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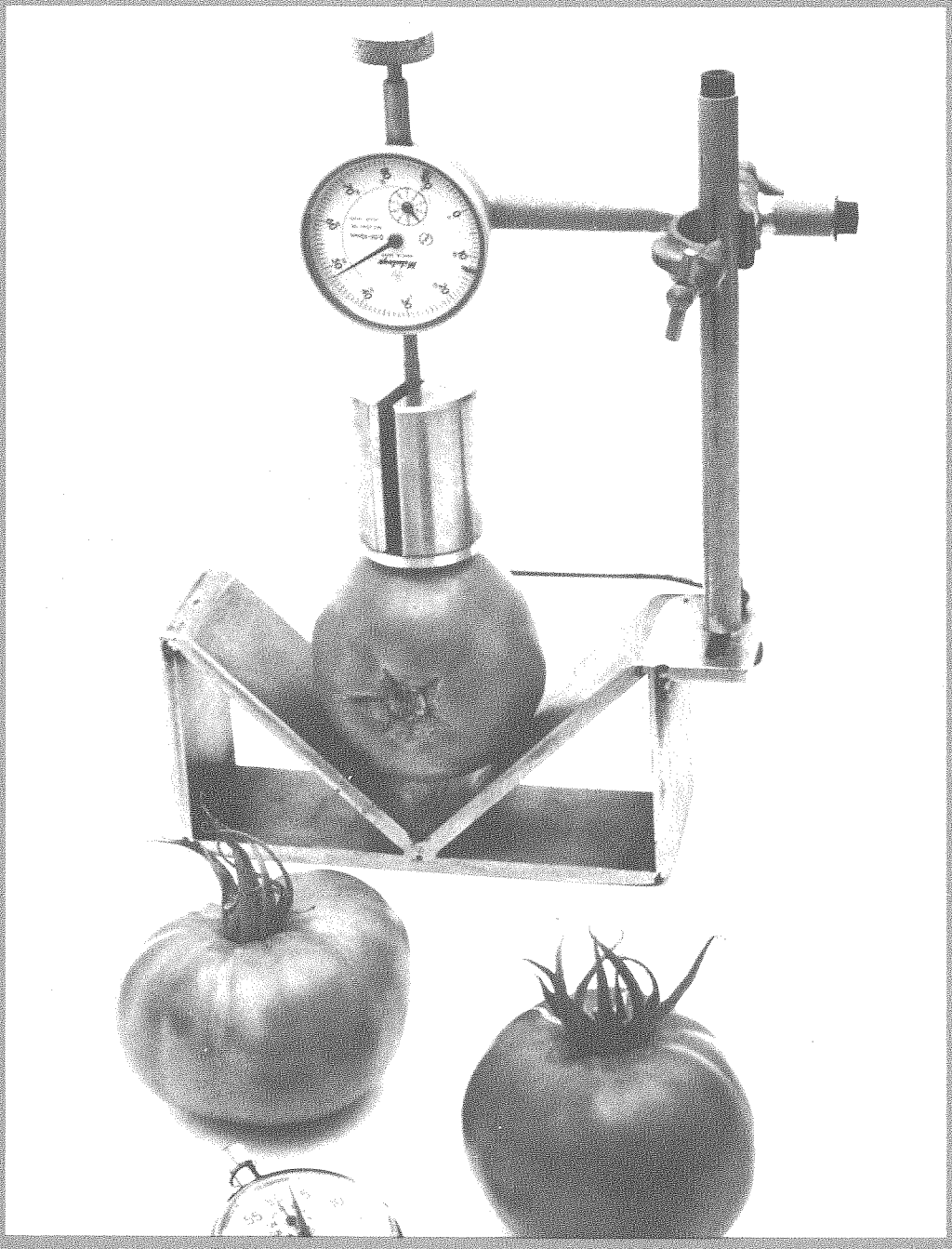

Determination of sensory quality in fresh market tomatoes

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Reducing sugars and acids contribute to the taste of fresh tomatoes but simple measurements of total soluble solids, pH, titratable acidity and electrical conductivity do not provide an adequate measure of sensory quality. A complex mixture of volatile (aroma) compounds interacts with the sugars and acids to give a characteristic tomato flavour. Although improvement in flavour can be achieved by breeding tomatoes with higher levels of sugars and acids, it is not yet possible to measure the contribution of the aroma compounds. Until this problem is solved it will be necessary to use taste panels for the evaluation of new cultivars.

Introduction

There has been an increased emphasis on improving the sensory quality of fresh market tomatoes in recent years, both in Australia and the USA. Media reports in Australia of consumer dissatisfaction with the taste of fresh tomatoes (Beattie *et al.* 1983) and a reported decline in the per capita consumption of fresh tomatoes in the USA (Stevens 1979) have stimulated research in this area. Much work has been done on the components of tomato fruit believed to influence taste and flavour and on how these components can be varied by breeding programs, horticultural practices and postharvest handling (Winsor 1979; Stevens 1981).

At present, evaluations of the sensory quality of tomatoes usually include the measurement of total soluble solids, pH, titratable acidity and the calculation of sugar to acid ratios. These methods are used routinely because they are widely reported to correlate well with sensory evaluation data (Simandle *et al.* 1966; Brecht *et al.* 1976; Al-Shaibani and Greig, 1979; Stevens 1979); they are simple, quick, inexpensive and suitable for a wide range of situations. For normal cultivars of commercially grown fresh tomatoes, total soluble solids range from about 4 to 6%, pH from 3.9 to 4.6, and titratable acidity from 9.7 to 14.6 m.e./100 ml of juice (McGlasson *et al.* 1983). The electrical conductivity of the juice expressed from tomato fruit has also been reported to correlate positively with taste panel results (Gormley and Egan 1982) and has been used as a quality indicator for tomatoes in Israel (Mizrahi 1981).

Volatile organic compounds are important in the flavour of fresh tomatoes (Kazeniac and Hall 1970) and so influence taste panel evaluations (Garrison, Ho and Ichimura 1981). There is no single volatile compound which gives a typical tomato aroma, but instead a complex interaction of tomato components is involved (Buttery *et al.* 1971). Of the large number of volatile compounds in tomatoes, a key group has been identified (Table 1) and the presence and concentration of some volatiles has been related to taste panel evaluation (Dirinck *et al.* 1976). Although a method for including the measurement of volatile compounds in routine tomato quality assessments has not been devised, this could lead to improved quality evaluation (Watada and Aulenbach 1979).

Quality is generally evaluated on the basis of measurements of composition and assessment by taste tests. Although a well-designed taste test with experienced panellists can produce very reliable information, it is difficult to compare taste panel evaluations obtained at different times, or by different workers, around the world. The composition measurements become points of reference for this work. Another factor limiting the use of

Table 1. Some key volatile aroma compounds in tomatoes and their flavour contribution

Compound	Flavour contribution
2-Isobutylthiazole	'Spoiled', 'vine-like', important contribution to fresh tomato flavour and enhances mouth feel
Cis-3-hexenal	'Green' and contributes to mouth feel
Trans-2-hexenal	Less fresh 'green'
n-Hexenal	'Green'
Trans-2-pentenal	Fresh 'green'
Methlysalycilate	Flat, insipid, refrigerated tomato juice flavour
Acetaldehyde	Cooked flavour
Linalool	Floral perfume
β-Ionone	'Violet-like', contributes to fresh flavour

(Kazeniac and Hall 1970; Stevens 1970)

taste panels is the large number of fruit required and hence the need for extensive field plantings (Winsor 1979). The costs of field trials and taste tests are high, due to the labour costs involved in harvesting, staffing and coordinating taste panels, and the costs of data processing. Clearly it would be of value if the need for routine taste panel assessments could be reduced (Jones and Scott 1983).

The use of simple measurements such as total soluble solids, pH, titratable acidity and electrical conductivity, without the back-up taste panel data, appears desirable considering the reported strong correlations between these simple measurements and taste panel results. One consideration, however, is the lack of definition as to what is actually reflected in these measurements. Total soluble solids is used to estimate sugar content, but sugars account for only 60 to 80% of the variation in tomato soluble solids (Davies and Hobson 1981). Similarly, titratable acidity, used to indicate free acid concentration, has been reported to correlate with potassium or total anion contents and with citric acid, but not malic acid or pH (Davies and Hobson 1981). Several components influence these individual measurements (Table 2) so it is difficult to know which of the tomato constituents affects eating quality.

An important objective of some fresh tomato breeding programs has been to increase the soluble solids concentration while maintaining a balance with acidity (Stevens, Kader and Albright 1979). However, the extent to which increased soluble solids levels is necessary is not clear (Jones 1982). Jones and Scott (personal communication) report results of taste panel evaluations of F_1 hybrids from high sugar and acid parent lines ranging from 6 to 7% soluble solids. They suggested that panellists' perception of sweetness reached a plateau and that above this level other flavour components become more important in breeding for improved tomato flavour. This is an important question, especially when plant breeders are considering other aims which may be in conflict with increasing the total soluble solids, such as decreasing the plant size and concentrating fruit set while maintaining high yield, firmness and other established fruit quality characteristics (Stevens and Rudich 1978; Hewitt and Stevens 1983).

Data accumulated on several new cultivars of fresh market tomatoes in recent experiments (Sumeghy *et al.* 1983; Nguyen *et al.* 1984) have enabled evaluation of the reported correlation between composition measurements and taste panel scores.

Table 2. Major constituents of red-ripe tomato fruit

Constituent	Concentration (g/100 g fresh weight)
Dry matter	5–7.5
Sugars	2.4–3.6
glucose	1.1–1.6
fructose	1.2–1.9
sucrose	<0.1
Acids	
citric	0.45–6.7
malic	0.20–0.30
dicarboxylic amino	0.10–0.15
ascorbic	0.025–0.037
Other amino acids, vitamins and polyphenols	0.050–0.075
Alcohol insoluble solids	1.35–2.02
proteins	0.40–0.60
pectic substances	0.38–0.52
hemicellulose	0.20–0.30
cellulose	0.30–0.45
Minerals	0.40–0.60
Lipids	0.10–0.15
Pigments	0.02–0.03
Volatiles	0.005–0.007

Source: (Davies and Hobson 1981a)

Field experiments

In the summer of 1981/82 and 1982/83, experiments on fresh market tomatoes were conducted at three different commercial production sites in NSW (Duranbah, Medowie and Richmond). In the first year, eight different cultivars were grown, including Flora-Dade as the standard commercial cultivar. In the second year six new cultivars were grown, plus Flora-Dade and Sunny. Sunny was the best cultivar from the first year (Sumeghy *et al.* 1983). The plants were all of the determinate type. They were grown on raised beds, covered with polyethylene mulch, and watered by trickle irrigation. Each cultivar was replicated four times in a randomized complete block design and the fruit were harvested over a three week period at each site.

Composition and sensory evaluation

Tomatoes for quality measurement were harvested at the breaker stage (first-colour at the blossom end, USDA colour stage 2, Anon 1975) and stored at 20 °C for six days until red-ripe, when all composition and taste evaluations were conducted. Up to 600 fruit from each site were used in the quality assessments. In the second year, no evaluations were conducted on fruit from Duranbah as persistent rain and the development of bacterial spot drastically reduced the yield of all cultivars.

The methods for the measurement of total soluble solids, pH and titratable acidity were described by Sumeghy *et al.* (1983). The conductivity of juice extracts was also measured in the 1982/83 experiment, using a conductivity meter standardized with saturated sodium chloride solution.

In the taste panel evaluations, fruit were judged by 24 experienced tasters for colour, flavour, texture and general acceptability. A 9-point hedonic scale was used, where 1 = very poor, 5 = satisfactory and 9 = very good. Only four cultivars were presented at a taste session so that three sessions, each including a standard (Flora-Dade in 1981/82, Sunny in 1982/83), were needed for fruit from each site. The difference between the scores given to a cultivar and those assigned the standard cultivar in the taste session were used to enable statistical comparisons between the tasting sessions. Scores for cultivars grown at different sites could not be compared directly as the spread in harvest dates did not allow a common sample of fruit of the standard cultivar to be included in each set of taste sessions.

