total	R*	16	10	4	2	0	0	0	0	0	0	48

* Total for reduced sets.

Table 4. Identity testing of honeys from non-characterized areas

Source of sample	Number of samples	Results when tested as originating in:							
		Australia		Canada		Mexico		United States	
		+VE*	−VE†	+VE	−VE	+VE	−VE	+VE	−VE
Argentina	8	0	8	0	8	0	8	0	8
Australia (mis)	18	1	17	0	18	0	18	0	18
India	5	0	5	0	5	0	5	0	5
Miscellaneous	11	0	11	0	11	0	11	0	11
Australia (E/C)‡	10	—	—	0	10	0	10	0	10
Canada	10	0	10	—	—	0	10	0	10
Mexico	10	0	10	0	10	—	—	0	10
United States	10	0	10	0	10	0	10	—	—
Total	82	1	71	0	72	0	72	0	72

* Significant above the 10% level of probability.
† Significant below the 1% level of probability.
‡ Eucalyptus/Clover.

areas. The honeys used for characterizing the areas were also tested but the results for the actual area have been omitted (they were all positive, as expected). The results of these tests are shown in Table 4. There were no 'don't know' verdicts.

Tests on binary mixtures of honeys

Tests were carried out, using a special version of the testing program, to determine the calculated minimum percentage of honey or adulterant which had to be mixed with a given honey so that the mixture was not identified as originating in the area of the predominant honey. Table 5 gives the minimum percentage of adulterant required to produce a non-identified result.

TABLE 5. Tests on mixtures of honey

Predominant honey source	Minimum percentage of adulterant to produce a non-identified result					
	Mixed with honey from:				Adulterated with:	
	Australia	Canada	Mexico	United States	Syrup	Treacle
Australia	—	10	15	10	45	15
Canada	1·0	—	5	35	7·5	2·5
Mexico	25	30	—	35	20	5
United States	40	1·0	45	—	35	7·5

Tests on floral and honeydew honeys

Bergner & Hahn (1972) reported amino acid analyses of honeys for twenty-six honeys from sixteen different floral sources and for fourteen honeydew honeys from six different honeydew sources. (Honeydew honey is honey made by bees from the sugary excretions of aphids and other insects.) There were insufficient numbers of samples in any of the individual groups for application of the method. However, there were sufficient numbers to test if floral and honeydew honey could be distinguished from each other. The results are shown in Table 6.

Discussion

Identification is a complex problem because it can never be certain. Non-identification can be established at a given probability level but actual identification can only be assumed because of the lack of contrary evidence. In some cases, where there is only a limited number of known possible identities, the identity of a sample can often be determined with a high degree of confidence. However, when the number of possible identities is large and the number of characterized groups is small there will always be an uncalculable risk that an identified sample belongs to a different but characteristi-

Table 6. Identity tests on flower and honeydew honey (Bergner & Hahn)

	Floral honey tested as		Honeydew honey tested as	
	Floral honey	Honeydew honey	Floral honey	Honeydew honey
Tested	16*	26	14	4*
Identified	10	3	11	4
Undecided	4	4	1	0
Not identified	2	19	2	0

* Ten samples were used to select the ratios.

cally similar group. It is for this reason that while the good results (Table 3) for correctly identifying samples from the characterized areas is encouraging, the most important results are those in Table 4 which show that only one, closely related, honey was incorrectly identified as originating from one of the characterized areas. This Australian honey had been previously identified, by a pollen analysis, to be a eucalyptus type (Davies, 1975a) but was not thought to be sufficiently similar to be included in that group.

Limitations of the method

Tables 5 and 6 demonstrate the limitations of the method, in general the testing method was fairly tolerant so that quite large amounts of adulterant were required to produce an effect. However, for commercial adulteration of one honey with another to be profitable it is likely that large amounts of the adulterant would be used. Occasionally the test was very sensitive but these results were caused by the presence of amino acids in the adulterating honey which were absent in all samples of the test honey. These peaks were small so that it is not certain that they would always be found in the adulterating honey. Thus, tests based on this type of result are not considered to be reliable.

The results from the Bergner & Hahn data show that most of the floral honeys could be distinguished from honeydew honey but that honeydew could not be distinguished from floral honey. This was because of the very large variation in the amino acid composition of the floral honeys and is possibly due to the larger number of floral honey sources. It would appear that the test method will only be successful when there is a chemotaxonomical justification for the formation of a group.

Bergner & Hahn considered that their data demonstrated that amino acid composition of honeys would not be useful for identifying honeys. However, this conclusion was based on consideration of ratios of amino acids with proline. Proline is not a good amino acid for forming ratios because it is normally present in large amounts and it is more prone to error than most other amino acids. There were also too

few samples from each source to determine the variability of amino acid ratios within a source.

The present work has shown that while there are variations in amino acid ratios between samples from the same source, the variation between sources is much larger.

Future work

While the application of amino acid analysis to honey identification has been demonstrated it is not recommended that the presently available data should be used as a basis for the four areas. This is because the samples used were from commercial sources, Davies (1975a) and it is not known how accurately they represent the areas. The extent of seasonal and annual variation also needs to be investigated.

Further applications

Amino acids are present in most foods and they have been proposed for determining authenticity in a number of cases: for citrus products, Lifshitz & Stepak (1971) and Vandercook & Price (1972); for syrups, Johnson, Corliss & Fernandez-Flores (1971); for fruit content, Goodall & Scholey (1975). It is possible that these and other commodities could utilize the method proposed for honey. The application of the method, to spearmint oils and animal fats, using variables other than amino acids has been previously demonstrated (Davies, 1975b). Thus, the method may also be of use for commodities which do not contain appreciable quantities of amino acids.

Acknowledgments

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Microbial succession in experimental soy sauce fermentations

F. M. YONG* AND B. J. B. WOOD

Summary

Conditions for the small-scale preparation of soy-sauce are described. The first stage of the fermentation, growing the mould *Aspergillus oryzae* on a mixture of soy beans and wheat flour, followed standard procedures. In the second stage, the soy mash or moromi stage, a variety of conditions were tested. Results showed that a two-step fermentation occurs, first lactic acid bacteria, then yeast growing in the mash. Good yeast growth in the very salty mash depends upon the provision of acidic conditions.

Introduction

Soy-sauce fermentation is a two-stage process. It has been practised in the East for thousands of years. For a recent review of its long and interesting history the reader is referred to Yong & Wood (1974). It is but one of a range of fermentations of vegetable foodstuffs in the Oriental cuisine (Wood & Yong, 1974) most of which are but little known in the Occident, although the work of Hesseltine (e.g. Hesseltine, 1965) has done much to correct this deficiency. The fermentation involves the biochemical activities of three types of micro-organism; viz, moulds, bacteria and yeasts. The first stage is done by growing *Aspergillus oryzae* or *Asp. soyae* on soy beans or a mixture of soy beans and wheat, depending on the local practice. The extracellular amylases and, more importantly, the proteases, hydrolyse the polysaccharides and proteins in the raw materials. When mould growth has reached the desired level, the mixture of soy bean and wheat covered by the mould mycelium (called Koji) is placed into 18% w/v sodium chloride solution. The mixture (soy-mash or moromi as it is normally called) undergoes lactic acid bacteria and yeast fermentations for at least one year at ambient temperatures to give a good quality product. In our studies we have employed a temperature of 40°C for the soy-mash fermentation, following the procedure advocated by S. T. Tan (personal communication). Under these conditions, a good quality soy-sauce is obtained in about one month.

* Present address: Singapore Institute of Standards and Industrial Research, 179 River Valley Road, Singapore 6, Republic of Singapore.

Authors' address: Department of Applied Microbiology, University of Strathclyde, George Street, Glasgow G1 1XW.

It has been firmly established that the soy-sauce fermentation is started by a mould. However, there are conflicting reports as to whether the lactic acid fermentation occurs before or after the yeast fermentation (Yong, 1971). It will be shown in this report that lactic acid bacteria will grow up rapidly in the soya-bean-wheat mixture after the addition of salt solution, hence lowering the pH. As the pH drops below 6·0 the bacteria start to decrease in number. Yeast fermentation will then begin when the pH of the mash is acidic enough (4·0–5·0) for the yeast to grow.

Materials and methods

Purity and source of chemicals

All inorganic chemicals used were of British Drug House 'Analar' Grade with the exception of the sodium chloride used for soy mash which was of 'Laboratory Reagent' Grade.

Soy beans (*Glycine max*) were generously donated to us by Clyde Oil Products Ltd of Glasgow.

The organic materials used were always of the highest grade readily available.

Microorganisms

(i) *Aspergillus oryzae* NRRL 1989
(ii) *Lactobacillus delbrueckii* NRRL B-445
(iii) *Saccharomyces rouxii* NRRL Y-1096

All the microorganisms used were pure culture strains.

Media and culture methods

The mould was maintained on rice–glucose agar, containing finely-ground, polished, long-grain rice (50·0 g), glucose (20·0 g), Oxoid No. 3 agar (20·0 g) and distilled water to 1 litre. Slopes of this medium were inoculated with mould spores, then incubated at 30° for nine days, by which time profuse sporulation had occurred. Stock cultures were then stored in the refrigerator for three months or until required.

The media used for the yeast and bacterium were variations on a basic broth with the composition malt extract (3·0 g), yeast extract (3·0 g), glucose (30·0 g), peptone (5·0 g), distilled water to 1 litre. The yeast was maintained on slopes of medium solidified with Oxoid No. 3 agar (25·0 g per litre), and with the glucose concentration reduced to 10·0 g per litre. The bacterium was maintained on basic medium plus sodium chloride (5·0 g per litre). For enumerating organisms from soy mash, agar (30·0 g), sodium chloride (50·0 g) and sodium propionate (2·5 g) were added to each litre of medium. In the bacterial count medium, the concentration of glucose was reduced to 3·0 g per litre, while for yeasts the medium was acidified to pH 4·5 with hydrochloric acid.

