Food Chemistry **12** (1983) 219–228

Distribution of Potassium, Calcium and Magnesium in Strawberry Fruits in Relation to Breakdown of the Sulphited Fruit

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(Received: 11 March, 1983)

ABSTRACT

Methods are described for the determination of the free and bound Ca^{++}, Mg^{++} and K^{+} in flesh and of these cations in achenes of strawberry fruits. A substantial amount of minerals was present in the achenes and the variation in mineral analyses of whole strawberries most probably reflects differences in the relative proportion of achenes to flesh. A negative correlation ($r = -0{\cdot}8$) was found between bound Ca^{++} and the survival of fungal polygalacturonase in liquor of sulphited fruit. The possible rôle of calcium in reducing enzymic softening of sulphited strawberries is discussed.

INTRODUCTION

Calcium has long been thought to be important as a cross-linking component between polygalacturonide chains in plant cell walls. Joslyn (1962) suggested that the softening of apple cell walls is due to the transfer of divalent metal ions, particularly calcium, from the wall into the cell. Calcium and magnesium have been reported to play a rôle in fruit firmness (Kertesz, 1951; Eaves & Leefe, 1962) and the antagonistic interaction between these divalent cations and potassium in plants,

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Food Chemistry 0308-8146/83/$03·00

including strawberries, is also well documented (Chiu & Bould, 1976). The present work was undertaken to determine the influence which the calcium, magnesium and potassium content of strawberries may have on the breakdown of sulphited fruit mediated by fungal pectolytic enzymes (Dennis & Harris, 1979). Although the variation in the incidence of breakdown of fruit from different sites was largely explicable in terms of the incidence of the appropriate fungal polygalacturonase enzymes (Dennis *et al.*, 1979), it was of interest to determine the variation in the cation content of fruit from different sites, especially in view of reports which suggest that Ca^{++} complexes with pectic substances, thus providing a resistance to enzymic degradation. Buescher *et al.* (1979) and Wills & Rigney (1980) have shown that Ca^{++} reduces the activity of polygalacturonase and can thus prevent softening of cucumber pickles and tomatoes, respectively. Preliminary experiments not only indicated a very large variation in the content of calcium, magnesium and potassium between samples but also that there were often large variations between replicates of the same fruit sample (see also Goodall, 1969 and Goodall & Scholey, 1975). Such variations appeared to be correlated with the relative proportion of flesh and achene in the samples. Thus, further work was undertaken to determine the cation composition of the flesh and achenes.

This paper reports the methods devised for this purpose and the data obtained for fruit from sites showing different degrees of breakdown in sulphite liquor.

MATERIALS AND METHODS

Materials

Fruit of the variety Cambridge Favourite was harvested from twelve commercial plantations used by Dennis *et al.* (1979) for their studies on the breakdown of sulphited fruit. The sound, healthy fruit used for analysis was selected from approximately 25 kg of commercially picked fruit at each harvest.

Sample selection and preparation

A 'core' was taken from each of twenty fruits using an 8 mm cork borer. The ends of the core (comprising skin and achene) were sliced off and stored separately from the remaining flesh (Fig. 1). All samples were

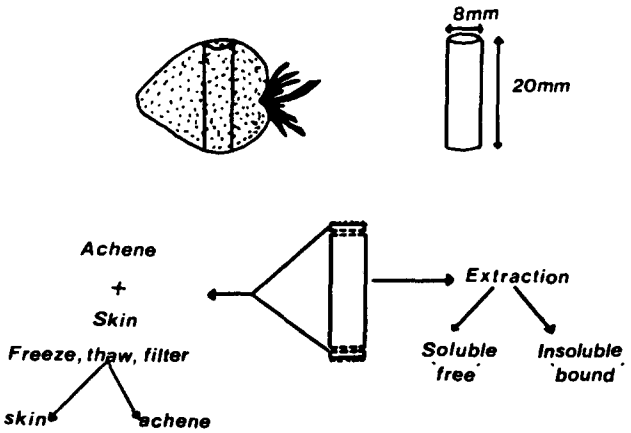

Fig. 1. Sampling method using a cork borer.