Results of quality measurements

In both years, the colour of all cultivars scored in the region was described as 'good'. The flavour, texture and general acceptability scores ranged only from 'poor' to 'satisfactory' in both years. The scores for general acceptability in 1982/83 (Fig. 1) show that the differences between cultivars and the overall quality range were small. However, differences in flavour between the cultivars were perceived by the panellists. The data for general acceptability scores in 1982/83, when standardized against Sunny (Fig. 2), show significant differences between the cultivars calculated by two-way analysis of variance. Fig. 2 also shows that there were no significant differences between the scores of those cultivars tasted twice in different sessions. Similarly, for the cultivars tasted twice in 1981/82 by the taste panel good reproducibility was obtained.

The composition measurements and standardized taste scores are presented in Table 3. The statistical differences shown by some quality measurements were not observed at every growing site; furthermore, the composition of the cultivars varied according to the environmental conditions at the sites, and between years. The yields in 1982/83 were much lower than in 1981/82, and this is reflected by the fruit composition. Both the cultivars Flora-Dade and Sunny had 20 to 30% higher concentrations of total soluble solids and titratable acidity at Medowie in 1982/83 compared to 1981/82.

The cultivars grown at Duranbah and Medowie in 1981/82 were not significantly different in flavour or general acceptability scores (Table 3). There were, however, differences in the levels of total soluble solids and titratable acidity at both sites, and for pH at Medowie. In contrast, at Richmond there were no differences in total soluble solids and few in pH or titratable acidity, but there were differences in general acceptability scores. As no differences were perceived in the flavour of the cultivars, these differences in acceptability may have been due to different texture scores, although some differences in the texture were also seen at the other sites. The 1981/82 data indicate little difference between the taste of the eight cultivars, while

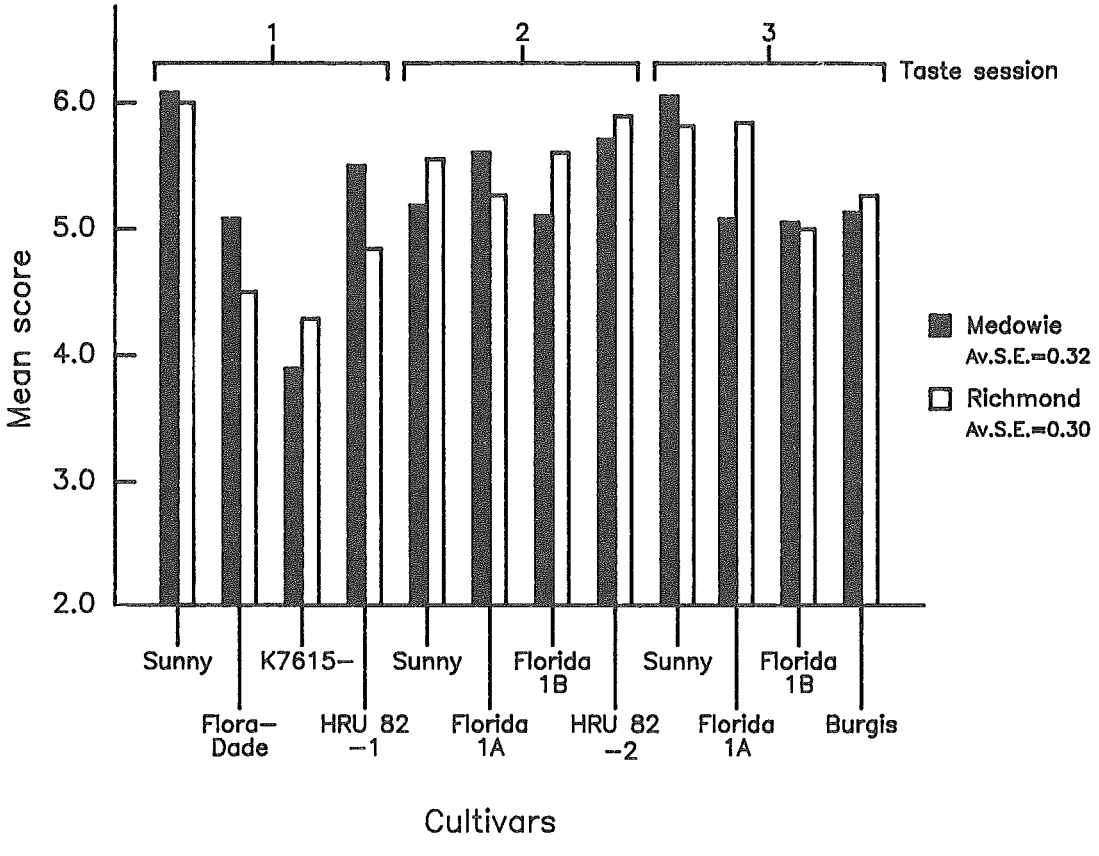

Fig. 1. The mean scores for general acceptability of tomato cultivars at three tasting sessions in 1982/83. Florida 1A and Florida 1B were evaluated twice and Sunny was included in each session.

there were differences in composition.

In 1982/83, the cultivars that were evaluated differed in taste scores for flavour, texture and general acceptability at both Medowie and Richmond, as shown in Table 3. At Medowie no differences were measured for the total soluble solids, few differences in pH and titratable acidity, while differences were found in conductivity. Similarly, at Richmond there were no differences in total soluble solids and pH, but greater differences indicated for titratable acidity. However, no significant differences were shown at Richmond for the conductivity. The 1982/83 data indicate differences in the flavour of the eight cultivars, while there were few differences in the composition.

Relationship between composition and taste panel evaluation

The composition measurements for the fourteen cultivars tested in 1981/82 and 1982/83 do not appear to closely reflect the sensory quality as determined by the taste panel. In particular, no relationships between the measurement of total soluble solids and eating quality were evident in either year. The differences in total soluble solids between sites for the same cultivar were often greater than the differences between cultivars at the same site. In 1982/83 the cultivars grown at Medowie were 20 to 25% higher in total soluble solids than the same cultivars grown at Richmond. Although such a rise in soluble solids seems important, a comparison of the scores for general acceptability (Fig. 1) suggests that the expression of taste characteristics has remained constant for each cultivar at both sites.

Broad trends may be seen for those cultivars scoring poorly or well in the taste panel results. The cultivar K7615-2-2-1-6-5,

Table 3. Mean value for composition and standardized taste panel scores for fresh market tomato cultivars

Site and cultivar	Total soluble solids %	pH	Titratable acidity (m.e./100 ml)	Conductivity (μs)	Flavour	Texture	General acceptability
1981/82							
Duranbah							
Flora-Dade	3.9 ab*	4.0 a	7.0 a	n.m.	0 a	0 b	0 a
Sunny	3.8 bc	4.2 a	6.6 ab	n.m.	0.08 a	0 b	0 a
GS393	4.0 ab	4.1 a	6.1 c	n.m.	0.62 a (0.58 a)**	0.92 a (1.42 ab)	0.67 a (1.08 a)
Duke	3.6 c	4.1 a	6.2 c	n.m.	−0.25 a	0.29 b	0.08 a
Calypso	4.2 a	4.0 a	6.4 ab	n.m.	0.29 a	−0.12 b	0.42 a
Tempo	4.2 a	4.1 a	6.3 bc	n.m.	0.13 a	0.08 b	0.08 a
Royal Flush	4.0 ab	4.2 a	6.8 a	n.m.	0.42 a (0.04 a)	0 b (0.67 ab)	0.37 a (0.58 a)
Full House	3.8 bc	4.2 a	5.2 d	n.m.	0.25 a	0.33 b	0.33 a
Meadowie							
Flora-Dade	4.5 b	3.9 b	8.4 a	n.m.	0 a	0 abc	0 a
Sunny	4.3 b	4.0 b	8.1 ab	n.m.	0.12 a	0.75 a	0.54 a
GS393	4.9 a	4.1 a	7.4 bc	n.m.	0.25 a (0.87 a)	0.54 ab (0.46 ab)	0.21 a (0.54 a)
Duke	4.4 b	4.1 a	7.5 abc	n.m.	0.17 a	0.29 ab	−0.29 a
Calypso	5.0 a	4.1 a	7.9 ab	n.m.	−0.29 a	−0.79 c	−0.75 a
Tempo	4.9 a	4.2 a	7.5 abc	n.m.	0.25 a	−0.38 bc	−0.08 a
Royal Flush	4.8 a	4.1 a	7.6 abc	n.m.	0.04 a (0.37 a)	0.67 ab (0.13 ab)	0.58 a (0 a)
Full House	4.8 a	4.2 a	6.8 c	n.m.	−0.21 a	0.58 ab	0.17 a
Richmond							
Flora-Dade	4.2 a	4.0 b	8.3 a	n.m.	0 a	0 abc	0 bcd
Sunny	4.4 a	4.1 ab	6.9 b	n.m.	0.42 a	0.33 ab	0.46 abc
GS393	4.4 a	4.2 a	6.8 b	n.m.	0.42 a (0.25 a)	0.29 ab (0 abc)	0.37 abc (0.42 bcd)
Duke	4.1 a	4.1 ab	7.5 ab	n.m.	0.33 a	0.21 abc	0.12 abcd
Calypso	4.4 a	4.0 b	7.0 b	n.m.	−0.21 a	−0.71 c	−0.58 c
Tempo	4.5 a	4.1 ab	7.2 b	n.m.	0.04 a	−0.58 bc	−0.33 cd
Royal Flush	4.4 a	4.1 ab	6.7 b	n.m.	0.83 a (0.17 a)	0.50 a (0.50 a)	0.71 ab (0.08 abcd)
Full House	4.4 a	4.2 ab	5.6 c	n.m.	1.08 a	0.87 a	0.91 a
1982/83							
Meadowie							
Flora-Dade	5.6 a	4.1 b	9.5 ab	6275 a	−0.96 b	−0.62 abcd	−1.08 d
Sunny	5.6 a	4.1 b	10.5 a	6141 ab	0 ab	0 ab	0 abc
Florida 1A	4.8 a	4.1 b	8.3 ab	5717 bc	0.83 a (−0.67 b)	−0.37 abc (−1.06 bcd)	0.17 ab (−0.71 bcd)
Florida 1B	5.1 a	4.2 a	8.0 ab	5693 c	0.25 ab (−0.92 b)	0.042 ab (−1.25 cd)	0 abc (−1.0 cd)
K7615-2-2-1-6-5	5.1 a	4.1 b	6.8 b	5556 c	−2.29 c	−1.71 d	−2.08 e
Burgis	5.5 a	4.1 b	9.3 ab	5746 bc	−1.0 b	−1.04 bcd	−0.17 de
HRU 82-1	5.4 a	4.1 b	9.5 ab	5850 abc	−0.62 b	−0.29 abc	−0.67 bcd
HRU 82-2	5.4 a	4.1 b	9.7 ab	5973 abc	0.58 a	0.46 a	0.62 a
Richmond							
Flora-Dade	4.7 a	4.1 a	7.9 a	5724 a	−1.46 c	−1.62 de	−1.5 de
Sunny	4.35 a	4.1 a	7.6 ab	5599 a	0 ab	0 a	0 ab
Florida 1A	4.1 a	4.1 a	6.4 c	5206 a	−0.46 ab (−0.12 ab)	−0.29 ab (−0.29 ab)	−0.46 ab (0 ab)
Florida 1B	4.4 a	4.1 a	7.1 ab	5585 a	0.083 a (−0.79 abc)	−0.33 ab (−0.67 bd)	−0.042 ab (0.83 bcde)
K7615-2-2-1-6-5	3.8 a	4.1 a	5.7 c	4973 a	−1.62 c	−2.42 e	−1.75 e
Burgis	4.3 a	4.1 a	6.4 c	5249 a	−0.67 abc	−0.5 ab	−0.33 abc
HRU 82-1	4.25 a	4.1 a	6.8 abc	5307 a	−1.0 bc	−1.25 bd	−1.08 cde
HRU 82-2	4.5 a	4.1 a	7.4 ab	5638 a	0.25 a	0.46 a	0.33 a