L. delbrueckii was sub-cultured into a 10 ml portion of broth, incubated at 40° for three to four days, then stored at 4° until required. Sub-culturing was carried out every two weeks. In order to obtain inocula for the soy mash stage of soy sauce fermentation, it was essential to 'train' the organisms to grow at high salt concentration; 'untrained' cultures did not grow when inoculated directly into media of high salt content. Steps of 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18% w/v salt were employed. At each transfer to medium of increased salt concentration, the organism showed an extended lag period before commencing growth. Thereafter sub-cultures into fresh medium of the same salinity displayed a normal lag period, but a reduced growth rate. The 'trained' culture was then routinely maintained in the 18% salt medium, incubating at 40° for five or six days then storing at 4°. Sub-culturing was done at weekly intervals.

The yeast was routinely maintained on slopes cultured at 30° for three days, then stored at 4°; sub-culturing was carried out every four weeks.

For use in the soy mash stage, the yeast was 'trained' by passage through portions of liquid maintenance medium containing gradually increasing amounts of salt. Steps employed were 2, 4, 8, 12, 15 and 18% w/v of salt. In the presence of salt, the pH of the medium had to be adjusted to 4·5 and the incubation temperature and period had to be increased to 40° and four to five days respectively in order to obtain satisfactory growth. 'Trained' cultures were maintained both on broths and slopes of MGYP + 18% salt, and were sub-cultured every two weeks.

pH Measurements

The pH value of soy-mash was determined using narrow range (B.D.H.) pH paper. All other pH measurements were made on a Pye pH meter.

Sterilization

Media for bacteria and yeast counts, and for growing up cultures to be used as inocula were sterilized by autoclaving at 15 p.s.i.g. for 15 min.

All apparatus was autoclaved at 15 p.s.i.g. for 15 min, except for materials made from Perspex, which were sterilized by soaking in 2% Domestos solution for 3 hr. The disinfectant was rinsed off with sterile water.

Preparation of Koji

Whole soy beans (60 g) were weighed into an Erlenmeyer flash and soaked in water (300 ml) for 12 hr at 30°; the water was changed every 3 hr. Next the hulls were removed from the soaked soy beans, then the beans were autoclaved at 10 p.s.i.g. for 1 hr to cook and sterilize them. The soy beans so prepared could be stored in the refrigerator at 3–4° for up to a week before use. This process, the treatment of the wheat flour and their mixing all follow current commercial practice as closely as possible.

Wheat flour (60 g) was placed in two Petri dishes (1·5 × 16 cm), covered and sterilized by dry heat at 150° for 12 hr.

The prepared soy beans and wheat flour were thoroughly mixed under aseptic conditions, until all the beans were covered with a layer of wheat flour, and then inoculated with mould spores as follows, employing sterile distilled water at all stages.

The conidia were rinsed from a slope culture of *Aspergillus oryzae*, and this suspension was filtered through a pad of sterile absorbent cotton wool in order to remove mycelium

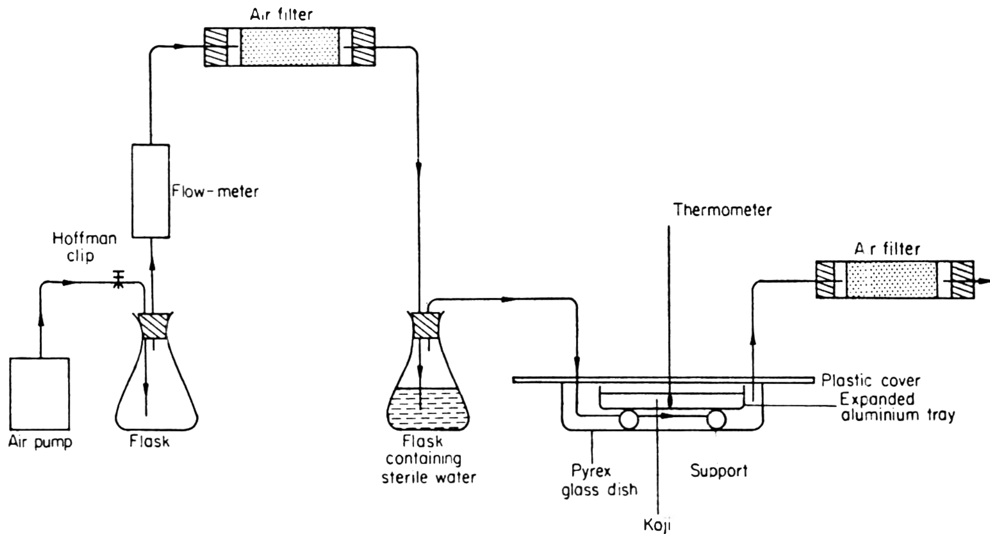

FIG. 1. Apparatus for the preparation of Koji. → Indicates direction of air flow.

fragments. The conidia were washed aseptically four times, centrifuged each time, then finally suspended in water at 2×10^8 spores per ml. Spore suspension (20 ml) was added to the mixture of beans and wheat, and the whole was thoroughly mixed. The inoculated mixture was transferred under aseptic conditions to sterile trays (14 × 10 × 3 cm deep) made of expanded aluminium.

The aluminium tray of Koji was placed in a Pyrex glass dish, and supported on two test-tubes as shown in Fig. 1. The whole apparatus, except for the air-pump, empty Erlenmeyer flask, and flow-meter had been previously steam sterilized.

The whole apparatus was placed in a 30°C constant temperature room and the Koji was incubated at this temperature for 72 hr. After 20 and 40 hr the Koji was mixed using a sterile spatula and aseptic procedures.

Incubation of soy-mash

Mature (i.e. 72 hr old) Koji (water content 35% w/v) was transferred aseptically into a flask. Sterile 18% w/v sodium chloride solution (240 ml) was added to it, and

lactic acid, *Sacch. rouxii*, *L. delbrueckii* were introduced as required. Organisms were added as fresh cultures of 'trained' cells grown on the appropriate medium (10 ml).

The soy-mash was thoroughly mixed with a sterile glass rod and the flask plugged with a sterile, non-absorbent cotton wool plug before incubating at 40° for one month. The flask was occasionally shaken to mix the contents, and was stirred thoroughly before samples were removed. Care was taken to minimise aeration when stirring or sampling.

Viable counts of microorganisms on soy mash

An approximate 1–2 g sample was taken under aseptic conditions and put into a tared, sterile, 50 ml Erlenmeyer flask using a flame-sterilized spatula. The exact weight of the sample was noted.

A sterile solution of peptone (0·1%) and sodium chloride (5%) was added to the sample. The flattened end of a sterile glass rod was used to break up the soy beans into very small pieces. Any soy-mash sticking to the glass rod was rinsed down with a little salt peptone solution. The flask was then stoppered with a sterile rubber stopper and shaken on a Griffin Flask Shaker (Serial No. 9020, by Griffin and George Ltd) operated at medium speed for 10 min in the cold-room. The sample was then transferred into a sterile 50 ml measuring cylinder and made up to 30 ml with salt peptone solution, and serial tenfold dilutions of the sample were then prepared, using salt peptone solution.

For bacterial counts, a diluted sample (1 ml) was introduced into each sterile Petri dish, followed by the addition of melted bacterial count agar medium (15 ml) at 50°. After mixing, the agar was allowed to solidify then another layer of liquid medium at 50° was poured over it. This was to encourage growth of lactobacilli by creating a microaerophilic condition. Triplicate plates were set up at each dilution.

For yeast counts, an inoculum of 0·05 ml for each dilution was introduced on to the surface of yeast count agar plates as five separate droplets. The agar plates had been previously dried at 37° for 3–4 hr.

The Petri plates for enumerating *L. delbrueckii* and *Sacch. rouxii* were incubated at 40° for five days and two days, respectively.

Under these conditions, the level of sodium propionate contained in the counting media was adequate to suppress growth of mould spores but had no effect on the viability of the yeast or bacteria, preliminary experiments having shown that mould spores remained viable throughout the moromi stage.

Results

In order to examine the interaction between the yeast and lactic acid bacterium, a number of different mashes were prepared, using soy mash prepared from the same batch of Koji. These were:

(i) uninoculated mash to serve as control;

(ii) mash inoculated with both organisms;
(iii) mash inoculated with yeast alone;
(iv) as (iii) but with pH adjusted to 4·5, with D-L- lactic acid;
(v) mash inoculated with lactic acid bacteria alone.

Samples from each batch were taken as required. At the same time, samples for chemical analysis were taken; the results of these studies will be reported in a later paper.

Changes in pH of soy-mash with no yeast or bacterium

Samples of soy-mash with neither yeast nor bacterium added were taken at the start and end of the fermentation period. They were plated out to test for the presence of yeast and bacteria. No microorganisms were detected.

The pH of this soy-mash began to decrease from 6·5 after four days' incubation at 40°. The pH reached 4·5 after eighteen days and thereafter remained steady at this value. This shows that even without the presence of any detectable bacteria or yeast the pH would still drop to 4·5 eventually, though this drop in pH was slower than in mash with yeast and/or bacteria present (as will be discussed later).

Viable counts of Sacch. rouxii *and* L. delbrueckii *in soy-mash inoculated with both these organisms*

The initial number of *L. delbrueckii* per gram dry weight of soy-mash was $6{\cdot}8 \times 10^6$ and it increased to $2{\cdot}5 \times 10^7$ after two days (Fig. 2). The number of bacteria remained at this level for only two days before it began to decrease. At the end of thirty-one days the viable count was about $1{\cdot}0 \times 10^4$, some 700 times lower than the counts at zero time.

Figure 2 also shows that from the start the number of viable *Sacch. rouxii* began to decrease from an initial count of $4{\cdot}6 \times 10^7$ until it reached a minimum of $1{\cdot}95 \times 10^5$ per gram dry weight of soy-mash at ten days. It then increased to $7{\cdot}55 \times 10^7$ on the fourteenth day of incubation and remained around this figure until the end of the fermentation period.

The number of *Sacch. rouxii* began to increase when the pH of the moromi had dropped to 5·0. The pH started to decrease after two days of incubation. This drop in pH occurred two days earlier than that of soy-mash without any bacterium or yeast.

Viable counts of Sacch. rouxii *in soy-mash with no* L. delbrueckii

Figure 3 shows that the number of yeasts in the soy-mash decreased from its initial number (of $2{\cdot}4 \times 10^7$ per gram dry weight of soy-mash) until the pH of the mash was 6·0. It then increased gradually until the soy-mash pH reached 5·5. From then on the yeast increased rapidly in number to attain a more or less steady figure of $1{\cdot}4 \times 10^8$ after thirteen days.