stored at −40 °C until required for analysis. The achenes were separated from the skin by preparing a slurry of the thawed end-slices and filtering it through muslin under vacuum. The achenes were blotted dry with filter paper and then dried *in vacuo* over P_2O_5 before being crushed in an agate mortar and further dried over P_2O_5. The flesh samples were allowed to thaw before macerating. Weighed samples (3 g) were homogenised with 20 ml of water, transferred to a centrifuge tube and centrifuged at 100 *g* for 15 min. The liquid phase was filtered through a Whatman GFC filter paper into a 50 ml flask and made up to the mark. This solution was analysed for the 'free' cations. The solid material was transferred to a beaker, dried at 105 °C overnight and used to analyse for the 'bound' cations.

Ashing of samples

Achene samples (0·1 g) were ashed in concentrated nitric acid and the final solution made up to 25 ml. The dried flesh solid was ashed at 500 °C and taken into solution by boiling with concentrated nitric acid (2 ml) and making up to 25 ml.

Atomic absorption spectroscopy

A Varian Techtron AA6 atomic absorption spectrometer was used to determine the concentration of the cations in suitable dilutions of the prepared solutions. A nitrous oxide/acetylene flame was used for the calcium analysis and all samples contained added potassium

(2000 μg/ml). Magnesium and potassium were determined using an air/acetylene flame.

Assessment of breakdown of sulphited fruit

Fruit samples were scored on an arbitrary 6-point scale as previously detailed (Dennis *et al.*, 1979) in which perfect fruit is given a value of 6 and completely broken down fruit a value of 1.

Polygalacturonase in sulphite liquors

The persistence of polygalacturonase in sulphite liquors was monitored by viscosimetry as previously described (Dennis *et al.*, 1979) and recorded as the number of weeks for which the enzyme could be detected.

RESULTS AND DISCUSSION

We considered it necessary to separate the minerals in the 'flesh' into 'bound' and 'free' as it would be the 'bound' form which could play a rôle in the resistance of the cell walls to attack by fungal enzymes. The analytical system used was thus designed to provide an estimation of the approximate proportion of 'bound' to 'free' minerals in strawberry flesh. The average results of the analyses for calcium, magnesium and potassium in triplicate samples of 'flesh' and duplicate samples of achenes for six batches of fruit are shown in Table 1. Calcium values on a further twelve batches of 'flesh' are given in Table 2. The elements in the achene were calculated as μg/g dry weight of achene. In order to calculate an approximate composition for whole fruit two factors had to be taken into account; the ratio of dried achene to flesh obtained by the sampling method and any bias created by the sampling method. The ratio of dried achene to flesh was found experimentally to be 0·009. Considering surface area to volume ratio for whole fruit, it can be shown (see Appendix) to a reasonable approximation that only 38% of the appropriate amount of skin is obtained by the cork borer method. Thus, a factor of 0·024 has been used to calculate the contribution of the achene to the composition of the whole fruit.

Substantial amounts of all minerals are present in the achenes and it is thus of importance that when determinations are made on 'flesh' samples there are no contaminating achenes. Variation in the mineral analysis of

TABLE 1
Mineral Content of Strawberry Fractions (μg/g)[a]

	Location, harvest specifications[b]					
	1,1	*1,2*	*4,1*	*6,1*	*8,1*	*13,1*
Calcium						
Free	69·0	89·0	73·9	62·4	69·2	80·0
Bound	33·1	33·2	44·4	27·4	22·4	17·7
Achene	5277	4829	4419	4676	5575	3330
'Whole'	229	238	224	202	225	178
Magnesium						
Free	38·5	43·3	48·5	64·9	47·3	40·9
Bound	10·9	20·8	16·8	19·9	13·2	12·8
Achene	1348	1790	1727	1551	1508	1284
'Whole'	81·8	107	107	122	96·7	84·5
Potassium						
Free	599	415	774	822	662	636
Bound	112	114	168	160	114	124
Achene	6356	5475	6634	6480	5360	4390
'Whole'	864	661	1102	1138	905	865
Breakdown score	5·0	5·0	5·0	4·0	4·0	1·0