* Means within each column, at each site, followed by the same letter are not significantly different ($P<0.05$)

** Values in parentheses are given for those cultivars which were evaluated twice in each experiment.

n.m. Not measured

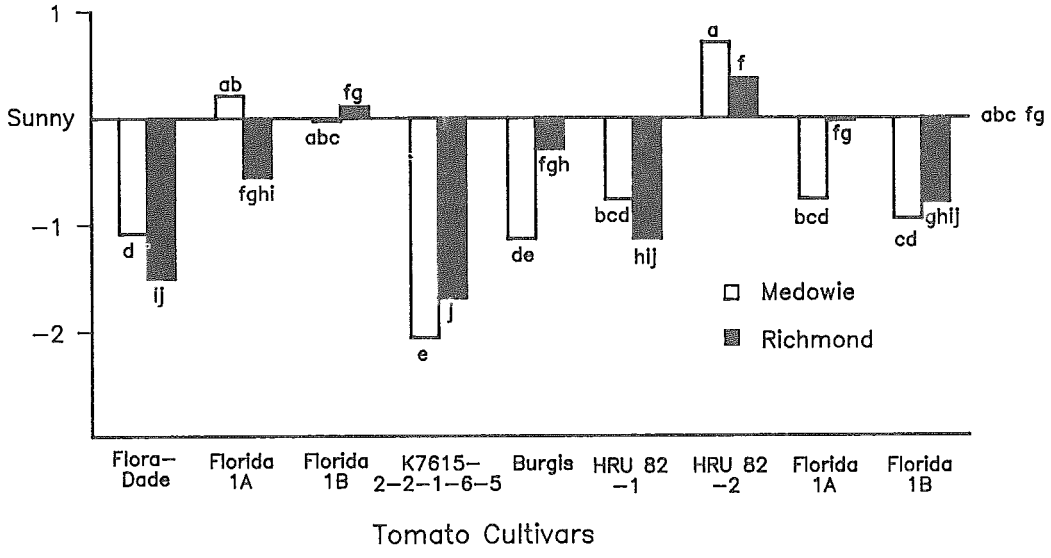

Fig. 2. Taste evaluation of general acceptability for each cultivar in comparison with Sunny. Florida 1A and Florida 1B were evaluated twice. Bars capped with the same letter "a, b, c, d and e" for Medowie and "f, g, h, and i" for Richmond, are not significantly different ($P<0.05$) as determined by Duncan's multiple range test.

which had the lowest scores in the taste tests at both sites in 1982/83, also had the lowest levels of total soluble solids and titratable acidity. HRU82-2 had the highest scores in 1982/83 and tended to have high levels of total soluble solids and titratable acidity. However, Flora-Dade in the same year was higher in total soluble solids and titratable acidity than HRU82-2 but scored very similarly to K7615-2-2-1-6-5 in the taste test.

To further investigate the relationships between composition measurements and the taste panel assessments, coefficients of correlation were calculated (Table 4). The measurements of total soluble solids, titratable acidity and conductivity were not significantly correlated with general acceptability scores. The pH and sugar acid ratios gave significant correlations at single sites only. The good correlation between taste panel scores for flavour and for texture with general acceptability indicates both factors are important in sensory quality. Correlation between the composition measurements and the flavour scores also showed no correlation with total soluble solids or conductivity. A significant correlation was found for pH, titratable acidity and sugar acid ratio at Richmond in 1981/82. This, together with the correlation between pH and general acceptability at this same site, indicates that the difference in acceptability scores seen at Richmond in 1981/82 may be due to fruit composition as well as the texture. The sugar-acid ratio was correlated with flavour at Richmond in 1981/82 and Medowie in 1982/83. The coefficient is negative in 1981/82 and positive in 1982/83 because the standard cultivar in 1981/82, Flora-Dade, was poorer in taste compared to Sunny, which was the standard for 1982/83.

The possibility of a better relationship between the composition measurements and the taste panel evaluation was investigated by the use of multiple regression. Equations including two to four composition variables and general acceptability did not give any significantly improved correlations.

Limitations of composition measurements

According to the analysis of data obtained in the recent local experiments, measurements of total soluble solids, pH, titratable acidity and electrical conductivity do not provide an adequate measure of tomato sensory quality. Similarly, Jones and Scott (1983; personal communication) using a multivariate analysis of sensory characteristics and the concentrations of acids and sugars in several cultivars were unable to account for a large proportion of

Table 4. Correlation coefficients for taste panel scores and compositional values with scores of general acceptability and for compositional values with flavour scores

Site	Duranbah 1981/82	Medowie 1981/82	Richmond 1981/82	Medowie 1982/83	Richmond 1982/83
			General Acceptability		
Total soluble solids	0.3175	−0.1913	0.0363	0.1073	0.2885
pH	0.0560	−0.1091	0.6843[A]	−0.0054	0
Titratable acidity	−0.2402	−0.1382	−0.5789	0.5787	0.2742
Sugar:acid ratio[B]	0.4083	−0.007	0.5463	−0.7389[A]	0.2171
Conductivity	—	—	—	0.2564	0.4120
Flavour	0.7487[A]	0.4761	0.9290[A]	0.9166[A]	0.7660[A]
Texture	0.8548[A]	0.8530[A]	0.8894[A]	0.8340[A]	0.8289[A]
			Flavour		
Total soluble solids	0.5019	0.0867	0.0935	−0.1068	0.2167
pH	0.0352	0.0301	0.6337[A]	−0.0830	0
Titratable acidity	−0.2512	−0.1228	−0.6948[A]	0.4314	0.2765
Sugar:acid ratio	0.5277	0.0863	0.6485[A]	−0.6755[A]	−0.3020
Conductivity	—	—	—	0.2442	0.3415

[A] The coefficient of correlation is significant at $P < 0.05$

[B] The sugar:acid ratio was derived by dividing measured level of total soluble solids by the titratable acidity.

the differences perceived in taste panel evaluations. In published accounts where correlations between these measurements and taste evaluations have been described, the cultivars had a wide range in composition and taste characteristics (Stevens, Kader and Albright 1979; Jones and Scott 1983 and unpublished data) or marked differences were achieved by growing a cultivar under different conditions (Bisogni, Armbruster and Brecht 1976). The potential for obtaining good correlations between composition measurements and taste panel evaluations was demonstrated by Jones (1982). In this work, additions of sugar and acid were made to standard samples of fresh tomatoes. Good correlations with taste panel evaluation and the levels of sugar and acid in the sample were shown only at the extremes. However, at intermediate levels of sugar and acid, additional factors influenced the taste scores. These factors interacted with the levels of sugar and acid perceived by the tasters, and also affected overall flavour perception.

The results of the 1981/82 and 1982/83 experiments demonstrated a poor correlation between taste panel scores and sugar content and there was a small range of intermediate sugar concentrations in the cultivars. At Duranbah and Medowie in 1981/82, cultivars were significantly different in total soluble solids but not in flavour or general acceptability. The concentrations of reducing sugars, fructose and glucose, were determined by h.p.l.c. for the eight cultivars tested at these two sites in 1981/82. No correlations were found between the level of reducing sugars (% fructose + % glucose) and general acceptability. There were no correlations between the ratio of glucose to fructose in each cultivar, and the general acceptability or flavour scores. There were also no correlations between the level of reducing sugars and the total soluble solids although the relationship was almost significant at Duranbah.

Fruit of non-ripening tomato mutants, *rin* and *nor*, were also analyzed by h.p.l.c. for reducing sugars in 1981/82. The concentrations of fructose and glucose in these unpalatable fruit were in the range determined for the eight normal cultivars (Table 5). Kopeliovitch *et al.* (1982) also found *rin* and *nor* fruit to have levels of reducing sugars, total soluble solids, pH and titratable acidity similar to normal cultivars.

Improving the sensory evaluation

The measurements of total soluble solids, pH and titratable acidity cannot be used to predict accurately the sensory quality of fresh market tomatoes. These measurements are useful as broad indicators of quality, particularly in evaluating samples with very different sugar and acid concentrations. To improve the evaluation of tomatoes and reduce the need for taste panels, additional measurements of fruit composition are

Table 5. The range in reducing sugar concentrations in the eight cultivars of fresh market tomatoes grown at Duranbah and Medowie and the concentrations found in *rin* and *nor* fruit (isogenic with Rutgers), determined by r.p.l.c.

	% Fructose + % glucose	Glucose/fructose ratio
1981/82 cultivars	1.81–2.82	0.80–0.93
rin	2.16	0.87
nor	2.00	0.94

necessary. There is not enough published work or any evidence from these field experiments to suggest that the measurement of electrical conductivity provides any additional information on sensory quality. Similarly, the analyses of reducing sugars is of limited value, particularly considering the results obtained for *rin* or *nor* fruit.

Defining and quantifying the other tomato components which may be important when sugars and acids are present in adequate levels, could provide a more complete measurement of sensory quality. An investigation of the contribution of volatile aroma compounds could fulfil this aim, since it is these fruit components which give the characteristic flavour to tomatoes (Buttery *et al.* 1971). A study on the heritability of some volatiles in different tomato cultivars and an investigation of the odour thresholds of these volatiles was reported by Stevens (1970). The results indicated that the differences in the volatile concentrations of the cultivars were great enough to contribute to differences in their flavour.

There are many problems in evaluating the contribution of individual volatiles to overall flavour and sensory quality. In aqueous solution, 2-isobutylthiazole was reported to taste spoiled, vine-like, and objectionable, but when added to a tomato product it gave an intense fresh tomato-like flavour (Kazeniac and Hall 1970). Similarly, other volatiles act unpredictably in combination with tomato components, by, for example, improving the mouthfeel, blending out harsh notes, flattening the overall flavour and producing different flavour effects at different concentrations. Much work must be done to overcome the problems associated with such complex interaction. Watada and Aulenbach (1979) demonstrated how information on volatiles can be used in conjunction with other composition measurements, such as total soluble solids, pH and titratable acidity. A multiple regression equation with these components showed an improved prediction of the acceptability of fresh tomatoes. The evaluation of volatile components might overcome the inadequacies found in the present compositional methods.

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National symposium on ionizing energy treatment of food

On 5–6 October 1983 more than 130 people from the food industry, academia and government met in Sydney to discuss recent developments in the use of food irradiation. The two best known applications are in the disinfestation of cereals, fruit and vegetables, and in the extension of storage life by reduction of the microbial load on a range of other foods.

Whilst the technology itself is not new, much work having been done in the 1950s and 1960s — including some at the Division, recommendations as to acceptable dose rates to ensure 'wholesomeness' of the foods have been made by international expert bodies in recent years. These have assisted national governments to formulate legislation permitting domestic and international trading in foods treated with ionizing energy.

Only a few days before the meeting, the USA, closely followed by Japan, unexpectedly decided to phase out the fumigation of citrus and sub-tropical fruits with ethylene dibromide (EDB) by mid-1984. This is likely to increase the need for an acceptable alternative, not only in the USA, but among its trading partners. In Australia, the National Health and Medical Research Council has also recommended that the use of EDB be phased out in export and domestic trade. Irradiation is an obvious choice, being proven safe. The challenge for Australia is its commercial viability — costs and logistics. These problems must now be faced and the Symposium participants resolved to request the Minister for Science, Mr Barry Jones, to liaise with the Ministers for Primary Industry and for Health to pave the way for Australia to adopt internationally acceptable codes of practice for the irradiation of foods. Whilst it is recognized that Australia's Federal/State system is a complicating factor in the adoption of uniform food legislation, clearly it would be desirable for Australia to not only support the Codex International General Standard for Irradiated Food and the Recommended Code of Practice for Operation of Radiation Facilities but to put them in the Model Food Act for subsequent incorporation in States and Territories legislation.