The soy-mash was tested for the presence of bacteria; none could be detected at any

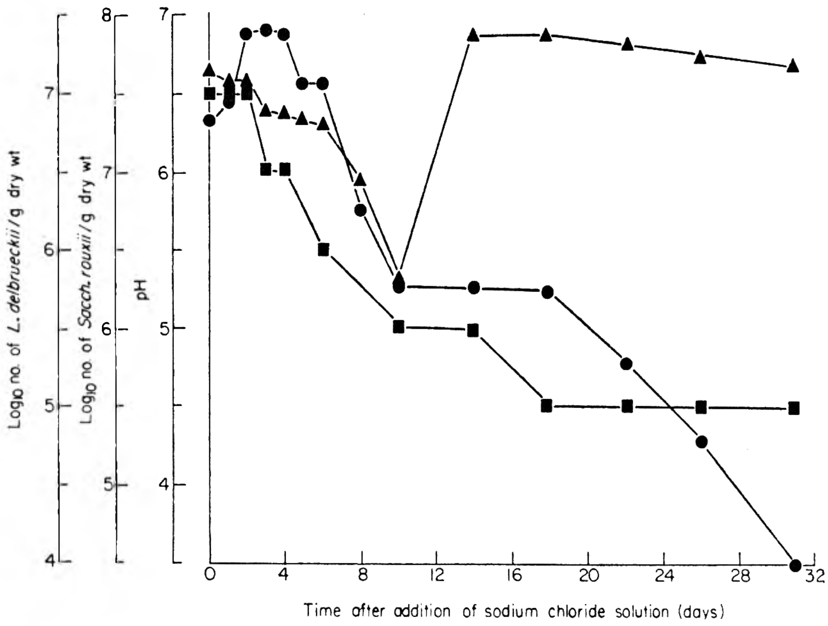

FIG. 2. Viable counts of *Sacch. rouxii* and *L. delbrueckii* in soy-mash inoculated with both of these organisms. ■ pH; ▲ *Sacch. rouxii*; ● *L. delbrueckii.*

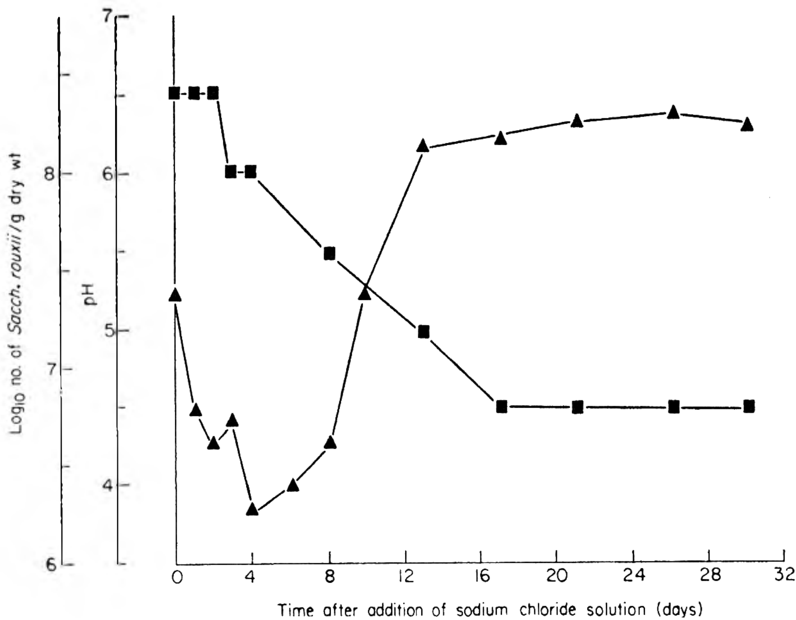

FIG. 3. Viable counts of *Sacch. rouxii* in soy-mash with no *L. delbrueckii.* Key as in Fig. 2.

stage. The pH of this mash behaved in a manner very similar to that of the uninoculated mash.

Viable counts of Sacch. rouxii *in soy-mash adjusted to pH 4·5 with (DL)-lactic acid and with no bacterium added*

For this experiment and the next one a much lighter inoculum was used in an effort to increase the sensitivity of the method. The yeast, after a lag period of one day, increased from $2{\cdot}25 \times 10^5$ per gram dry weight of soy-mash on the day of inoculation to $7{\cdot}0 \times 10^6$ on the second day. The number of yeast cells then increased more gradually, finally reaching a steady value of $1{\cdot}4 \times 10^7$ after twelve days (Fig. 4). The pH of this mash stayed steady throughout the fermentation.

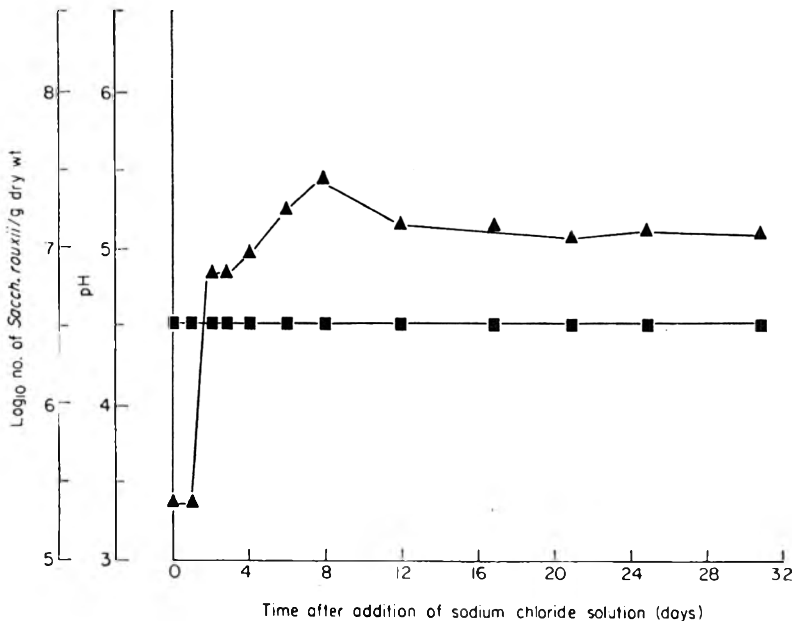

FIG. 4. Viable counts of *Sacch. rouxii* in soy-mash adjusted to pH 4·5 with lactic acid and with no bacteria added. Key as in Fig. 2.

Viable counts of L. delbrueckii *in soy-mash with no yeast added*

The *L. delbrueckii* increased to about ninety times its original number ($3{\cdot}8 \times 10^4$ per gram dry weight of soy-mash) after four days, as shown in Fig. 5. It remained at $2{\cdot}7 \times 10^6$ for only four days before it began to decrease until finally there were only $5{\cdot}6 \times 10^3$ organisms per gram dry weight of sample at the end of thirty-one days.

The fall in pH of the soy-mash began after only one day's incubation and reached a constant value of 4·5 after twelve days. *L. delbrueckii* alone caused a more rapid drop in

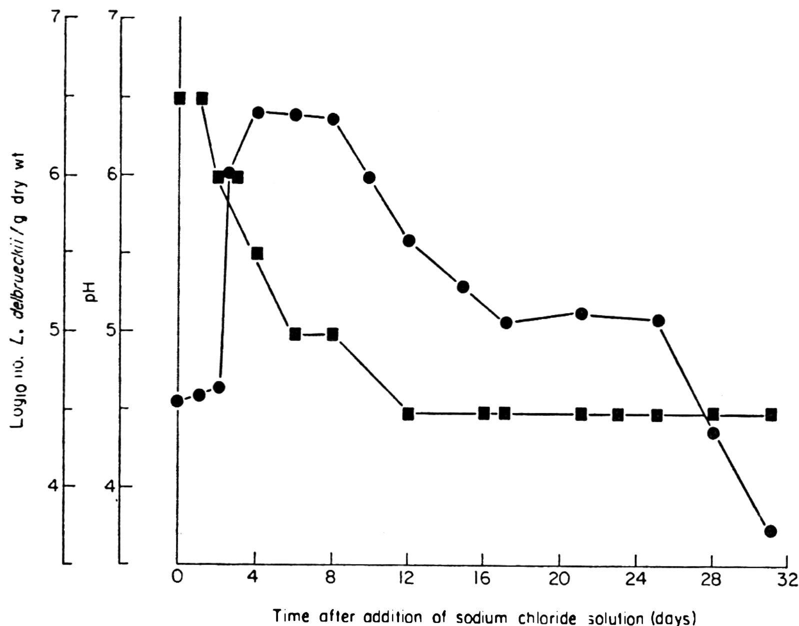

FIG. 5. Viable counts of *L. delbrueckii* in soy-mash with no *Sacch. rouxii.* Key as in Fig. 2.

pH of the soy-mash than occurred when either yeast alone or yeast and bacteria were used to inoculate the soy-mash, despite the fact that a much lighter inoculum had been used in the former case, suggesting that there may be a degree of competition between yeast and bacterium when both are present.

Discussion

The pH of soy-mash was found to drop even though there was neither yeast nor bacteria present. The presence of *L. delbrueckii* only accelerated the drop in pH. Further investigations are necessary before an explanation can be offered as to why the pH of the soy-mash dropped to 4·5 even though neither yeast nor bacterium was added.

It is of course not possible to state categorically that no bacteria or yeast had adventitiously entered the mash to which no microorganisms had been deliberately added. The media which we used for enumerating microorganisms were necessarily selective. We had previously tested five different formulations of media, using samples of soy-mash brought from Singapore as the source of microorganisms (Yong, 1971). We found that only the media and conditions described above gave high counts of bacteria and yeast respectively combined with adequate suppression of unwanted organisms (*Aspergillus oryzae* in both cases, yeasts on the bacterial count plates). We consider it unlikely that organisms which could not be detected on our media would yet grow

significantly in the soy-mash, whose combination of poor gas transfer and high salinity render it a highly selective medium in its own right. Clearly the conditions employed will ensure that the enzymes produced by the fungus will survive intact in the soy-mash, but we do not know the extent to which they are active under these highly saline conditions. None of the extracellular enzymes known to be produced by the mould produce significant amounts of acid. At present the least unlikely explanation of this acidification would seem to involve the activity of intracellular enzymes from the moribund mould mycelium, an area on which we have no evidence either way. During the Koji stage, however, the pH moves slowly upward from an initial value of 6·55, reaching 7·3 after seventy-two hours' incubation; further incubation results in the formation of ammonia (which gives a disagreeable flavour to the finished soy sauce) and the pH finally reaches 7·5.

