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Extractability of calcium from apple fruit and apple leaf tissue and the occurrence of bitter pit

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SUMMARY

A method was developed to extract calcium from carefully sampled apples of the same diameter (7·00±0·15 cm) and leaves using solutions of different extraction strength. The correlation between the extracted quantities of Ca from these tissues and the occurrence of bitter pit was no better than the correlation between total Ca and the incidence of bitter pit. Bitter pit was significantly correlated (r=0·92) with both the K/Ca ratio and the (K+Mg)/Ca ratio (both in equivalents). The correlation coefficients for the relationships between bitter pit and the extracted quantities of Ca, total Ca, K/Ca and (K+Mg)/Ca in fruit tissue were much higher than those derived from leaf tissue.

BITTER PIT, a physiological disorder of apple fruits, is still an important storage problem. Many investigators have found significant correlations between the disorder and the Ca content, the K/Ca and/or (K+Mg)/Ca ratio in the fruits (Garman and Mathis, 1956; van der Boon *et al.*, 1968; van der Boon, in press). The correlations were somewhat higher when the fruit/leaf ratio of the tree and the regularity of the fruit/leaf ratio among trees within the orchard were also taken into account (van der Boon, in press). Sometimes, however, fruits showed an incidence of bitter pit which differed from that expected from their (K+Mg)/Ca ratio, and the correlation between K/Ca or (K+Mg)/Ca ratios and the incidence of bitter pit has led to many contradictory conclusions about the relative importance of K, Ca and Mg (Bünemann, 1972). Principal component analysis indicated that Ca and mean fruit weight (more bitter pit with increasing fruit size) played a predominant role in the incidence of the disorder in Cleopatra apples (Martin *et al.*, 1975), Jonathan apples (Ratkowsky and Martin, 1974) and in Merton Worcester apples (Lewis *et al.*, 1977).

A considerable reduction in bitter pit is obtained when apple trees are sprayed with Ca salts, but van der Boon *et al.* (1968) and van Goor (1971) found that the correlation between the Ca content of the fruits and the occurrence of bitter pit, compared with the control, diminished or disappeared altogether in fruits from the sprayed trees. This may reflect an alteration in the amount of physiologically active Ca in fruit since apples from trees sprayed with $Ca(NO_3)_2$ contained a higher proportion of water-soluble Ca (Perring and Plocharski, 1975).

Wieneke and Führ (1973) found crystals containing Ca in apple fruit and fruit stem, and Stebbins *et al.* (1972) in apple fruit and leaf petiole. After having made corrections for differences in fruit/leaf ratio, fruit size and the mineral composition of the apple, van der Boon (in press) found that high temperatures in August resulted in an increased development of bitter pit. This could be caused by a decreased mobility of Ca in the fruit stem, as Chang *et al.* (1968) reported for the stalks of the leaves of *Nicotiana tabacum*, or by increased fixation of Ca in the fruit, or both. Fractionation of Ca in apple tissue showed a stronger fixation of Ca with increasing fruit age; the solubility of Ca in fruit stem tissue also decreased as the fruits ripened (Wieneke, 1974).

In our investigation we tried to discover the portion of Ca present in apple fruits and leaves that is physiologically active. Such a fraction might show a better correlation with the occurrence of bitter pit than that obtained with total Ca.

MATERIALS AND METHODS

Leaves and fruits were sampled at harvest from Cox's Orange Pippin apple trees from 16 different orchards. The orchards were selected in the ex-

pectation that a wide range of bitter pit occurrence would be found after storage, and the trees had not been sprayed with Ca salts. Seventy apples per orchard were collected for chemical analysis and 210 apples per orchard to determine the incidence of bitter pit after storage. The diameter of the selected apples ranged from 6·85 to 7·15 cm. Storage was at 4°C for a period of 10 weeks and then at 20°C for two weeks. The occurrence of bitter pit and breakdown (browning and softening of the apple, especially in the apical half) was then determined after paring and cutting.