Immobilized enzyme systems*

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Enzymes can be utilized to promote chemical reactions under mild conditions in sensitive biological systems such as foods. Immobilizing the enzymes offers the possibility of a wider and more economical exploitation of the highly specific functions of these natural catalysts.

Enzymes

Without enzymes there would be no life as we know it. They are the catalysts which enable all the complex chemical reactions that are necessary for the survival and growth of living organisms to be performed within the narrow range of temperatures where life is possible. Enzymes are proteins, i.e. they are long chains of polymerized amino-acids. The order in which these amino acids are arranged in the chain or chains of an enzyme determines the three-dimensional structure which the molecule assumes to form its active centre and which in turn is responsible for its particular catalytic ability. The catalytic activity is highly specific with respect to the reaction catalyzed. Being true catalysts, enzymes are not generally permanently altered during the reactions they catalyze and, provided there are no inhibitors present, each enzyme molecule will continue to perform its catalytic function as long as conditions are favourable and unchanged substrate molecules remain. Thus they are very efficient and small amounts of enzyme are able to transform large quantities of substrate. A good example of the efficiency of action of enzymes is the coagulation of milk by rennet during the manufacture of cheese. Approximately 1g of chymosin, the major milk-clotting enzyme extracted from calf vells, is capable of causing the coagulation of about 23 000 l of milk in 30 min at 30 °C.

*A talk presented to the Southern Branch of the Australian Institute of Food Science and Technology, Melbourne, July 1982.

To achieve this the 1g of enzyme must catalyze the splitting of about 100 kg of κ-casein in the milk, i.e. at a ratio of 1:100 000. If we could extract the enzyme from the coagulated milk, it could be used to coagulate a further 23 000 l and so on.

It is this high level of efficiency and the high degree of specificity they possess that make enzymes such useful aids in many processes, enabling the modification under mild conditions of one constituent in a complex multi-component system such as exists in most foods. For example, the level of lactose in milk can be reduced by treatment with β-galactosidase, an enzyme that catalyzes the hydrolysis of the disaccharide to its two constituent monosaccharides, glucose and galactose, without affecting the fats, the proteins or the minerals in the milk.

The application of enzyme technology to food processing is by no means a recent development. Cheese, fermented dairy products such as yoghurt, leavened bread, beer and wine are all examples of the ways enzymes were being used in food processing before we knew of their existence. Today a wide range of enzymes with well-characterized catalytic functions is available to the food technologist. A major deterrent to their widespread application as aids to processing is their cost. Designed by nature as highly efficient catalysts, they are synthesized in very small quantities so that their isolation involves separation from large amounts of inactive material. In addition, the enzyme preparation used to effect a desired change must be free from other enzymes that might be present in the source material and which could adversely affect other components in the system being treated. For instance, in the example mentioned earlier, if the β-galactosidase preparation used to treat the milk contained a protease or a lipase, the milk would coagulate or become rancid during the treatment. Furthermore, if added

to foods, enzyme preparations must be free of any harmful products, such as toxins, allergenic factors etc., that might be present in the biological materials from which they are derived. Purifying enzyme preparations to the degree necessary is often a costly operation, although only small amounts of enzymes are required due to their inherent efficiency of action and this to some extent offsets the high cost of producing them.

Enzymes are heat-sensitive proteins and lose their catalytic capabilities when the three-dimensional structures in the protein which determine these properties are permanently disrupted by heat. Thus the extent of the enzymic action can be controlled by heating the mixture when the required level of product(s) has been reached, provided the material being treated with the enzyme is not itself heat-sensitive. An extra cost factor may be added to the process by the heating.

Immobilized enzymes

Clearly it would be a great advantage if we could recover the expensive enzyme after it has performed its task and use it again. In this way the amount of material processed per unit mass of enzyme would be greatly increased. Efforts to realize this aim have led to the development of immobilized enzyme technology. The concept is a simple one. The enzyme is first attached to, or trapped within, an insoluble carrier so that it can be removed from the mixture when the enzyme treatment has progressed to the required extent. An additional advantage of such a system is that it opens the way to continuous processing whereby the material to be treated is passed through a reactor containing the immobilized enzyme. The extent of the enzymic reaction is then controlled by the rate of flow through the reactor since this determines the time the enzyme is in contact with its substrate. The lack of mobility of the bound enzyme is offset by a high ratio of enzyme to substrate in the reactor.

A great deal of work has been done over the past 15–20 years on immobilized enzymes, although the first report of an artificial immobilized enzyme system was in 1916 (Richardson 1974). The term 'artificial' really applies here because we are simply mimicing nature with this technology. In their natural environments, i.e. in living organisms, enzymes are localized by binding or entrapment so that the reactions they catalyze can proceed in the correct sequence and in the right place. Many different enzymes have been immobilized by various techniques to a wide range of carriers and the effects of immobilization on the kinetic properties of the resultant conjugates elaborated. In some cases the catalytic activities of enzymes change only little, following immobilization, while other bound enzymes exhibit lower activities, sometimes less than 1% of the activity of the corresponding free enzyme. Often an immobilized enzyme is able to perform its catalytic function over a broader range of pH values, while at the same time it becomes less sensitive to heat. The particular method employed to immobilize the enzyme and the size of the substrate on which it acts, are factors which affect the catalytic efficiency of the bound form.

Methods of immobilization

Enzymes possess binding sites which interact with groups on the substrate to orient it in the correct position relative to the active centre so that this region of the molecule can then perform its catalytic function. The method of immobilization adopted must leave the binding site(s) and the catalytic centre accessible to the substrate. A relatively simple method of immobilization is to physically trap the enzyme within a porous matrix (Fig. 1a). This method is only effective if the substrate is small enough to pass through the matrix to the enzyme within. As the active centre and binding site(s) are not affected, enzymes immobilized in this way usually retain high levels of activity, unless the rates at which the substrate enters or the products leave the matrix are much less than the rate of enzymic reaction.

Direct covalent attachment to the carrier is possible by utilizing reactive side-chain groups of amino-acids in the enzyme molecule such as the amino group of lysine, the carboxyl groups of aspartic and glutamic acids, the hydroxyl groups of serine and threonine, the phenolic group of tyrosine and the imidazole group of histidine. These are reacted with suitable groups on the carrier to form a chemical linkage between the two. The type of side-chain group on the enzyme chosen to link it to the carrier should not be one which is essential for binding its

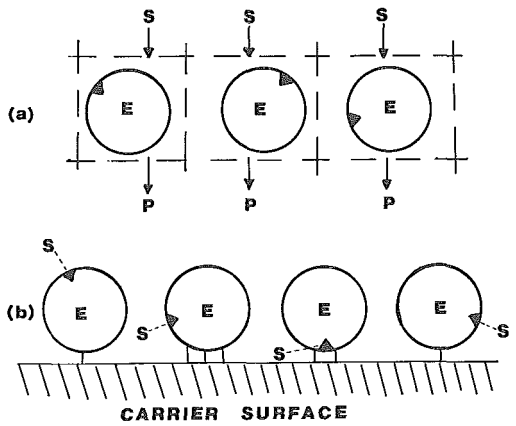

Fig. 1. Schematic illustration of an enzyme (E) immobilized (a) in a porous matrix which is permeable to the substrate (S) and products (P) and (b) by covalent bonding to a solid surface. The dark triangle represents the active centre. Accessibility of the substrate to the active centre can be lost during immobilization.

substrate, or one which is involved with the catalytic function of the active centre, as groups in these regions tend to be more reactive than similar groups in other parts of the molecule. Hence, they are likely to participate in the immobilization procedure and the bound enzyme will then not be active.

Because an enzyme generally contains a number of residues of each type of amino-acid distributed along its protein chain, and each of these can react, it is unlikely that the point of attachment will be the same for each enzyme molecule. Also some molecules are likely to be attached at more than one point. Since the proximity of the point(s) of attachment to the active centre or binding site of the enzyme will influence the accessibility of the substrate, which in turn affects the catalytic activity, a decrease in activity can occur in the immobilized enzyme preparation (Fig. 1b). The larger the substrate the more its access to the bound enzyme is likely to be restricted and hence the more the activity of the latter will be depressed when it is immobilized. Spacers can be inserted between the carrier and the enzyme to allow more freedom of movement and better access for the substrate. With direct coupling of the enzyme, the extensive surface available in a porous carrier such as controlled-pore glass, can be exploited to bind large amounts of enzyme per unit mass of carrier. In order to take advantage of the quantity of enzyme bound, however, the substrate must be able to freely penetrate the pores in the carrier.

Being proteins, enzymes carry a net electrical charge, which is determined by the number of negatively- and positively-charged amino-acid side-chains they contain. At a given pH value the net charge can be negative or positive depending upon its amino-acid composition. This property can be used to immobilize enzymes, by binding them to a support which has groups carrying an electrical charge that is opposite to the net charge on the enzyme. These ionically-bound preparations are useful for treating non-charged substrates in conditions of low ionic strength. Highly-charged substrates and charged ions in the solution compete with the enzyme for the charged sites on the carrier and displace it. Cross-linking the enzyme molecules after adsorption will stabilize the enzyme on the support. Adsorption onto carriers such as silica and alumina, which bind proteins much less strongly than highly-charged adsorbents, followed by cross-linking has resulted in stable immobilized enzyme preparations.

So far the immobilized systems described have had the enzyme bound to or trapped within a suitable insoluble carrier. Developments in the field of membrane technology have resulted in a different system for performing continuous enzyme reactions. Known as a membrane reactor, it combines the fast reaction of free enzymes with their continued use. In this process, the enzymic reaction is performed on one side of a porous membrane, the permeability of which allows the reaction products to pass through it but which prevents passage of the enzyme. Thus the products are continuously removed, while the enzyme is retained to act on fresh substrate flowing into the reactor.

Commercial applications of immobilized enzymes

Immobilized enzyme systems are currently being employed in commercial processes. In Italy milk is treated with β-galactosidase to reduce the level of lactose for consumption by people suffering from lactose intolerance (Pastore 1978). The milk is circulated through a reactor packed with the enzyme entrapped within cellulose acetate fibres until

70-80% of the lactose is hydrolyzed. It is necessary to carry out the process at a temperature of 8 °C or below to limit the growth of bacteria in the milk during the treatment, although this increases the time required for conversion of the lactose.

In a Japanese process, amino-acylase, an enzyme which catalyzes the hydrolysis of acyl groups attached to amines, is immobilized by adsorption onto diethyl aminoethyl cellulose or Sephadex and is used to facilitate the separation of L-amino acids from their biologically-inactive D-forms (Tosa *et al.* 1969). A racemic mixture of the two forms is acetylated and passed through a bed of immobilized amino-acylase where only the L-form is deacetylated, allowing chromatographic separation of the two. With time the enzyme is gradually displaced from the adsorbent (about 40% in 4 weeks) and this has to be replenished. The system gives a ten-fold increase in the efficiency of utilization of the enzyme, compared with a batch type operation using free enzyme.

Another commercial application of an immobilized enzyme system, again in Japan, is a process for the production of food sweeteners, which involves the isomerization of glucose to the much sweeter sugar, fructose, using the enzyme glucose isomerase (Takasaki *et al.* 1969). The enzyme used is an intracellular enzyme produced by the microorganism *Streptomyces albus* and can be liberated by disrupting its cells mechanically or by autolysis. Rather fortuitously it was found that heating the cells for 10 min at a temperature of around 70 °C stabilizes them against autolysis but does not affect the activity of the glucose isomerase. Thus the enzyme is conveniently retained within the cells, which are permeable to the glucose, and these are simply trapped in a filter bed through which the glucose solution is passed to effect the isomerization to fructose.

Two processes in the USA, one employing glucose isomerase bound to DEAE-cellulose ionically and the other with the enzyme adsorbed on a porous ceramic carrier, are currently being used for the commercial production of high-fructose syrups. The enzymic isomerization of glucose to fructose is the world's largest and most successful application of immobilized enzyme technology to date (Weetall 1976).