It has been suggested that the laboratory reagent grade sodium chloride might make an acidic brine, but if that were so, one would expect the pH to be low immediately after mixing the brine and Koji, gradually rising as buffering agents such as proteins diffused out from the solid particles of Koji. This is the reverse of what was actually observed. A satisfactory explanation of the observed decrease in pH therefore eludes us at the present.

When *L. delbrueckii* was inoculated into the soy-mash it grew up quite rapidly to reach a maximum number of organisms, and stayed at this maximum value for a short time, during which the continued production of acid was indicated by the continuing decrease in pH value. As the pH approached 4·5 the viable number began to decrease rapidly, presumably due to the accumulation of acid metabolic products in the soy-mash.

As the pH of the soy-mash dropped to below 5·5 the yeast began to increase in number rapidly until it reached a maximum value. It remained at this level up to the end of the fermentation period. In the soy-mash which had its pH adjusted to 4·5 the *Sacch. rouxii* did not show an initial decrease in numbers. However, there was a lag period of one day before it grew up vigorously.

The above results show that the yeast will only grow up vigorously in the soy-mash when the pH has dropped to below 5·5. Onishi (1957) has also found, using a defined medium with a high concentration of sodium chloride, that the 'soy-yeasts' could only grow when the pH lay between 4·0 and 5·0.

Sakaguchi (1959a, b) found that the role of *Pediococcus soyae* in soy-mash is entirely concerned with the production of acid. Similarly *L. delbrueckii* accelerates the drop in pH of soy-mash inoculated with it. Putting together the results published by Japanese workers (Onishi, 1957; Sakaguchi, 1959a, b) and those obtained by the authors, it is now possible to specify the sequence of microbial flora in soy-sauce fermentation. The mould grows up in the Koji stage and its growth is terminated by the addition of 18% w/v sodium chloride solution to the Koji. Growth of the mould is presumably inhibited by a combination of the high salt concentration and the anaerobic conditions

which must rapidly develop in the soy-mash. Mould spores retain their viability in the soy-mash and if sodium propionate was omitted from the media used for enumeration of the yeast and bacteria, which contained only 5% sodium chloride, they would readily germinate and rapidly overgrow the plate.

Lactic acid bacteria will grow up rapidly in the freshly prepared soy-mash, lowering the pH, and thus permitting yeast growth to begin.

It may reasonably be asked if our conditions have much in common with those found in a traditionally conducted soy sauce fermentation. We had hoped to determine if this was so by examining samples of authentic soy-mash brought by air from Singapore. Unfortunately, despite every effort to keep them cool during the journey, the samples (which were intended to represent different stages in the fermentation) had evidently continued fermenting and the samples showed a similar pattern in the numbers of yeasts and bacteria, both types of organism being high in a sample taken from a week-old soy-mash (10^8 yeasts, 7×10^9 bacteria per gram dry weight of mash) and declining steadily to much lower numbers (500 yeasts, 10^7 bacteria per gram dry weight of mash) in samples taken from a five-week-old mash. Among the yeasts no differences in colonial form were observed, but on the bacterial count plates two types of colony were observed, about 90% being small lenticular colonies, yielding very short Gram-positive rods; the remaining 10% of colonies were rather larger and yielded Gram-positive cocci.

It will thus be seen that our results on the laboratory-made soy-mash were in reasonable accord with those from the samples of authentic soy-mash.

Another line of evidence which may be adduced to support the contention that the results reported here are germane to what happens in the normal soy sauce fermentation is provided by organaleptic assessment of the quality of the material produced in our fermentation. In the time available for this work we were not able to conduct formal and properly designed taste-panel experiments, but one of us (Y.F.M.) is familiar with it as an everyday article of diet, and we also secured the view of other oriental students at Strathclyde University. There was broad agreement that all of the fermentations involving yeast gave a good quality product. Generally the sauce from the yeast plus lactic acid bacteria was regarded as the best product, with the one from the mash soured with lactic acid then fermented with yeast, coming a very close second. The materials from a lactic acid bacteria fermentation without yeast, and from the sample not inoculated with any organism at all were alike described as lacking any soy sauce aroma or real character, being bland, inoffensive, characterless and generally like much of the inferior grades of soy sauce which are widely sold in the Occident.

Finally, we may ask what light these results can throw on events in the traditional process, where the new soy-mash is either primed with a little of an old mash, or is even left to develop a microbial flora from chance contamination. Our results show that an old mash is likely to harbour but few lactic acid bacteria, thus delaying the development of conditions favourable for yeast growth, although this is likely to be compensated for in part at least by the self-souring of the mash which we observed

in the uninoculated samples. Clearly, therefore, this factor allied with the rather lower and certainly more variable incubation temperature experienced by the mash will go a long way towards explaining why we could get an acceptable soy sauce in so much shorter a time than is apparently needed in the traditional process.

Acknowledgments

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The ultrastructure of unmodified and chemically-modified tapioca starch granules as revealed by the freeze-etching technique

J. E. ALLEN,* L. F. HOOD* AND M. V. PARTHASARATHY†

Summary

The ultrastructure of tapioca starch granules was studied using the freeze-etching technique in an attempt to overcome the problems associated with the other methods of preparation for electron microscopy. A chemically-modified tapioca starch was also studied to ascertain whether chemical modification affected granule structure. Variations in fracture faces were observed in both modified and unmodified granules suggesting that organization within the granules was not homogenous. Particles were present on granule fracture faces. The size range of the particles was 4 to 10 nm in the unmodified and 6 to 15 nm in the modified granules. These observations were similar to those found by others in freeze-etched starch granules and must be considered to be of some structural significance. In contrast to the fracture face, the outer surface of the granules was smooth and particles were not evident.

Introduction

The structure of the starch granules has been of interest to the biologist in studying granule synthesis and to the food scientist in evaluating the textural properties of starches in processed foods. During cooking, starch granules swell and the viscosity of the suspension increases. By chemically modifying the starch prior to cooking, its swelling and viscosity-affecting properties can be changed (Wurzburg & Syzmanski, 1970). Srivastava & Patel (1973) demonstrated that different cross-linking reagents, such as sodium trimetaphosphate, epichlorohydrin and phosphorous oxychloride, under different pH conditions had an effect on pasting viscosities of tapioca starch. The effects of cross-linking can be quite marked and in some cases can inhibit gelatinization (Strivastava & Patel, 1973). Although there have been some chemical studies, there have been few reports on how modification affects the ultrastructure of the tapioca starch granule. Hood and associates (Hood, Seifried & Meyer, 1974; Hood & Seifried,

Authors' addresses: *Department of Food Science and †Section of Genetics, Development and Physiology, Cornell University, Ithaca, NY 14853, U.S.A.

1974) noted the changes in the cooking and storage of tapioca starch/milk gels but also highlighted the problems involved with the preparation of this material for electron microscopy.

The ultrastructure of isolated starch granules has been difficult to discern, partially because of methodology limitations. Starch is not easily infiltrated by embedding media and artifacts can occur such as granule shrinkage (Hood *et al.*, 1974) and folding (Mussulman & Wagoner, 1968; Gallant & Guilbot, 1971) when using the thin sectioning technique for transmission electron microscopy studies. Scanning electron microscopy also has its limitations (Chabot, Hood & Allen, 1976). It is difficult to achieve routine resolution better than 20 nm with this material.

The freeze-etching technique was used by Mühlethaler (1965) and Leonard & Sterling (1972) for the study of potato starch granules. Freeze-etching reveals some structural characteristics which are not revealed by any other microscopy technique. Fracturing reveals the interior of the granule and etching exposes some outer surfaces.

In this paper, we report on the application of the freeze-etching technique to the investigation of the ultrastructure of both unmodified and chemically-modified tapioca starch.

Materials and methods

The starches used were an unmodified tapioca starch and a modified tapioca starch, hydroxypropyl distarch phosphate (Stein, Hall & Co. Inc., NY, U.S.A.). An 8% (w/v) suspension of each starch was prepared at 4°C in either glass-distilled water, 25% glycerol in glass-distilled water or 100% glycerol. Each suspension was centrifuged for 4 min at 750 ***g*** and three-quarters of the supernatant was removed. The precipitated granules were transferred in droplets onto Balzers bored gold specimen holders, frozen in liquid Freon '12' and stored in liquid nitrogen.

Freeze-etching was carried out in a Balzers BA360M instrument according to the method of Moor & Mühlethaler (1963). The specimens were fractured at −102°C; etching times were 10 sec for the material frozen in water, and 2 to 4 min for the material frozen in 25% glycerol. The material frozen in 100% glycerol was shadowed immediately after fracturing.

The platinum/carbon replicas were cleaned with 70% sulphuric acid overnight, rinsed twice with distilled water, cleaned again for 3 hr with 5% sodium hypochlorite solution, and rinsed three times with distilled water. The replicas were then mounted on formvar-coated 75 × 300 mesh copper grids and viewed with a Philips EM201 electron microscope at 60 or 80 kV.