[a] Results for achene are on a dry weight basis; all others are on a fresh weight basis.
[b] See Denis *et al.* (1979).

whole strawberries most probably reflects differences in the relative proportions of achenes to flesh. Data in Table 1 for whole fruit are in general agreement with those of Goodall & Scholey (1975). The proportion of bound to free minerals in the 'flesh' varied according to mineral and also between batches of fruit. Generally, there was a greater proportion of bound Ca^{++} (range 18 to 49 %, average 31 %) than bound Mg^{++} (range 22 to 32 %, average 25 %) and bound K^{+} (range 15 to 22 %, average 17 %). A negative correlation ($r = -0{\cdot}6$, $P < 0{\cdot}01$) between free K^{+} and Ca^{++} was observed and confirms the antagonistic interaction between these cations reported for strawberry plants by Chiu & Bould (1976).

One-way analysis of variance showed that there was a significant difference ($P < 0{\cdot}05$) between the mean values for bound calcium in different breakdown groups using the complete data averaged in Table 1. No other significant relationships between breakdown and the mineral

TABLE 2
Additional Results for Calcium Content of Strawberry Flesh
(μg/g Fresh Weight)

Location, harvest specifications[a]	*Free calcium*	*Bound calcium*	*Breakdown score*
5,1	54·1	53·1	4·0
7,1	75·7	29·6	6·0
11,1	76·8	35·8	5·0
12,1	78·4	25·5	1·0
14,1	92·2	33·6	5·0
15,1	105·0	36·6	6·0
5,2	66·1	29·4	1·0
7,2	89·7	51·9	1·0
11,2	61·8	32·5	4·0
12,2	68·4	41·0	1·0
14,2	64·0	38·0	4·0
15,2	73·8	37·0	6·0

[a] See Denis *et al.* (1979).

content were detected and so only calcium analyses were performed on further batches of fruit (Table 2). However, when all the data were included in the analysis of variance the difference between the mean values for bound calcium in different breakdown groups was not significant ($P > 0{\cdot}10$).

Previous work (Dennis, 1978; Dennis & Harris, 1979; Dennis *et al.*, 1979) showed a clear relationship between the presence of polygalacturonase and breakdown of the fruit. There is also published evidence (Buescher *et al.*, 1979; Wills & Rigney, 1980) of interaction of Ca^{++} and polygalacturonase which led us to test if there was evidence for a correlation between breakdown and bound calcium in samples where the presence of the enzyme had been detected. These data (Fig. 2) ($r = -0{\cdot}8$, $P < 0{\cdot}01$) suggest that the presence of bound Ca^{++} in the cell walls of strawberry 'flesh' affects the susceptibility of the fruit to enzymic breakdown by affecting the persistence of the enzyme. Buescher *et al.* (1981) recently showed that Ca^{++} does not completely inhibit pectinolytic softening of cucumbers, but greatly reduces the rate of softening, while Bateman & Lumsden (1965) predicted that resistance to maceration caused by Ca^{++} could be overcome with increased