A number of smaller-scale, but nevertheless commercially viable, operations utilizing bound enzymes are being employed, principally in Japan, for the production of materials for use in the pharmaceutical and cosmetic industry. Compounds such as aspartic acid, L-citrulline, 6-amino penicillanic acid and urocanic acid are produced continuously using reactors consisting of bacterial cells immobilized in polyacrylamide. In each of these instances of successful application, the immobilization procedure is a simple one and the substrate is small.

Possible future applications

Work being conducted currently on the use of immobilized enzyme systems in commercial processes indicates that further applications are likely in the near future. For example, bound enzymes have a potential application in the production of sweeteners from cheese whey.

Cheese making is essentially a de-watering process which is initiated by the proteolytic cleavage of a single peptide bond in a stabilizing component, κ-casein, in the milk protein system. The splitting of this component triggers off the coagulation of the milk and the spontaneous shrinking of the resultant gel to expel its liquid. Thus the bulk of the protein and fat are obtained in a concentrated form, which is cheese. The remainder of the milk, the whey, contains most of the water, soluble proteins, minerals and lactose with the latter accounting for about 70% of the dissolved solids. Disposal of the large amounts of this by-product presents difficulties. The introduction of legislation prohibiting the discharge of untreated whey into streams and rivers and the costs associated with the treatment of whey to prevent pollution, have resulted in considerable effort being devoted to seeking methods for recovering and utilizing its components, thereby reducing its potential for pollution, while at the same time increasing the financial return from the milk.

The proteins in the whey can be isolated by heat treatment or ultrafiltration for use in a variety of foods and the lactose obtained by crystallization. However, uses for lactose are limited. Although it is the equal of sucrose as a source of energy, its solubility and sweetness are so low as to preclude its use as a sweetener in the way that sucrose has become accepted. Glucose and galactose on the other hand, are much more soluble than lactose and much

sweeter, about 70% as sweet as sucrose. Hydrolyzing the lactose to glucose and galactose provides the means for producing sweeteners with potential applications in confectionery, jams and canned fruits.

A continuous system for the production of glucose-galactose syrups from ultrafiltered, de-ionized cheese whey, employing β-galactosidase attached to porous silica for the conversion of the lactose, has been evaluated on a semi-commercial scale (360-500 l/h) in France and England (Dohan *et al.* 1979). The enzyme reactor was operated for 16–20 hours per day, 5 days per week over a period of 6 months. A conversion level of 80% was maintained throughout the trials by increasing the temperature of reaction to compensate for the small loss of activity observed in the bound enzyme. It was concluded from the trials that the process was ready for commercial exploitation. Encouraging results have also been obtained from pilot-scale trials using β-galactosidase in a membrane reactor to hydrolyze the lactose in ultrafiltrate from milk.

Other potential applications for immobilized enzymes in the food industry have been studied and show promise of success (Olson and Richardson 1974). Starches and proteins can be hydrolyzed continuously using immobilized amylases and proteases respectively. Since starches and proteins are large polymers, the technique of immobilization by entrapment within a matrix cannot be used for enzymes intended to treat these materials unless they are first partially degraded to permit access to the enzyme. A membrane reactor has been used to hydrolyze starch with amylases. Proteases covalently bound to a copolymer of ethylene and maleic anhydride have been used during the manufacture of beer to hydrolyze protein constituents, which would otherwise aggregate on chilling and produce a haze in the product.

Another area where a considerable effort has been devoted to utilizing the potential advantages of immobilized enzymes is in the manufacture of cheese. As mentioned earlier, the manufacture of cheese commences with a specific and limited proteolysis of the κ-casein in the milk. The use of a bound-enzyme reactor to effect the necessary proteolysis affords the possibility of developing a continuous milk-coagulating system for integration into the cheese-making process. Due to the particular sensitivity of the peptide bond in κ-casein it is cleaved quite rapidly at 15 °C while at this temperature the subsequent clotting of the milk is extremely slow. Thus by passing the milk through an immobilized protease reactor at this temperature the enzymic treatment can take place without the danger of the milk coagulating in the reactor. The treated milk then gels almost immediately when subsequently warmed to 30 °C.

The susceptible bond in κ-casein is rapidly split by a wide range of proteolytic enzymes but many of these are unsuitable for conventional cheese-making as their general proteolytic activity is so high that the small amount retained in the cheese curd produces excessive breakdown of the proteins present and thereby has a deleterious effect on the flavour and/or structure of the final cheese. However, such proteases could possibly be utilized in the immobilized form as they would be retained in the reactor and play no part in the maturation of the cheese.

A major constraint placed on the action of an immobilized protease in such a system results from the fact that the casein proteins in milk are assembled into large complex structures termed casein micelles, each of which contains many thousands of protein molecules. Although these complexes are sufficiently porous to allow penetration of proteases such as chymosin and pepsin to cleave the κ-casein located within them, the action of an immobilized enzyme is limited to the κ-casein that is accessible at the surface of the complex or free in the serum of the milk. Nevertheless, the use of pepsin bound to porous glass has been reported to yield a milk gel with physical characteristics very similar to those of a gel formed by the free enzyme (Ferrier *et al.* 1972). In another study, it was reported that cheese prepared by using an immobilized protease from *Bacillus subtilis* to initiate coagulation differed little from control cheese made with rennet extract (Ohmiya *et al.* 1979). In both cases, the activity of the immobilized enzyme preparations decreased rapidly when in contact with the milk.

Analytical applications

In addition to applications for processing biological material, enzymes are being used increasingly in analytical procedures, particularly clinical analysis, where their

high catalytic capabilities and precise specificities can be employed to advantage (Gray *et al.* 1977). A suitable enzyme is chosen to catalyze the transformation of the component being sought.and the concentration of one of the products of the enzymic action is determined by a suitable method. For example, glucose oxidase catalyzes the oxidation of glucose to gluconolactone with the concomitant formation of hydrogen peroxide. By determining the hydrogen peroxide formed, a measure of the glucose originally present is obtained. Combinations of enzymes may be needed to provide suitable products for measurement. For instance, lactose can be determined by using β-galactosidase to hydrolyze it to glucose and galactose and glucose oxidase to oxidize the glucose formed. The concentration of hydrogen peroxide then indicates the level of lactose in the sample.

As the enzymes used in these analytical techniques must be highly purified to ensure the selectivity of the analysis, and hence are costly, there exists a considerable incentive for employing these catalysts in an immobilized form. More analyses can be performed per unit of enzyme and immobilization offers the possibility of integrating the enzymic reaction into an automated procedure. Many such methods are in use today.

Immobilized enzymes have been combined with newly-developed selective electrochemical sensors to form what are termed enzyme electrodes. These are still in the early stages of development and the results to date suggest that they will greatly facilitate the analysis of biological materials. The enzyme is bound to or trapped in a porous membrane attached to the detector which responds to one of the products of the enzymic action. An example is an enzyme electrode consisting of urease immobilized on an ammonium ion electrode for determining urea in biological fluids. For the greatest accuracy the sensor should be as selective towards the product of the enzymic reaction as the enzyme is towards its substrate. So far this has not been achieved completely but considerable progress has been made in the field of selective electrochemical sensors and it will not be long before a number of highly accurate and specific enzyme electrodes are available. Another technique which shows great promise is to combine a bound enzyme with a very sensitive thermistor and measure the heat produced during the enzymic reaction (Mossbach and Danielsson 1981). This obviates the need for a specific method to detect the reaction products.

The number of enzymes known and characterized, and in many cases isolated and purified, is now over 2000 with new ones being continually added to the list as we discover more about the chemical reactions taking place in living organisms. To date, we have exploited the unique properties of only a relatively small fraction of these natural catalysts. As they become more readily available and cheaper through, for example, the use of microorganisms for their production, combined with modern fermentation technology and genetic engineering, the range of applications will increase and immobilized enzyme systems will play an increasing part in the exploitation of their remarkable capabilities.

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Some flavours which industry could well do without
Case studies of industrial problems*

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Introduction

Every year the Australian food industry receives complaints from consumers concerning off-flavours in processed and packaged foods. Fortunately many of these are only isolated cases, but on occasions major problems are encountered which may involve a day's production or even a whole season's harvest. The chemicals responsible for these off-flavours are often volatile organic compounds which can be present in the food at concentrations of less than 1μg kg^{-1}. Because of their low concentrations and the complexity of food flavours, the identification of compounds responsible for off-flavours is an exacting and, often, time-consuming exercise. Furthermore, the determination of the origin of such compounds often requires maximum cooperation between the analyst, members of the food industry and occasionally the primary producer.

Off-flavours in foods derived from microbial spoilage, fungal sources and oxidative breakdown have always been a problem, only incompletely alleviated by modern methods of food handling. In the past, consumers accepted these undesirable flavours as a part of life, and either silently discarded the item or disguised the offending flavour by the addition of strongly flavoured additives. Today, through intense competition within the food industry and through the education of the consumer, notably that he does not have to accept products of inferior quality, food manufacturers have become very sensitive to off-flavour problems in their products. Moreover, in the past 30 years many new synthetic chemicals have been introduced into the environment and some of these have resulted in the indirect introduction into foodstuffs of potent new classes of off-flavours. In the next decade, as these synthetic chemicals show up in a wide variety of raw materials, as either non-degraded compounds or metabolites, the food industry could find itself confronted with a whole new range of off-flavour problems.

Modern foods, processed or fresh, occasionally are affected by off-flavours described as medicinal, disinfectant-like, mouldy, earthy, catty, painty or solvent-like, faecal, metallic and cardboard-like. These off-flavours may be introduced into the food by absorption from the air, from water used in processing, from packaging materials and from the environment in which the food has been grown, processed or stored. Many of the compounds causing off-flavours are man-made chemicals, and for this reason this paper will concentrate principally on undesirable flavours derived from synthetic chemicals. It will briefly describe techniques used to identify such compounds, discuss the types of compounds most frequently encountered and indicate some simple precautions that can reduce the risk of incorporating these compounds into food.

Techniques

In the Food Research Laboratory, North Ryde, techniques developed to study the chemical composition of food flavours have been adapted to the isolation and identification of compounds responsible for off-flavours. The procedure involves the initial isolation of a concentrated flavour

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extract, followed by fractionation of the extract into its individual components. Adsorption of headspace volatiles onto porous polymers (Murray 1977) provides an excellent isolation and concentration procedure which yields extracts free from solvent contamination. This technique is normally preferred over other procedures; however, on occasions extraction of the volatiles into a suitable solvent, using a modified Likens and Nickerson vapour extraction apparatus (Schultz *et al.* 1977), can be favoured where contamination with solvent does not invalidate the identification procedure. Fractionation of the extract is normally achieved by high-resolution capillary gas chromatography (Murray and Whitfield 1975) although with some problems high performance liquid chromatography may be preferred (Sha and Stanley 1983).

The next step, the identification of the specific component(s) responsible for the off-flavour, can present a most difficult assignment. Fig. 1 shows the position in the gas chromatogram of the three chemicals, present in minute quantities, responsible for a mouldy off-flavour in dried fruit. Gas chromatography combined with the sniffing of the effluent gas has to date proved to be the most efficient and reliable technique for the detection of such compounds in complex mixtures (Whitfield *et al.* 1982a, 1983a). If the sniffing procedure is unsuccessful, the extract is fractionated by gas chromatography or by high performance liquid chromatography and the resultant fractions are dissolved in water and the solutions assessed for the off-flavour by taste and odour (Bemelmans and te Loo 1976). Occasionally, when an off-flavour is obscure, it is necessary for a representative of the company concerned to work with the analyst and assist in defining the odour and taste responsible for the offending flavour, most usefully by participating in the sniffing runs.