Results

Fracture faces of both unmodified and chemically-modified granules differed

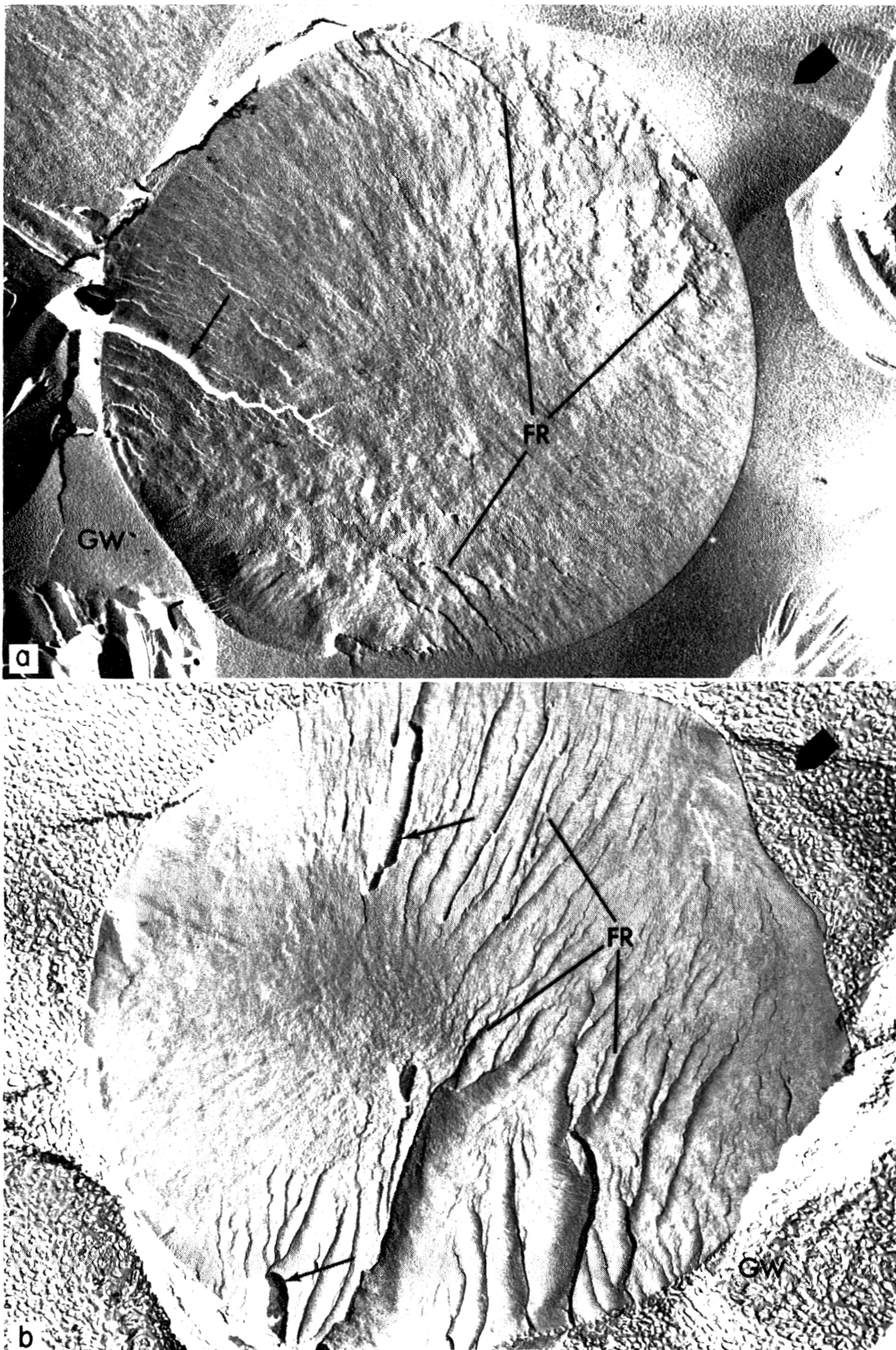

PLATE 1. (a) Fracture face of a modified tapioca starch granule in 25% glycerol/water (GW) showing a few small fracture ridges (FR). The large white line (arrowed) is a tear in the replica. The marker in the top right corner indicates the shadowing direction. ×9200. (b) Fracture face of a modified tapioca starch granule in 25% glycerol/water (GW) showing large fracture ridges (FR). The arrows mark those ridges where the replica has folded. The marker in the top right corner indicates the shadowing direction. ×9200.

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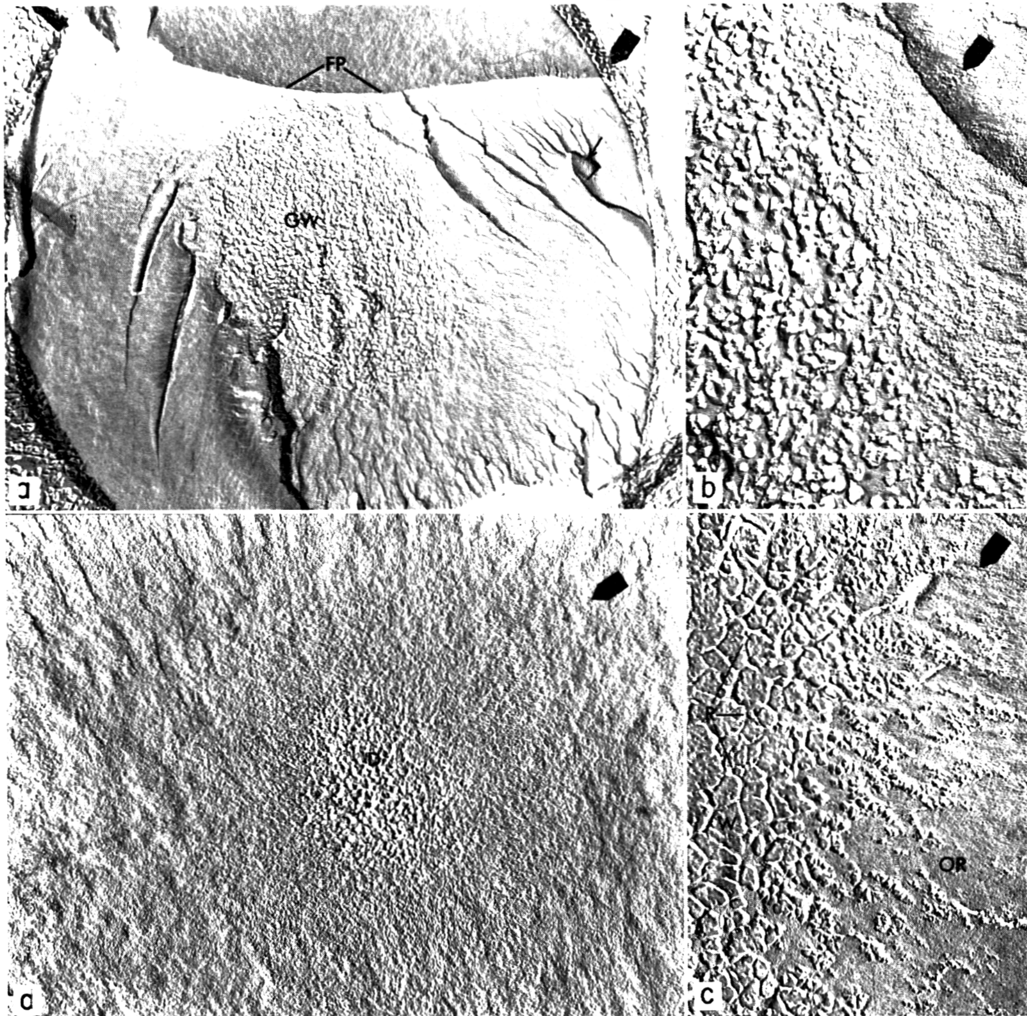

PLATE 2. (a) Fracture face of a modified tapioca starch in 25% glycerol/water (GW). A change in the fracture plane is evident at (FP). The central pattern of glycerol/water indicates its infiltration into the granule. A fracture ridge has caused a fold in the replica (arrow). The marker in the top right corner indicates the shadowing direction. ×4980. (b) Higher magnification of the central pattern on the fracture face of the modified tapioca starch granule seen in (a). The marker in the top right corner indicates the shadowing direction. ×15 000. (c) Fracture face of modified tapioca starch granule in water (W), showing the transition from the outer regions of the granule (OR) to the central reticulate network (R). The marker in the top right corner indicates the shadowing direction. ×15 480. (d) Fracture face of a modified tapioca starch granule in water showing the central region of depressions (D). The marker in the top right corner indicates the shadowing direction. ×16 200.