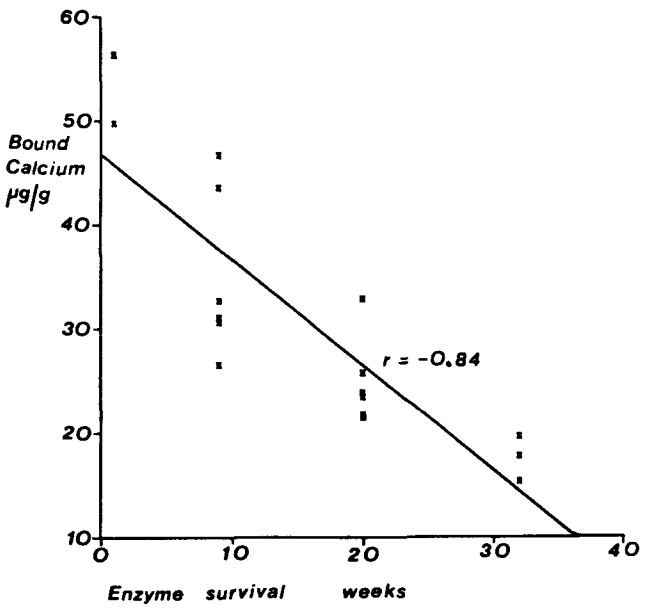

Fig. 2. Correlation of bound calcium with persistence of polygalacturonase activity. (Data from Tables 1 and 2 excluding samples with no detectable enzyme activity at harvest time.)

polygalacturonase. In the case of sulphited strawberries, the polygalacturonases are slowly inactivated by the acid pH (*ca.* 3·0) of the sulphited liquor (Harris & Dennis, 1979). Thus, a further reduction of activity by Ca^{++} would be expected to result in a further reduction in breakdown of the fruit.

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APPENDIX: ESTIMATION OF THE BIAS CAUSED BY THE CORK BORER SAMPLING METHOD IN THE PROPORTION BETWEEN ACHENE AND FLESH

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Sampling a strawberry by using a cork corer to remove a cylindrical core passing through the centre produces a biased proportion between seeds and flesh.

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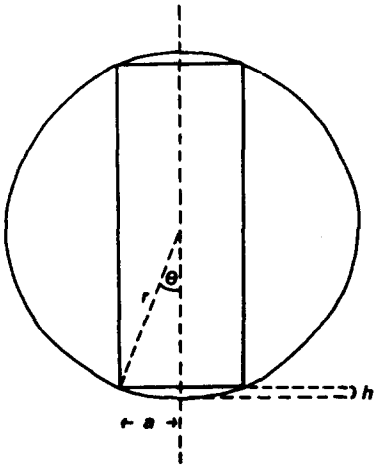

Fig. A1. Idealized geometry of cork borer sampling method.

Approximating a strawberry as a sphere it is reasonable to take the surface area as being proportional to the quantity of achene. The flesh sampled is the cylinder produced when the end caps are removed from the core which has been bored out.

Considering a sphere of radius r and a core of radius a, the height of each end cap, h, is given by $h = r(1 - \cos\theta)$ where $\sin\theta = a/r$, as shown in Fig. A1.

The combined surface area of the two end caps (A) is, by Archimedes' Theorem, $2h(2\pi r)$. The volume of the cylinder, V, is $2(r - h)(\pi a^2)$. Hence $A/V = 2/(r(1 - h/r)(2 - h/r))$. For a whole sphere the area to volume ratio is $3/r$. Therefore, for a sphere of any given radius, the sampled A/V ratio is in a proportion, P, of $2/(3(1 - h/r)(2 - h/r))$ to the ratio for a whole sphere. Table A1 gives some calculated results for different a/r ratios.

TABLE A1
Percentage of Surface Area Sampled by Cork Borer

a/r	h/r	A/V	$P\%$
0·1	0·005 0	1·00 8/r	33·6
0·2	0·020 2	1·031/r	34·4
0·3	0·046 1	1·073/r	35·8
0·4	0·083 5	1·139/r	38·0
0·5	0·134 0	1·238/r	41·3
0·7	0·285 9	1·634/r	54·5

For a strawberry (average diameter 20 mm), using an 8 mm borer, the a/r ratio is 0·4 which gives a value of 38 % of the proportion of the whole sphere. It will be noted that this percentage is only slightly affected by changes in the a/r ratio.

ACKNOWLEDGEMENTS

The authors are grateful to Mr D. E. Walters for statistical advice, to Mr R. Stansfield for preparation of the Appendix, to Mr R. P. Davis for sampling the fruit and to Mr D. P. Coe for skilled technical assistance.