Once the compound responsible for the off-flavour has been detected the task of establishing its structure begins. The maximum quantity of such compounds

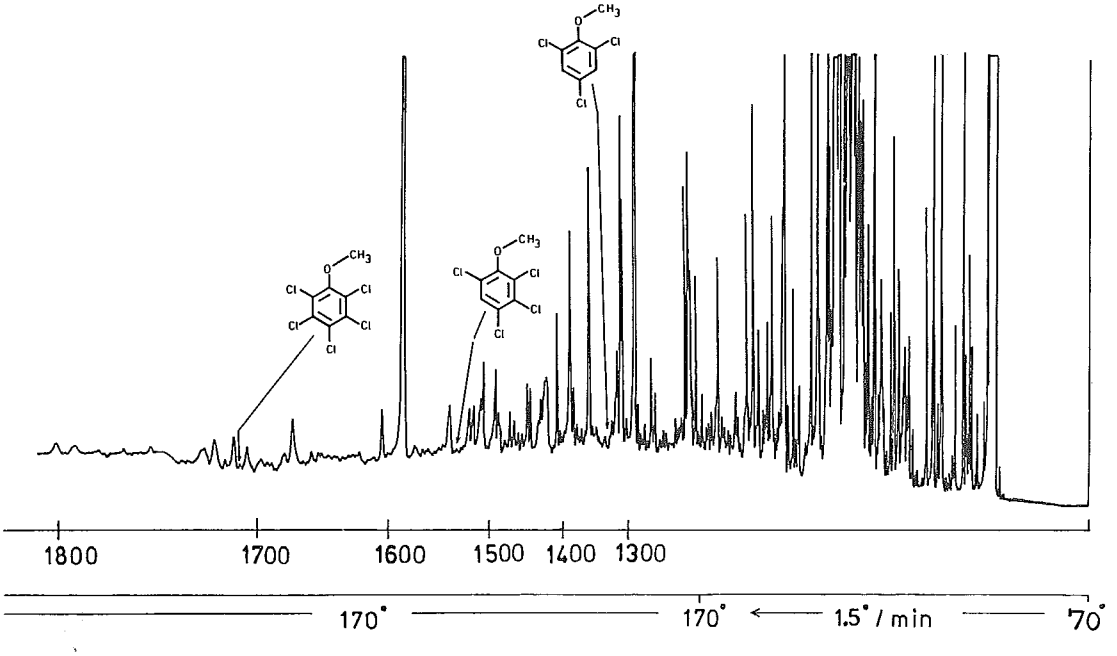

Fig. 1. Gas chromatogram of steam volatile extract of dried fruit with a mouldy off-flavour. Cause of off-flavour: 2,4,6-trichloroanisole and 2,3,4,6-tetrachloroanisole. Pentachloroanisole is also present but at a concentration below its recognition threshold. Capillary column — stainless steel WCOT (150 m long, 0.75 mm i.d.) coated with Silicone OV-101. Carrier gas — Helium, flow rate 5 ml min^{-1}. Conditions — oven temperature: temperature programmed 70° to 170°C at 1.5°C min^{-1} then held at 170°C for 3 h.

which the analyst can expect to isolate is 1 μg and the only instrumental techniques which can provide definitive structural data with this quantity of material are mass spectrometry, reaction gas chromatography and Fourier transform ^{1}H-nuclear magnetic resonance and infrared spectroscopy (Whitfield *et al.* 1982b). Fortunately, structures have been established for many off-flavour compounds which can then be identified by high-resolution gas chromatography and mass spectrometry alone, by reference to the authentic compound (Whitfield *et al.* 1983b).

Once the identity of the compound has been established, very sensitive techniques based on combined gas chromatography-mass spectrometry can be applied to detect the offending compound in other food samples and estimate its concentration. For example, mass chromatography, which offers the ultimate in selectivity, has been used to monitor the occurrence of trimethylarsine, a compound with an intense garlic flavour, in species of Australian prawns (Whitfield *et al.* 1983a); multiple ion monitoring, which provides maximum sensitivity, is currently routinely used in the U.S.A. to establish the concentration of 2-methylisoborneol, a compound with an intense earthy-mouldy flavour, in municipal water supplies (McGuire *et al.* 1983).

Types of compounds responsible for off-flavours

The compounds responsible for off-flavours belong to many and varied chemical groups and only a few examples can be presented here. Synthetic compounds and their metabolites that are known to cause major problems in industry are shown in Fig. 2. The chlorophenols, of which 6-chloro-2-methylphenol (6-chloro-*o*-cresol) (Patterson 1972) is a notable example, are usually responsible for medicinal or disinfectant-like flavours; their methyl ethers, such as 2,3,4,6-tetrachloroanisole (Engel *et al.* 1966), produce mouldy and musty off-flavours in foods at extremely low concentrations. Paint, plastic and printing ink flavours are mainly due to the presence of benzenoid hydrocarbons, such as styrene and naphthalene, and compounds which contain ester or ketonic functional groups, for example, methyl vinyl ketone. Catty flavours are essentially due to sulphur-containing compounds, such as

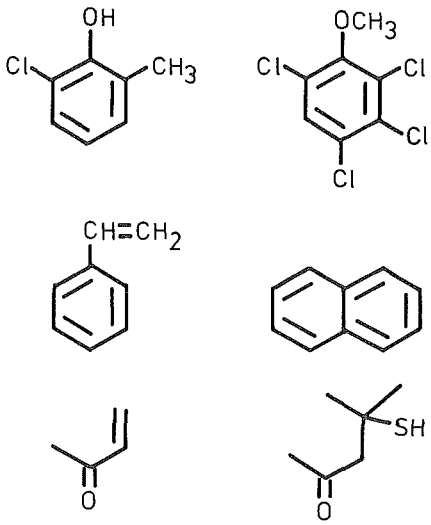

Fig. 2. Synthetic compounds frequently associated with off-flavours.

4-mercapto-4-methylpentan-2-one (Patterson 1969). Some compounds responsible for off-flavours derived either from microbial contamination or as a result of oxidative decomposition are shown in Fig. 3. Faecal flavours can be due to the presence of the bacterial metabolite skatole (Whitfield *et al.* 1982b), and earthy flavours as a result of contamination of food with the *Actinomycetes* metabolites, geosmin, 2-methylisoborneol and 3-isopropyl-2-methoxypyrazine (Whitfield *et al.* 1983b). Cardboard flavours can be due to saturated and unsaturated aldehydes, such as (2*E*), (4*E*)-octa-2, 4-dienal, and metallic flavours to unsaturated ketones, such as (5*Z*)-octa-1, 5-dien-3-one (Swoboda and Peers 1977); both types of carbonyl compounds are produced in foods by oxidative processes.

Over recent years the most frequent and serious problems referred to this Laboratory by the Australian food industry have been due to trace quantities of chlorophenols in packaged, canned and bottled foods and chloroanisoles in cold stored meat and packaged dried foods. The off-flavours produced by these compounds are generally easily detected by most consumers and will always lead to complaints from fastidious customers. With both chlorophenol and chloroanisole contaminations, two types of problems can be distinguished. The first type

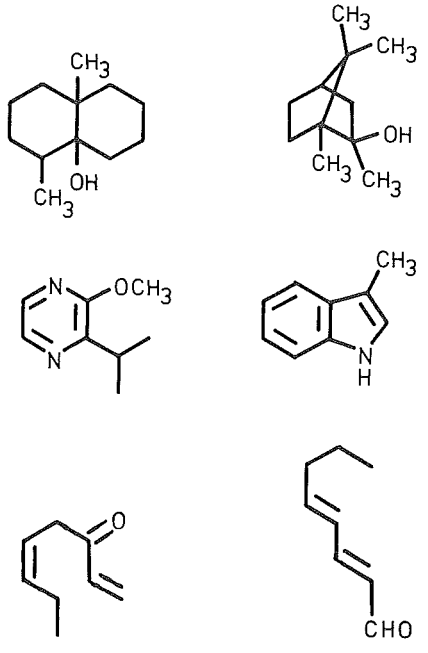

Fig. 3. Naturally occurring compounds frequently associated with off-flavours.

arises from in-plant reaction between chlorine and phenol, both fairly common components of the food processing environment; this reaction yields only the monochlorophenols, the 2,4- and 2,6-dichlorophenols and 2,4,6-trichlorophenol which in turn can yield the corresponding chloroanisoles on microbial methylation. The second type of problem results from contamination with industrially-prepared chlorophenols and the related chloroanisoles, and such contamination is readily distinguished by the occurrence of pentachlorinated and tetrachlorinated compounds which can only arise when very special conditions are used for the reaction between chlorine and phenol. The compounds responsible are not, however, easily identified or their origins determined, as shown by the following examples of industrial problems recently investigated in the North Ryde Laboratory.

Chlorophenols and disinfectant-like off-flavours

Analytical techniques

At low concentrations chlorophenols are difficult compounds to identify and estimate unless a derivative is prepared. For this reason, in our early investigations, extracts containing chlorophenols were treated with the methylating reagent diazomethane before analysis by combined gas chromatography - mass spectrometry (cf. Gee *et al.* 1974). Today, the chlorophenol components of the extracts are usually acetylated to give the acetate derivatives before analysis (Korhonen and Knuutinen 1983). However, as a result of the introduction of special gas chromatography columns (White and Parsley 1979) and C-18 reverse phase column packings for high performance liquid chromatography (Sha and Stanley 1983), some analyses can be performed on extracts without derivatization.

In-plant chlorination of phenols as a source of chlorophenols

A frequent cause of chlorophenol contamination in foods to which water has been added results from the reaction of phenolic compounds with heavily chlorinated mains water, the phenols being introduced as in-plant contaminants. At some concentrations aqueous chlorine solutions can react spontaneously with simple phenols, such as phenol and the cresols, to produce compounds with intense disinfectant-like flavours (Burttschell *et al.* 1975). For example, phenol can yield a mixture of 2,4- and 2,6-dichlorophenol and 2,4,6-trichlorophenol (Fig. 4), any one of which will cause off-flavours in foods at very low concentrations, since they have taste thresholds in water of 0.3, 0.2 and 2 $\mu g\ kg^{-1}$ respectively (Dietz and Traud 1978).

Plastic fittings and resins as a source of phenol — In our experience the most troublesome sources of phenol in the processing line are phenol-based resins and paints used as protective coatings for the surfaces of liquid storage tanks and water filtration vessels. When the resin has not been suitably cured, or when the formulation has left a slight excess of free phenol, this surface can become an embarrassing source of off-flavours after contact with chlorinated mains water. Chlorophenol contamination can sporadically occur when chlorinated water is left in contact with plastic fittings and hoses containing free phenol. Sporadic off-flavours may then develop since food in contact with the initial stream of water will be affected more than subsequent batches where the

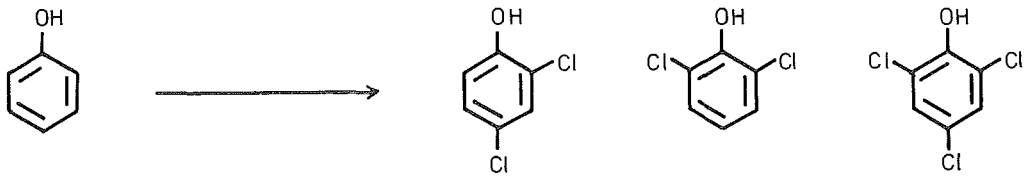

Fig. 4. In-plant production of chlorophenols.

level of chlorophenols may be negligible due to dilution with fresh water.

Two problems, involving a canned carbonated beverage and a reconstituted fruit juice, serve to illustrate how simply chlorophenol off-flavour problems can develop in processing plants. Examination of the off-flavoured products showed that each contained trace quantities of 2,4- and 2,6-dichlorophenol. Based on the assumption that these compounds were derived from in-plant chlorination, a search was made for the source of the free phenol. With the beverage this was traced to a newly-installed plastic fitting and with the reconstituted juice to a phenol-based resin used to protect the surface of a filtration vessel; removal of the fitting and the vessel from the processing line overcame the problem.