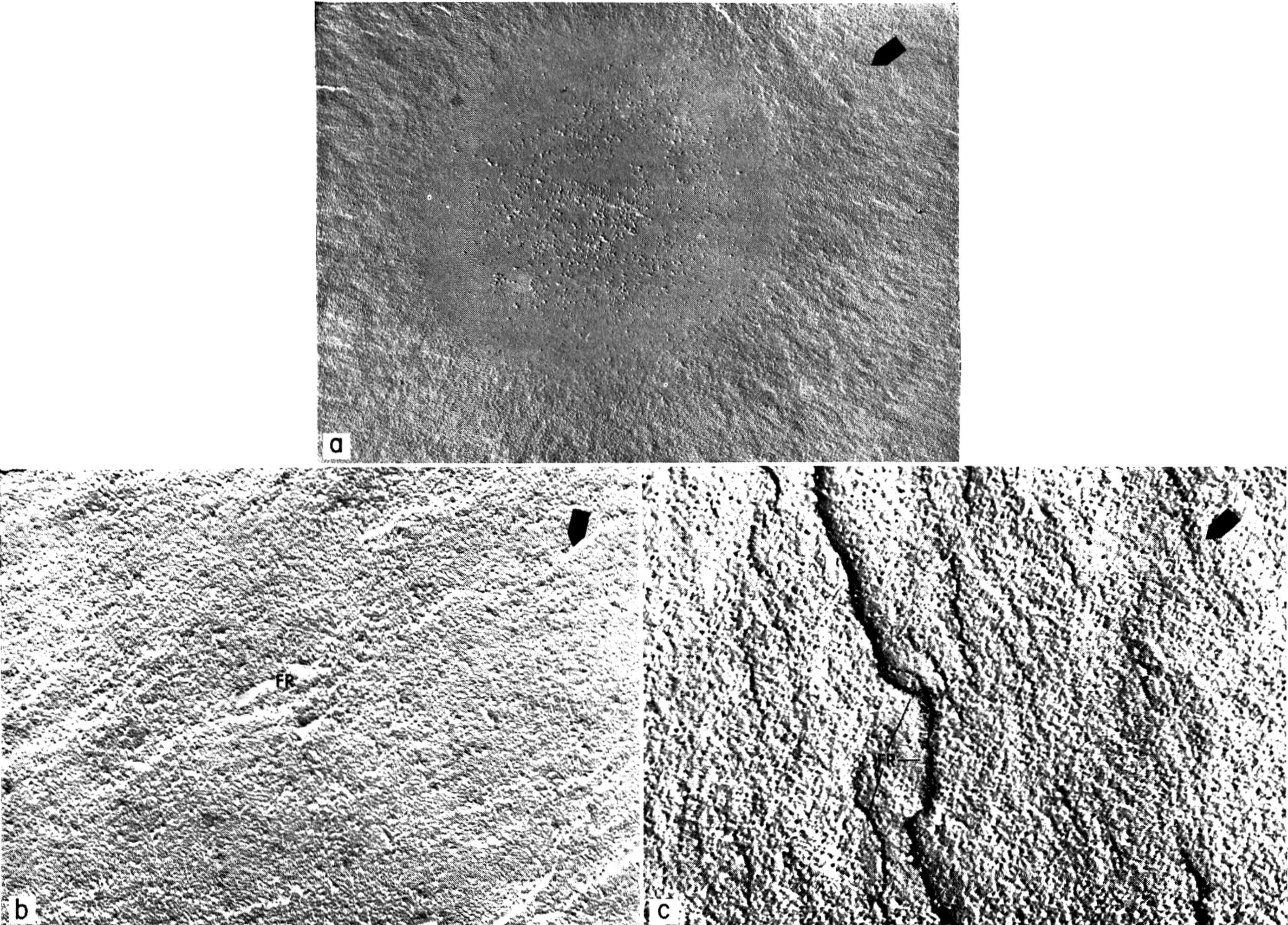

PLATE 3. (a) Fracture face of an unmodified tapioca starch granule in 25% glycerol/water showing the central region. The marker in the top right corner indicates the shadow direction. × 12 000. (b) Particulate fracture face and small fracture ridges (FR) of an unmodified tapioca starch granule in water. The marker in the top right corner indicates the shadow direction. × 53 400. (c) Particulate fracture face and fracture ridges (FR) of a modified tapioca starch granule in water. The marker in the top right corner indicates the shadowing direction. × 53 400.

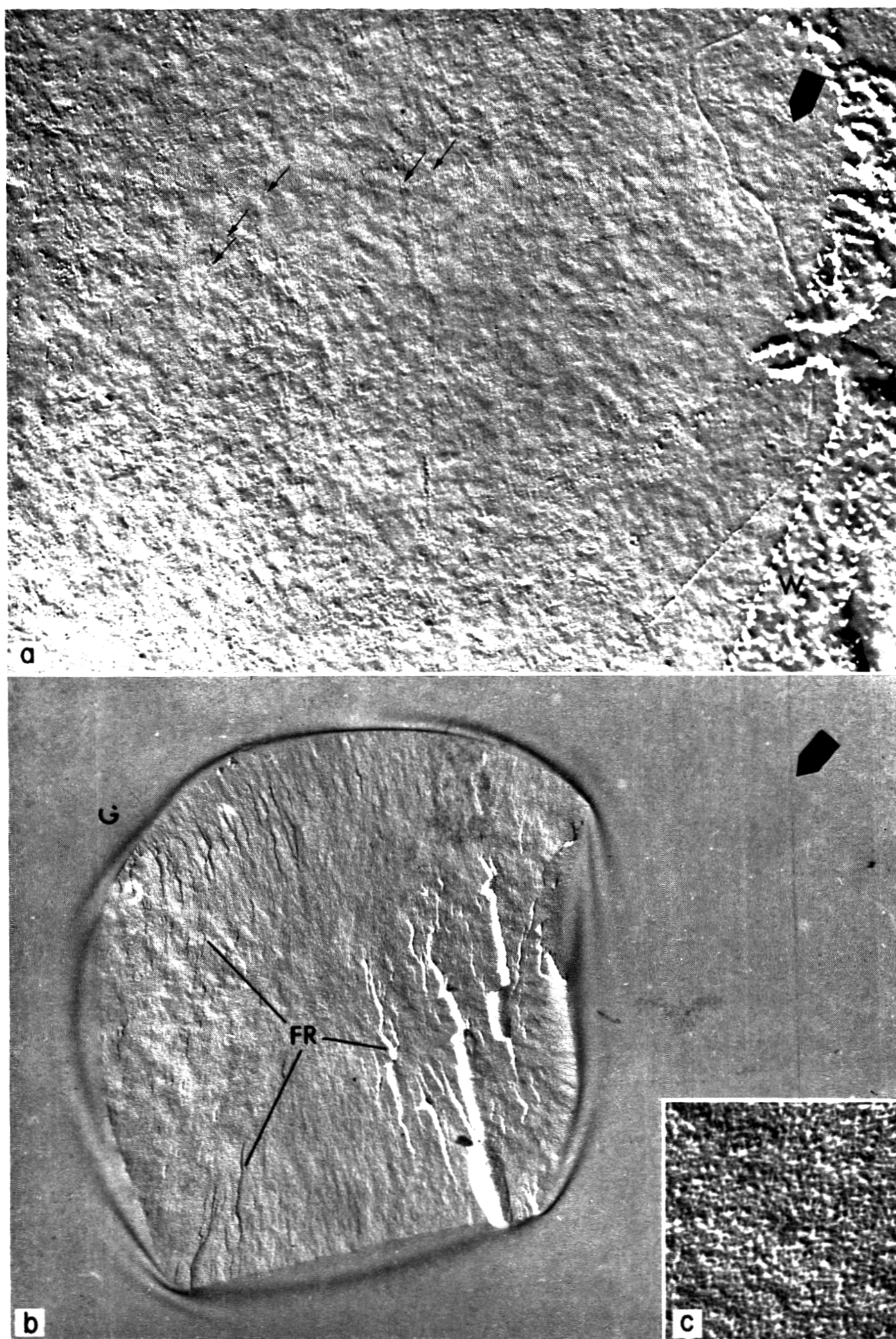

PLATE 4. (a) Outer surface of a modified tapioca starch granule in water (W), revealed after etching, showing the faint parallel lines (arrowed). The marker in the top right corner indicates the shadowing direction. × 32 000. (b) Unetched fracture face of unmodified tapioca starch granule in 100% glycerol (G), showing particulate surface and fracture ridges (FR). Some ridges have caused the replica to tear (arrow). The marker in the top right corner indicates the shadowing direction. × 7600. The insert (c) is a high magnification of the particulate fracture face of this granule. × 59 200.

morphologically. In some, the fracture faces revealed only a few fracture ridges (Plate 1(a)) whilst in others, ridges were larger and more prominent (Plate 1(b)). It is common for the morphology of glycerol/water to vary. Although all the starch samples were frozen in liquid freon, some variation probably existed in freezing rates throughout the sample. This would lead to small differences in the freeze-etch pattern of glycerol/water. Although we have observed these differences, the number and size of the fracture ridges in the granule were independent of the morphology of the glycerol/water.

All the fracture faces of the granules had a fine particulate appearance, regardless of the medium in which the granules had been frozen. Some of the modified granules showed the presence of glycerol/water in the central region which had the same freeze-etch pattern as that which surrounded the granule (Plate 2(a)). There was no distinct boundary between the frozen glycerol/water in this central region and the surrounding starch material in the rest of the granule (Plate 2(b)). However, when the modified granules were frozen in water alone, the ice in this central region was enclosed by a reticulum (Plate 2(c)). The reticulum network was continuous with the surrounding granule material and had the same particulate fracture face. The area of ice enclosures within the reticula appeared to become smaller as they neared the unaffected outer regions of the granule. In some of the modified granules there was only a small area of depressions in the central region of the granules when frozen in water (Plate 2(d)).

The unmodified granules exhibited a different central region than observed in the modified granules, regardless of the medium in which they were frozen (Plate 3(a)). The central region appeared to be smooth and about 3–5 μm in diameter. No water or glycerol/water was evident in the central region. A few particles were evident in this region but were not as uniformly distributed as in other areas of the granule's fracture face.

Outside the central region, both types of granules had a fine particulate appearance (Figs 8 and 9). The size range for the particles was 4 to 10 nm for the unmodified granules and 6 to 15 nm for the modified granule.

The outer surfaces of both modified and unmodified granules were similar. In contrast to the fracture faces (Figs 1–9), the outer surfaces did not have a particulate appearance but seemed to be slightly undulated with faint parallel lines visible in most areas (Fig. 10).

Discussion

The ridges observed on most of the fracture faces (Figs 1–5, 8, 9 and 11) may not be structural features of the granule. In yeast cells Moor (1966) identified the fracture ridges as an artifact formed during fracturing as a result of the differing fracture characteristics of the cell components. The fracture ridges on the fracture face of the apparently compact starch granule may indicate the presence of structural variations within the granule which might not otherwise be visible. Thus the existence of several

types of fracture faces (Figs 1 and 2) suggests that the starch granule is not structurally homogeneous. A relatively smooth fracture face (Fig. 1) probably results from a fracture plane of less resistance within the granule. On the other hand fracture faces with large prominent fracture ridges (Fig. 2) suggest a fracture of planes or areas of higher resistance within the granule.

Theoretical models for starch granule formation suggest that growth is by apposition producing either an 'onion-like' layering or a radial layering (Badenhuizen, 1969; Banks *et al.*, 1973; Finkelstein & Sarko, 1972; Hall & Sayre, 1971; Sterling, 1968). In this study no distinct layering was observed in any of the fractured tapioca granules.

Particulate fracture faces have also been demonstrated in potato starch granules by Mühlethaler (1965) and by Leonard & Sterling (1972). The size range of the particles (5 to 15 nm) in tapioca starch is similar to that found in potato starch granules. A particulate appearance has been noted in freeze-etched starches in the red alga *Rhodymenia pseudopalmata* (Pueschel, 1975) and in *Smilax aspera* leaf and storage starches (Ferri, 1974). Ferri (1974) suggested that these particles could be a resublimation artifact produced on the fracture face of the starch granule. However, in the present study, a particulate fracture face was routinely obtained from unmodified tapioca starch granules frozen in 100% glycerol where no water was present and there was no etching (Plate 4(b)). Since the size and distribution of the particles observed here were similar to those in earlier studies on starch (Ferri, 1974; Leonard & Sterling, 1972; Mühlethaler, 1965; Pueschel, 1975), it is likely that the particles have some structural significance within the starch granule. The slight increase in the particle size range in the modified granules could be a result of cross-linking between or within the starch molecules caused by the chemical modification process.

The particles observed on the fracture faces could also be the microfibrils viewed in cross-section that others have observed (Sterling, 1971; Leonard & Sterling, 1972; Sterling, 1974). However, there was no indication on any of the fracture faces examined of any microfibrils that were longitudinally oriented. Thus there is little evidence to suggest that the microfibrils reported in potato starch are present in tapioca starch.

A characteristic of all starches is their morphology and birefringent properties when viewed with the optical microscope. Tapioca starch granules have a distinctive birefringent pattern when viewed through cross polarizers. The intersect of the polarization cross corresponds to a central or near-central zone that is more translucent than the rest of the granule when it is viewed with brightfield or phase-optics. Modification of the starch causes some of the granules to lose their birefringent properties. This would indicate some irreversible change taking place in the central region during the modification process. This would permit the infiltration of water into this region and the subsequent formation of a reticulate network. The effect of water infiltration of granules during cooking will be described in a subsequent paper.

Acknowledgment

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Technical note: Precursors of carbonyls in chapaties

S. S. ARYA, K. S. PREMAVALLI AND D. B. PARIHAR

Introduction

A major portion of the wheat produced in the Indian subcontinent is consumed in the form of chapaties which are highly relished by all segments of the population. The chapati baking quality of various wheat varieties grown in India have been investigated to some extent (Austin & Ram, 1971) but information on various factors that influence the flavour and aroma of chapaties is scanty. High sugar and diastatic activity influence the sweet taste of chapaties (Singh & Bailey, 1940; Uprety & Abrol, 1972) whereas high tyrosinase activity has been shown to affect the darkening in the whole-meal dough and thereby the colour of chapaties (Abrol & Uprety, 1970).

Carbonyl compounds have been shown to constitute the major part of the aroma fraction in chapaties (Kannur *et al.*, 1974). The changes in carbonyls that take place during storage of preserved chapaties have also been reported previously (Kannur *et al.*, 1974). However, no information is available about the chemical components and the reactions involved in their formation in chapaties.

Materials and methods

Wheat starch and lipids

Starch was prepared by the cold water washing method (Wolf, 1964). The moist starch was extracted with methanol followed by a chloroform-methanol (2 : 1) mixture to remove adhering protein and lipid residues. Lipids were extracted from wheat flour (straight run 90–95% extraction) by the method of Tsen, Levi & Hlynka (1962). All the solvents used were carbonyl free and were distilled before use.

Chapati baking

The chapaties were baked from wheat starch (100 g) and water (60 ml) both with and without amino acids (0·015 mol; glycine, alanine, leucine, isoleucine and phenylalanine), sugar (0·015 mol; glucose or ribose) and wheat lipids (2·0 g). These sugar and lipid levels were selected because 90–95% extraction flour contains 2–3% total sugars and 2–2·5% total lipids. Starch dough of moderately stiff consistency was prepared by hand and 45 g pieces were rolled in circular discs of 15 cm diameter and baked on a

Authors' address: Defence Food Research Laboratory, Mysore-10, India.

hot plate maintained at 200 ± 15°C for 2 min (1 min each side). Similarly chapaties were also prepared from wheat flour (90–95% extraction).

Isolation and separation of carbonyls as 2 : 4-dinitrophenyl hydrazones (DNPHs)

Freshly baked chapaties were powdered and 50 g samples were extracted with carbonyl free chloroform (200 ml). The chloroform extracts were refluxed with 1% 2 : 4-dinitro phenyl hydrazine (DNP) solution in 2 N hydrochloric acid for 2 hr. The chloroform layer was repeatedly washed with 2 N hydrochloric acid to remove unreacted DNP followed with water and dried over anhydrous sodium sulphate. The above procedure was essentially that of Johnson & El-Dash (1969) employed for the isolation of bread carbonyls. Dried extracts were concentrated, made to a known volume and DNPHs separated on silica gel-G plates, activated at 120°C for 2 hr using carbon tetrachloride : hexane : ethylacetate (10 : 2 : 1) as irrigating solvent. The coloured bands were scraped off and repeatedly extracted with chloroform. The colour intensity and absorption maxima were determined using a Perkin-Elmer Model 124 spectrophotometer. Total carbonyls in chapaties were determined by the method of Henick, Benka & Mitchell (1954) using carbonyl free benzene for extraction. The concentration of amino acids in water extracts of wheat flour and chapaties were determined by measuring the free amino groups by trinitrobenzene sulphonic acid method (Satake *et al.*, 1960).

Results and discussion

A typical chromatoplate showing the resolution of carbonyls in chapaties from different doughs is depicted in Plate I. It is interesting to observe that carbonyls of chapaties baked from only wheat starch or wheat starch and glucose remained near the base line and gave a blue colour with alcoholic potassium hydroxide spray, an indication of mostly dicarbonyls. These combinations had no detectable faster moving monocarbonyls except very feeble bands corresponding to acetaldehyde and acetone. Various monocarbonyls formed from the thermal degradation of carbohydrates have been reviewed by Fagerson (1969) and include homologues of aldehydes and ketones having one to six carbon atoms and furfural derivatives. But these do not seem to be formed in appreciable quantities from glucose or starch during chapati baking.

Mechanisms of formation of dicarbonyls from sugars have been discussed by Hodge (1967). Dicarbonyls arise from the fission products of 2-ketoses with subsequent dismutation and dehydration leading to pyruvic aldehyde, diacetyl, acetoin, etc. The DNPHs of these compounds remained near the base line in the solvent system employed in the present study.

Chapaties baked from starch, glucose (or ribose) and glycine, alanine, leucine, isoleucine and phenylalanine had major carbonyl bands corresponding to formaldehyde, acetaldehyde, valeraldehyde, isovaleraldehyde and phenyl acetaldehyde respectively.

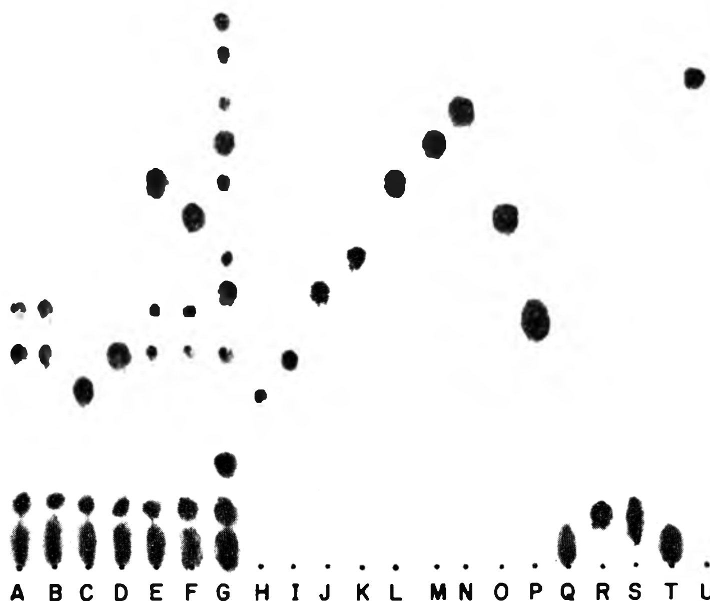

PLATE 1. TLC showing the separation of DNPHS of carbonyls of chapaties prepared from starch (A), starch + glucose (B), starch + glucose + glycine (C), starch + glucose + alanine (D), starch + glucose + leucine (E), starch + glucose + phenylalanine (F), and starch + glucose + lipid (G); H to U, DNPHS of pure compounds; formaldehyde (H), acetaldehyde (I), propanal (J), butanal (K), pentanal (L), Hexanal (M), 2,4-decadienal (N), phenylacetaldehyde (O), acetone (P), diacetyl (Q), hydroxymethyl furfural (R), pyruvic aldehyde (S), acetoin (T) and nonanal (U).

(*Facing p.* 544)

These carbonyls are formed from the respective amino acids by Strecker degradation. This is further supported by the decreases in the concentrations of amino acids in model systems and of the free amino groups in the water soluble fraction of wheat flour during chapati baking (Table 1). The concentrations of free amino groups in salt soluble and

TABLE 1. Percentage decrease in amino acids during baking

Amino acids	Percentage decrease
Glycine	9·2
Alanine	9·5
Leucine	22·9
Phenylalanine	22·1
Wheat flour water solubles*	17·0

* Per cent decrease in amino groups.

70% alcohol soluble proteins did not change significantly during chapati baking, and therefore these may not be involved in the formation of carbonyls. The losses in amino acids during chapati baking varied from 9 to 22%. Comparatively losses were higher in the combinations containing leucine and phenylalanine than containing glycine or alanine. The relative concentration in those of the respective Strecker's aldehyde was also higher in the combinations containing leucine and phenylalanine (35–37%) than the glycine and alanine combinations (19–22%). The concentrations of total sugars has been found to decrease during the baking stage in chapati preparation (Uprety & Abrol, 1972) indicating their involvement in the sugar–amino acid interaction. Formation of carbonyls from sugar–amino acids interactions during bread baking has been well documented (Linko & Johnson, 1963; Salem, Rooney & Johnson, 1967). The composition of various carbonyls produced during bread baking is influenced by the relative concentrations of various amino acids in the dough, and the same is true in chapaties also as observed in the present study in model systems.

The carbonyl composition of chapaties baked from starch and wheat flour lipids is more complicated (Plate I) and consisted of both saturated and unsaturated aldehydes. The major bands identified included acetaldehyde, propanol, butanal, pentanal, hexanal, decadienal and some unidentified spots. All these compounds may result from the degradation of unsaturated fatty acids and have been reported previously in peroxidized lipids. Preliminary investigations on the changes in fatty acids during chapati baking indicated significant losses in the linoleic and linolenic acids (3–4%) of wheat flour lipids, indicating their involvement in the generation of carbonyl compounds. Comparatively wheat flour lipids seem to exert a very pronounced effect on the carbonyl composition of chapaties, in not only generating a large number of additional aldehydes,

but also in retaining the volatile carbonyls formed by other reactions. This is clearly brought out in Table 2 wherein the total carbonyls in chapaties baked from starch-

Table 2. Total carbonyls in chapaties baked from starch, glucose, amino acids and lipids

Ingredients	Total carbonyls*
Starch	1·04
Starch + Glucose	1·16
Starch + Glucose + Leucine	1·76
Starch + Glucose + Lipid	5·92
Starch + Glucose + Lipid + Leucine	8·64

* Expressed as mg of valeraldehyde per 100 g chapati powder/30% moisture.

glucose-leucine-lipids is higher than the sum of the carbonyls of chapaties baked from starch-glucose-leucine and starch-glucose-lipids respectively. The majority of the carbonyl compounds in normal chapaties (Kannur *et al.*, 1974) and those prepared in isolated system from different combinations of ingredients have been found to be similar. This indicates similarity of chemical components and the reactions involved in their formation in both the systems investigated.