Boiler water as a source of phenol — Another occasional source of phenols and chlorophenols in food plants is steam used for heating brines and syrups by direct injection. A disinfectant off-flavour in cans of sliced fruit recently presented a most interesting problem which illustrates the essential need for industry-analyst collaboration. Initially the company suspected a plastic fitting as the source of the off-flavour, and preliminary trials showed that chlorinated water in contact with this item gave a strong chlorophenol taste reminiscent of the off-flavour. Investigations showed that the water contained a mixture of 2,4- and 2,6-dichlorophenol and 2,4,6-trichlorophenol, that the fitting contained free phenol and that the above chlorophenols had deposited on its surface. In the product itself only 2,4,6-trichlorophenol was detected in minute concentrations. Removal of the fitting from the line did not, however, stop the contamination.

Subsequent in-plant studies showed that the only products affected by the off-flavour were those which were subjected to live steam injection during the pre-process sterilization procedures. A closer examination of the process showed that a phosphate chemical, which contained lignin sulphonate, was routinely added to the boiler water to control hardness. Lignin sulphonate is a non-homogeneous product from the sulphite liquor wastes of paper mills; its major component is a complex phenolic compound which may give rise to polyphenols on heating at high pH but, more important, it may contain, as components, simpler phenols which yield phenol itself under these conditions. Laboratory experiments showed that when the phosphate additive was autoclaved for 1 h at pH 10.5 to 11 the steam distillate produced a chlorophenol-type off-flavour when treated with water containing 8 to 10 mg l^{-1} of chlorine. 2,4- and 2,6-Dichlorophenol and 2,4,6-trichlorophenol were detected in this solution and phenol in the initial steam distillate, providing the link between the lignin sulphonate and the off-flavour compounds. The off-flavour problem ceased with the replacement of the phosphate additive by one free of lignin sulphonate.

Algaecides as a source of chlorophenols

Another instance of chlorophenol contamination in foods to which water has been added involved the accidental introduction of an algaecide into a processing line. When the company first detected a disinfectant-like off-flavour in one of their canned products, a source of chlorophenols was looked for in the factory. It was soon established that the most likely source was sodium pentachlorophenate used as an algaecide in the plant's refrigeration condenser water. The algaecide was found to contain in addition to pentachlorophenol significant concentrations of 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol (Fig. 5). The same three chlorophenols were subsequently isolated from the off-flavoured product. Pentachlorophenol at concentrations below

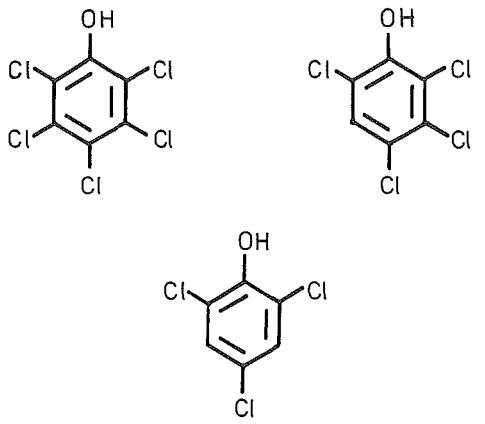

Fig. 5. Chlorophenols frequently present in algaecides, fungicides and anti-sapstain preparations.

30 μg kg^{-1} does not produce a recognizable off-flavour but the other two compounds can be detected by taste at a concentration of only 2 μg kg^{-1}. Quantitative studies indicated that the compound responsible for the off-flavour was 2,3,4,6-tetrachlorophenol.

In-plant investigations showed that, as a result of a loss of water pressure, coupled with a series of interrelated mechanical faults, a small quantity of refrigeration condenser water had been sucked back into the water supply to the processing line. Modifications to the plumbing in this section of the factory made certain that this series of events would not occur again.

Fungicides as a source of chlorophenols

The ubiquity of pentachlorophenol in the industrial environment and its contamination by highly flavoured impurities has resulted in its involvement in a wide range of off-flavour problems in stored and packaged dried foods.
Pentachlorophenol mixtures are used as fungicides in wood preservatives, adhesives and glues and in anti-sapstain preparations. The chlorophenols present on the surfaces of treated timber may be sorbed into foods through the packaging material from such items as pallets, shelving and timber frames and even from the walls of store-rooms and warehouses. Adhesives used in packaging materials, for example, cardboard boxes and paper bags, can also be a source of serious chlorophenol contamination. It has also been claimed (Goldenberg and Matheson 1975) that "off-flavours" may be introduced into foods from packaging materials either as a result of the presence of preservatives added to re-pulp waste paper mix or to compounds associated with printing inks in waste paper.

Two recent studies serve to illustrate how easily chlorophenols are introduced into foods. In one, evidence indicated that chlorophenols isolated from bulk chocolate stored in a warehouse were derived from pallets treated with a wood preservative. In the other, packets of biscuits stacked on end in cardboard boxes were found to possess a disinfectant-like off-flavour; for example, only biscuits at each end of the packet were affected by the off-flavour. Since much higher concentrations of the chlorophenols were found in the lids and bases than in the sides, the adhesives used in the final assembly of the boxes were the logical source of the contamination.

Agrichemicals as a source of chlorophenols

Other potential sources of chlorophenols in foods are chlorinated agrichemicals such as 2,4-dichlorophenoxyacetic acid (2,4-D), which frequently contain a variety of chlorophenols as major impurities (Fig. 6). Although food contamination from these sources may be expected to occur by direct contact and sorption of the chemicals by the agricultural raw material, we have never established such a relationship. However, airborne contamination by agrichemicals has been encountered in a problem involving soft

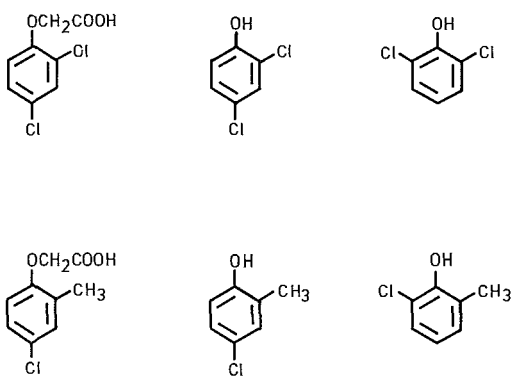

Fig. 6. Chlorophenol impurities frequently present in herbicides.

drink cans stored in a warehouse inadvertently sited near an agrichemical factory. A manufacturer of soft drinks experienced a sporadic medicinal-type off-flavour in several of his products over two successive summers. The company had successfully demonstrated that the off-flavour was not in the product before canning and that the off-flavour regularly occurred in only a small proportion of cans on the processing line. Analysis of the affected product failed to establish the identity of the off-flavour compound although all concerned with the problem agreed that it was probably a chlorophenol. The company eventually established that empty cans which had been incompletely covered by the cardboard spacers when stacked on pallets often gave an off-flavoured product. On a visit to the warehouse where some of the cans were stored in the off-peak periods it was noted that the neighbourhood smelt strongly of chlorophenols and that an agrichemicals plant was situated nearby. When wind blew from the direction of the plant towards the warehouse, principally during the spring and early summer months, volatile chemicals were blown into the storage area where they could be sorbed onto the surfaces of exposed cans. The can suppliers subsequently ceased using the warehouse for storage and the off-flavour was no longer detected. Contaminated cans were reclaimed by restoving to drive the odour out of the linings where it had been sorbed. In addition, many cardboard spacers had to be destroyed as it was proved that they could be a continuing source of contamination. Although not identified in the off-flavoured product, 6-chloro-2-methylphenol is suspected to be the compound responsible for the medicinal off-flavour. This compound is the most potent chlorophenol yet identified and can be detected in water at a concentration of only 8 x 10^{-5} μg^* kg^{-1} (Griffiths and Land 1973). 6-Chloro-2-methylphenol and 4-chloro-2-methylphenol are frequently major impurities in the herbicide 4-chloro-2-methylphenoxyacetic acid (Fig. 6) which at that time was being used in formulations produced by the agrichemicals plant.

* 1 microgram (μg) = 10^{-6} gram (g)
1 nanogram (ng) = 10^{-9} gram (g)
1 picogram (pg) = 10^{-12} gram (g)

Chloroanisoles and mouldy-like off-flavours

Analytical technique

Chloroanisoles may be detected in foods by their aromas at extremely low concentrations and for this reason a highly sensitive instrumental technique is essential for their identification and estimation. High-resolution gas chromatography coupled to a mass spectrometer operating in the multiple ion monitoring mode has, to date, proved the only facility with the selectivity and sensitivity to analyse these compounds in foods. For example, in recent analyses of wine affected by a musty off-flavour the minimal detectable quantity of 2,4,6-trichloroanisole was 2–5 pg*, corresponding to a concentration in wine of only 2–5 ng* kg^{-1} (Buser *et al.* 1982).

Chlorophenols as a source of chloroanisoles

Chloroanisoles, which impart a mouldy off-flavour to foods, are readily produced by fungal methylation of the parent chlorophenols (Fig. 7). Fungicides, wood

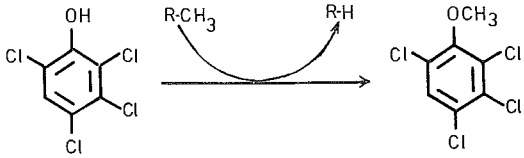

Fig. 7. Fungal methylation of chlorophenols.

preservatives and anti-sapstain preparations containing chlorophenols (Fig. 5) are therefore frequent sources of the extremely potent chloroanisoles (Fig. 8) and indeed any source of chlorophenols may under suitable conditions give rise to chloroanisoles. Studies carried out at the Agricultural Research Council, Food Research Institute, Norwich, U.K., showed that many fungi, including *Aspergillus* and *Penicillium* species, can methylate chlorophenols (Curtis *et al.* 1974). Methylation is one of several biological processes avilable to microorganisms for the detoxication of chlorophenols (Cserjesi and Johnson 1972). Some chloroanisoles produced by the methylation of pentachlorophenol-based fungicides include 2,4,6-trichloroanisole, 2,3,4,6-tetrachloroanisole and pentachloroanisole (Fig. 8). These compounds can be detected as off-flavours in water at concentrations of

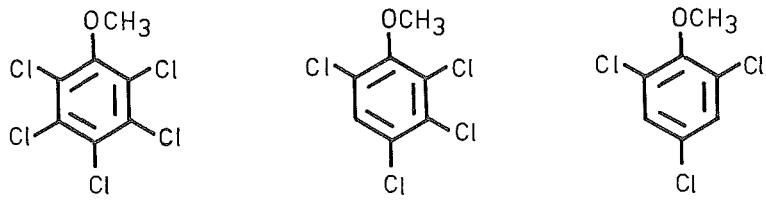

Fig. 8. Major chloroanisoles produced by fungal methylation of pentachlorophenol fungicides.

3×10^{-5}, 4×10^{-3} and 4 $\mu g\ l^{-1}$ respectively (Griffiths 1974).

Adhesives as a source of chlorophenols. — The sodium salt of pentachlorophenol, which frequently contains as impurities 2,4,6-tri- and 2,3,4,6-tetrachlorophenol, has been used extensively in starch and animal protein based glues to provide antimicrobial protection during their manufacture, storage and service life (Freiter 1979). The first problem we encountered involving chloroanisoles, which were apparently derived from chlorophenols in adhesives, concerned dried fruit exported from Australia to Europe. The product was reported to possess a mouldy off-flavour on arrival. This off-flavour was particularly apparent when the fruit was used in the production of baked products such as buns and speciality breads. The fruit had been packaged in cardboard boxes and the product shipped overseas in metal containers. Only a few boxes per container were affected and only a small proportion of the containers shipped developed the problem. No complaints had been received concerning fruit packaged at the same time but sold in Australia.

Analysis of the off-flavoured fruit led to the isolation of three compounds with distinctive mouldy aromas, subsequently identified as 2,4,6-trichloroanisole, 2,3,4,6-tetrachloroanisole and pentachloroanisole. Initially it was suspected that the contamination was occurring before packaging, but the analyses of all materials used in the production and handling of the product failed to identify a source of the offending compounds. In the laboratory, however, it was noticed that the off-flavour in the fruit was strongest at the upper layer of each of the affected boxes examined. Analysis of the boxes showed that they contained the three chloroanisoles and that the concentration of these compounds was far greater in the lids than in either the sides or bases. Furthermore, the three corresponding chlorophenols were also in the boxes but at about 100 times the concentration of the chloroanisoles. Again, the concentration of these compounds was greatest in the lids of the boxes. Analysis of the adhesives used in the construction of boxes and in the sealing of the packed boxes showed that some of these contained high concentrations of the chlorophenols. The link between the chlorophenols and chloroanisoles was provided by storing samples of cardboard contaminated with chlorophenols under conditions of high water activity for several weeks. In some cases the distinctive aroma of the chloroanisoles was readily detected. Subsequent examination of the inner corrugated surfaces of a box contaminated with chloroanisoles led to the isolation of several *Aspergillus* and *Penicillium* species (Pitt, unpublished data). As these groups of fungi have been shown to methylate chlorophenols in wood shaving litter in chicken runs (Curtis *et al.* 1974), this finding strengthens the connection between chlorophenols and chloroanisoles in the packaging material.

A hypothesis may therefore be proposed to account for the sporadic occurrence of the mouldy off-flavour in the exported product. The chlorophenols introduced into the boxes by way of the adhesives are the precursors of the off-flavour compounds. The conversion of these phenolic compounds to the chloroanisoles may then be achieved by microbial methylation. It is well known that, when boxes are taken from a warm warehouse and placed in a cold metal shipping container, moisture will condense on the roof and may then precipitate onto the uppermost surfaces (Middlehurst and Parker 1974). Cardboard at high water activity and a temperature of about 25–35 °C provides conditions most conducive to the growth of fungi with the ability to methylate chlorophenols (Curtis *et al.* 1974).

As a variation on this source, multi-walled paper sacks were recently shown to be

responsible for the contamination of imported cocoa powder, part of a shipment having a particularly disagreeable off-flavour. The powder itself had a distinctive chlorophenol aroma and beverages prepared from it had an intense mouldy off-flavour with a strong disinfectant after-taste. Analysis of the cocoa showed that it contained 2,4- and 2,6-dichlorophenol and 2,4,6-trichlorophenol together with the corresponding chloroanisoles. As the tainted material appeared to occur in only one particular section of the sacks near the side seams, it was likely that the source of the contamination was the glue used in sealing. Seam sections of the suspect sacks were found to contain 2,4- and 2,6-dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol, together with the corresponding chloroanisoles, in excess of one hundred times the concentration present in sacks which contained cocoa of acceptable flavour. Examination of all sacks in the shipments showed that the problem was associated with produce from only one supplier; the solution was simply to discard all material from this supplier.

In-plant chlorination as a source of chlorophenols. – Quite a different source of chloroanisole contamination was encountered in an investigation of a mouldy off-flavour in meat exported from Australia. The problem was brought to us after several overseas laboratories had failed to identify the compound responsible for the off-flavour. The uncooked meat had no distinguishing aroma; however, after frying, the meat had a characteristic mouldy aroma and was quite inedible. Analysis of the meat eventually showed that it was contaminated with trace quantities of 2,4,6-trichloroanisole (CSIRO Division of Food Research 1982). Determining the origin of this compound proved a difficult task. It was first traced to a block of four chillers in one meatworks. These chillers had a mouldy aroma and the chloroanisole was present in condensate on the refrigeration coils. Condensate was observed to drip occasionally onto the carcasses where the off-flavour compound was readily absorbed into the fatty tissue. A false ceiling was built to overcome this source of contamination. However, although the intensity of the off-flavour decreased, the problem still existed since carcasses held in these chillers for extended periods had an intense mouldy off-flavour. Analyses of the atmospheres taken from within the chillers and immediately outside them indicated that the chloroanisole was present in both areas in minute concentrations. The insulation within the walls of the chillers was eventually identified as the likely source of contamination since this material was heavily contaminated with 2,4,6-trichloroanisole. It was therefore suggested that the water which periodically flooded the cavity walls transported the chloroanisole through the relatively porous brick walls to the floor of the cold store, situated below the chillers, where it accumulated in the ice. Once the compound had reached the outside wall of the chillers, it was easily picked up by the air stream and circulated through the whole area where it was readily absorbed by the hanging carcasses. The method of formation of the off-flavour compound is uncertain. However, it is possible that it was produced in a two step synthesis involving inadvertent chlorination of phenol, present as a minor component in the insulation material, by chlorine used in the sterilization of the chiller rooms and corridors; the product, 2,4,6-trichlorophenol then underwent fungal methylation to give 2,4,6-trichloroanisole. The flavour problem ceased with the closing down of the contaminated chiller complex. As refurbishing the existing facility would not guarantee the complete removal of the source of the chloroanisole, the decision was made to build a new chiller cold-store complex.

Precautions which may prevent contamination with chlorophenols and chloroanisoles

From the above examples it is obvious that the Australian food industry is currently confronted with a major screening program if it is to prevent the accidental introduction of odorous chemicals into its products. However, the following precautions may assist processors in reducing the incidence of contamination with such compounds as the chlorophenols and chloroanisoles. Processors may find the following list of precautions of value:

(a) Where chlorinated water is used in the processing line, or anywhere in the plant, thoroughly check all newly installed equipment and building materials for sources of phenolic compounds. Do not accept the

installer's assurance.

(b) Where plant water is recycled to the boilers, make certain that it does not contain simple phenols or chlorophenols. In particular, establish that boiler water additives and waste water from fruit and processing lines cannot provide a source of phenol when heated for prolonged periods in the boilers.

(c) Make certain that any chlorophenol-based algaecides and disinfectants used in the plant cannot be accidentally introduced into the processing line.

(d) Take precautions to prevent stored packaged foods from coming in contact with timber previously treated with chlorophenol-based wood preservatives and anti-sapstain preparations.

(e) Check that the levels of chlorophenols in packaging materials, including adhesives, are below concentrations that may cause off-flavour problems. This may conveniently be achieved by storing the food with the packaging material for 48 h and assessing the food for off-flavour by a taste panel (Standards Association of Australia 1983).

(f) Take care that packaged products are not stored in contact with materials containing chlorophenols, even at extremely low concentrations. The product can become contaminated with highly odorous chloroanisoles if storage conditions favour the growth of moulds able to methylate chlorophenols.

(g) Choose insulation materials for the walls of chillers and cold-stores with great care, making certain that they do not contain phenol and have not been treated with chlorophenol fungicides.

(h) Do not store containers and bulk supplies in warehouses sited near chemical plants.

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News from the Division

Appointments

Dr J. H. B. Christian, Chief of the Division and Mr J. F. Kefford, Honorary Research Fellow, have become Member of the Board of Trustees and Executive Secretary respectively of the Australian Branch of the International Life Science Institute (ILSI).

ILSI is a non-profit organization developed to sponsor scientific research in food and nutrition, conduct international meetings and forums, act as a repository for scientific information, and to promote the rapid communication of new scientific findings. Based in Washington, D.C., ILSI is comprised both of industry and non-industry representatives. Non-industry members include university scientists, members of scientific foundations and government officials. Financial support comes from member companies which pay a basic membership fee and contribute to the support of ILSI activities.

Mr Ross Allen, Senior Laboratory Craftsman (Carpenter) at FRL has been appointed Apprentice Master and Co-ordinator for the Northern Region (NSW/Qld/ACT/NT), CSIRO.

The Organization employs on average nearly 100 apprentices and the two Apprentice Masters are responsible for providing Divisions with advice and assistance in the selection, induction, training, rotation and counselling of apprentices. They further advise CSIRO's Technical and Trades Staff Development Advisory Committee on the overall approach to apprentice training.

Collaborative programs

Dr J. K. Raison of FRL's Plant Physiology Group is visiting the USA under the US/Australian Agreement for the Advancement of Science, and working at Stanford with Dr J. Berry in the Plant Biology Division of the Carnegie Institution of Washington, on the detection of membrane lipid phase changes in living tissues.

From April 1984 Dr Raison will spend some five weeks on a collaborative research program with Dr Norio Murata at the University of Tokyo School of Biological Sciences. This is a joint program between the Australian Academy of Science and the

Japanese Society for the Promotion of Science, which has already enabled Dr Murata to visit the PPG for three weeks during 1983. The research is on the role of membrane lipids in chilling injury in plants.

Retirements

Dr R. P. Newbold

Dr Bob Newbold retired from the Division on 19 October 1983. At the time of his retirement Bob Newbold was Senior Principal Research Scientist and leader of the Metabolic Studies Group at MRL, having completed a long and productive series of studies on the biochemical behaviour of post-mortem muscle.

Dr Newbold was born in Wellington, New Zealand and obtained his first science qualifications in that country. He then studied in the United States and obtained the degree of Ph.D. in Comparative Biochemistry from the University of California.

During his working life, Dr Newbold worked as a chemist and later as a biochemist with both the New Zealand Department of Scientific and Industrial Research and the New Zealand Department of Agriculture. His studies ranged from investigations on the effect of animals on pasture growth and composition, the conservation and preservation of pastures and the digestibility of pastures, hay and silage, through to research on the dehydration of meat. Dr Newbold's interest in the biochemistry and physiology of muscle received a stimulus when he was awarded a National Research Council of Canada Post-Doctoral Fellowship, and his researches in Canada led, on his return to New Zealand, to his establishing a program of basic research on meat quality.

On the 23 December 1958, Dr Newbold was offered appointment to the Division of Food Research (then the Division of Food Preservation and Transport) as a Principal Research Officer, to be in charge of muscle biochemistry studies, a position which he formally assumed on the 19 May 1959. From that time he studied muscle biochemistry at the Division's headquarters at North Ryde until January 1967, when he transferred to the newly built Meat Research Laboratory at Cannon Hill, Queensland. Here, with his own research group and with some distinguished visiting scientists from overseas, Dr Newbold studied a number of aspects of post-mortem glycolysis, especially as it is influenced and controlled in muscle through the uptake and release of calcium by the sarcoplasmic reticulum. These very fundamental studies were later to be of great value in helping explain cold-induced toughening of meat and the effectiveness of electrical stimulation of muscle in the prevention of this occurrence. His contributions to muscle biochemistry resulted in his election to membership of the New York Academy of Science.

Dr Newbold was very widely consulted by his colleagues, who recognized in him an enormous store of detailed understanding of the behaviour of muscle post-mortem. If information about some rather obscure aspect of muscle was required, he had an extensive, well-indexed reference system, from which they appeared to invariably manage to extract a relevant reference.

His many friends at MRL wish Bob and his wife Marjorie a long and happy retirement.

DJW

D. C. Rose

With the retirement in November 1983 of Doug Rose, FRL's glassblower since 1953, the Division farewelled one of a disappearing race of skilled craftsmen. When he began his work as an apprentice glassblower there were no such things as trade courses in glassblowing nor an apprentice scheme for glassblowers. The few countries which had such schemes included the United Kingdom and Germany. Doug learnt his trade from glassblowers who had trained in Europe. He leaves behind some fine examples of his technical expertise and artistry as he was particularly skilled in the construction of vacuum equipment and also in the manufacture of glass-to-metal-to-ceramic seals. During his 30 years' service he was very much involved with the research staff in the design of equipment using glass components.

For the past several years, as the needs for glassblowing at FRL decreased due in part to automation and developments in plastics, Doug offered his services to other Divisions in the Sydney area and these Divisions were most grateful for the help he gave them. Although Mr Rose plans eventually to leave Sydney, he has agreed to continue his services on a part-time basis for the time being.

ARJ

R. P. Newbold — Some selected publications

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