Acknowledgments

Thanks are due to Dr H. Nath, Director, Defence Food Research Laboratory, Mysore, for his encouragement.

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Book reviews

Starch and its Components. By W. BANKS and C. T. GREENWOOD.
Edinburgh: Edinburgh University Press, 1975. Pp. xi + 342. £10.00.

The authors have drawn largely on their own extensive researches on starch and its components, amylose and amylopectin. A clear picture is presented of the current state of knowledge in this complicated area which should be easily understood by the large majority of food scientists. The text covers the fractionation of starch into its components amylose, amylopectin and the intermediate forms and the characterization of these molecular species. This includes detailed studies by enzymic hydrolysis, viscosity determinations and iodine binding measurements. The latest theories on the structures of amylose, amylopectin and the intermediate material are presented and discussed in relation to previous ideas.

The main flow of the text from the details of the components to the nature of the actual starch granules is interpreted by a chapter on starch degrading enzymes. This chapter is particularly helpful in interpreting the results of studies on starch by various workers using enzymes from different sources. It helps to outline the mode of action and degradation pattern of the many types of starch degrading enzymes.

Finally the text describes the authors views, on the nature of the structure and organization of the sub-units within the starch granules and the biosynthesis of the granules themselves. Complex areas such as gelatinization phenomena, crystalline structures, varietal differences in size and shape are discussed in the most detailed manner yet published.

This text is clearly superior to the earlier books published on starch chemistry and should become the standard reference book for students and researchers for several years.

R. C. E. GUY

Water Relations of Foods. Ed. by R. B. DUCKWORTH.
London: Academic Press, 1975. Pp. xvi + 716. Price £17.50.

This book consist of thirty-five papers presented at the International Symposium held in Glasgow in September 1974.

The first section of the book, covering six papers, deals with the nature of water in its various forms and basic interactions with carbohydrates, phospholipids, proteins and other constituents of biological systems. This is followed by methods and techniques used in the study of water in foods, e.g. sorption techniques, differential thermal analysis, differential scanning calorimetry, dielectric measurements, nuclear magnetic resonance techniques and electron microscopy.

The third section consisting of five papers, is devoted to the behaviour of micro-

organisms in relation to water activity. This is followed by two sections, each of three papers, on the influence of water on enzyme action, and the effect of water on non-enzymic chemical changes, including non-enzymic browning, free radicals and oxidative reactions.

The sixth section deals with water relationships during freezing and thawing.

The final section, consisting of seven papers, deals with various aspects of the condition and properties of water in relatively moist food materials. Excellent and thorough author and subject indices are also included.

The list of authors reveals that some of the most distinguished international food scientists contributed papers to this symposium. The overall standard of the book is extremely high and it must be regarded as one of the most substantial contributions to the literature food science which has been produced recently. It will, undoubtedly, be a major source of reference for many years to come. The success of the symposium and the book must be attributed, in large measure, to the editor and his enthusiasm for this subject. Food scientists will enjoy reading this book.

S. D. Holdsworth

Protein Nutritional Quality of Foods and Feeds. Ed. by M. Friedman. New York: Marcel Dekker, 1975. Part 1: Pp. xx + 626. £27.50. Part II: Pp. xx + 674. £27.50.

Mendel Friedman has achieved the impossible task of editing the papers from a symposium (A.C.S. Symposium on Chemical and Biological Methods for Protein Quality Evaluation, Atlantic City, 1974) so as to produce a very readable volume, instead of the collection of isolated contributions which normally result from symposium proceedings. The magic formula is to include invited papers in addition to the verbal presentations. This gives continuity so that the reader is automatically led on to the next chapter which follows a logical pattern.

The fifty-five well referenced chapters occupy a total of 1300 pages requiring to be bound in two parts. The first part is devoted to assay methods for whole proteins and specific amino acids and covers chemical, biochemical, physical, enzymic, microbiological and biological techniques; of special note is a chapter devoted to the theoretical estimation of the available energy of amino acids and proteins. Four chapters are concerned with the interpretation and modification of protein efficiency ratio determinations so as to provide more precise information about nutritional quality, a topic very much to the fore with the advent of food products from unconventional sources.

The second part can be classified under 'commodities': the protein quality and content of cereals, pulses, horsemeat, fish, mushrooms, leaf protein, cottonseed, dairy products and potatoes are covered, and the alterations which occur as a result of plant breeding and processing are reported. Although the major intention of the symposium

was to assess protein value for application to human nutrition, individual chapters refer to work undertaken with pigs and ruminants.

With this volume to hand, the reader has a most comprehensive up to date coverage of all aspects concerning the nutritional value of proteins and it will be many years before another publication rivals this 'bible'. The tragedy is that the price restricts purchase to libraries whereas the nutritionist, food scientist, agriculturalist and home economist would benefit greatly from having a personal copy. It is not only an ideal reference work but is written in such a uniform clearly expressed style that the reader is encouraged to peruse aspects of protein nutrition, such as coeliac disease or the estimation of available methionine, which may be outwith their particular interest.

K. MARY CLEGG

Fresh Meat Technology. By E. KARMAS.
New Jersey: Noyes Data Corporation, 1975. Pp. xi + 282. US$36.00.

Few books can have been given a more misleading title. The information it contains consists of abstracts from over 150 U.S. patents concerning meat appearing between 1960 and 1974, information which by no means covers the subject of fresh meat technology nor does it adequately cover the advances in that time since it omits those which were not deemed patentable or which appeared in the patent literature of the rest of the world.

The patents are dealt with in considerable detail but without any attempt at critical evaluation. Thus the most extravagant claims, many of which have already been examined and reported on in the journal literature, appear without comment and some of the scientific explanations which appear are unsound.

The book provides a useful summary of the ground covered by the U.S. patent literature and should be of value to research and development alike; its value would have been enhanced and its cost reduced by a more critical approach and by the inclusion of the rest of the world's patents, at least those in English.

D. N. RHODES

Single Cell Protein II. Ed. by S. R. TANNENBAUM and D. I. G. WANG.
Cambridge, Mass.: M.I.T. Press, 1975. Pp. ix + 707. US$20.00.

This book represents a collection of the papers presented at the second international conference on Single Cell Protein (SCP) held at the Massachusetts Institute of Technology in 1973. The book is wholly in English although the contributors were drawn from many different parts of the world. The printing has been accomplished by photographing directly the author's typescript which for this reader provides a clear and perfectly readable text.

There are some thirty-five chapters in all without any division into sections. The first

two chapters deal with SCP in general. These are followed by six chapters concerned with selected aspects of the theory of and technology involved in SCP production. The next sixteen chapters contain perhaps the most interesting and most useful material in the book. Practically every major SCP process under development at that time is described here, only the so-called 'Symba' process developed in Sweden appears to be missing. Apart from the industrial processes based on hydrocarbons as substrate, several processes utilizing carbohydrates in the form of waste materials are also described. Nutritional and toxicological aspects of single cell protein are discussed in the next eight chapters followed by two chapters on SCP marketing. The final one describes the role of international agencies.

The 1973 M.I.T. conference was held at a time when interest in and hopes for SCP were at their highest. Since that time, major changes in the world economic situation have occurred and thinking about the role and potential of SCP has undergone some revision. Although two large SCP plants have been constructed in Europe it seems unlikely that the editorial comment in this book, 'that before the end of the '70s many hundreds of thousands of tons of industrially produced protein would go into animal feeds and perhaps even human food', will be fulfilled. Some of the economic forecasts and profitability estimates contained in the book have clearly been overtaken by events. The chapter on marketing SCP in low income countries now seems outdated. The SCP industry at present seems to be going through a period of reassessment and marking-time until the economic position clears but there appears to be no doubt to those concerned that SCP will have a role to play in providing feed and food in the future.

Single cell protein has proved a favourite subject for conference organizers in recent years and other symposia papers on this theme have been published. However, this book probably presents the most comprehensive guide to the subject. This volume together with the proceedings of the first M.I.T. conference on single cell protein would give the general reader a good overall view of the topic and provide a useful reference text for those involved more deeply in the subject.

A. Tolan

Books received

A Guide to the Vitamins. Their Role in Health and Disease. By John Marks. Lancaster: Medical & Technical Publishing, 1975. Pp. xii + 208. £6.50.

A critical review of the present state of knowledge of vitamins and their importance in health and disease.

Second Book of Food and Nutrition, 3rd edn. By W. Mathews and D. Wells. London: Home Economics, 1976. Pp. x + 255. £2.50.

An updated edition of a well established introductory text.

Concise Guide to Food Legislation. By D. PEARSON.
Weybridge: University of Reading, National College of Food Technology, 1976. Pp. 38. £1.30.

A compilation of standards, regulations and official reports governing the nature and quality of foods. This will be a most welcome guide through an ever more complex field.

Report of the Government Chemist, 1975.
London: H.M.S.O., 1976. Pp. 158. £2.10.

As in previous years the annual report of the laboratories of the Government Chemist contains a considerable amount of information which will be of interest to food technologists.

A Manual of Methods for the Bacteriological Examination of Frozen Foods.
By L. P. HALL.
Chipping Campden: Food Preservation Research Association, 1975. Pp. 85. £5.35.

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Edited by Marvin L. Speck, Ph.D.

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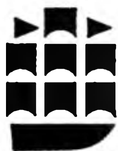

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Abbreviations. Abbreviations for some commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular. Wherever possible the metric SI units should be used unless they conflict with generally accepted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

SI UNITS

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

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in^2	= 645·16 mm^2
square foot	ft^2	= 0·092903 m^2
cubic inch	in^3	= 1·63871 × 10^4 mm^3
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·5461 l
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= 2·76799 × 10^4 kg m^{-3}
dyne		= 10^{-5} N
Calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 T°C + 32

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

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