The apples for chemical analysis were washed with deionised water, dried with a towel and frozen at *c.* −20°C. Subsequently the apples were cut into pieces and freeze-dried in a Weinkauf, model L2, apparatus, followed by grinding in a Starmix MXC 500. Bradfield (1977) reported that drying caused an alteration in the distribution of the Ca fractions in strawberry leaves, and so time-consuming freeze-drying is necessary to prevent such conversions. After ashing the powder at 675°C for 6 h, total Ca and Mg were determined by atomic absorption spectrophotometry; K was determined using a flame photometer after ashing the powder at 400°C for 6 h. Using a modification of a method of Faust *et al.* (1968), we carried out the following extractions in the freeze-dried tissue to fractionate Ca:

1) deionised water extraction (water soluble fraction);
2) 2M NaCl and 3) 2M NH_4Cl extraction (fraction 1 and exchangeable fraction, associated principally with proteins and pectins);
4) 2M HAc extraction (fraction 2 or 3 and Ca bound to phosphates and carbonates;
5) 2M HCl extraction (fraction 4 and Ca bound to oxalates).

Our procedure was as follows:

Freeze-dried apple tissue (20g) was shaken on a Bühler shaking apparatus, type SM 2, for 1 h with 200 ml of the relevant solution in a 500-ml erlenmeyer flask at 20°C. The extract was then centrifuged (M.S.E. 18) and 18 500 *G* and 15·5°C for 1 h. The supernatant was filtered (MN 680 m) at 20°C. Of the filtrate, 100 ml was pipetted into a platinum dish and evaporated to dryness. The dishes with dried NH_4Cl filtrate were pre-ashed on a flame to prevent damage to the muffle furnace by the released fumes. After evaporating and pre-ashing, the dishes were placed in a muffle furnace at 675°C for 6 h. The ash was allowed to dissolve in 2 ml 25% HCl for at least 0·5 h. The solution was transferred to a filter with hot deionised water and filtered into a volumetric 100-ml flask. The filter was washed thoroughly with hot deionised water. After cooling, diluting to the mark and mixing, an aliquot was pipetted into a volumetric flask of 50 ml. The Ca concentration in the solution was measured with a Perkin-Elmer 305 atomic absorption spectrophotometer after adding 5 ml 2% $SrCl_2$, diluting to the mark and mixing. The concentration of HCl, $SrCl_2$ and also NaCl, when using 2M NaCl as extraction solution, in the standard solutions was normalized to the concentration in the sample solution.

About 500 leaves, comprising the third and fourth leaves from the bases of newly-formed shoots, were taken per sample also at harvest. The leaves were washed twice with deionised water for 1 min, partially dried between filter paper and frozen at *c.* −20°C. The leaves were then freeze-dried in a Weinkauf, model L 2, apparatus, and ground in a Starmix MXC 500. Extracts of leaf powder were made as with the powder of the freeze-dried fruits using deionised water, NH_4Cl, HAc and HCl. The extraction solution, 2M NaCl, was however substituted by 50% ethanol (in water) since this extracts less Ca than water. In the leaves total Ca, Mg and K was determined in the same way as in the fruits.

RESULTS AND DISCUSSION

A test was carried out, using multiple regression equations with one Ca determination and all six Ca analyses, respectively, as independent variables. After evaluating the effect of one variable the mean square ascribable to the five remaining Ca factors was determined. This variance with five degrees of freedom (df) was tested by the F-test against an error variance with nine df (obtained from the regression on all six Ca determinations), but the mean square ascribable to the five remaining Ca factors was never significantly different from the error variance for all six determinations, the highest F values being 1·32 (leaves) and 2·33 (fruits). Thus one Ca determination gave as much information about the occurrence of bitter pit as any of the fractions. Perring and Plocharski (1975) also failed to find a difference between Ca extracted by water or 75% ethanol from two Cox's Orange Pippin samples

which differed in bitter pit incidence.

About 55 to 60% of total Ca was extracted from the fruits by water (Table I). By using 50% ethanol/water (the weakest extracting solution used) about 8–11% of total Ca was extracted from the leaves, whereas with deionised water about 15–24% of total Ca was extracted (Table II). In the experiments of Bowen *et al.* (1962) about 40% of total Ca in tomato leaves was present as pectate with the greater part of the remainder as free Ca^{2+} ions, but only about 10% of total Ca was biologically active in incubated apple fruit tissue (Faust and Klein, 1974).

Wiersum (1974) has also suggested that only a small proportion of the total Ca in the protoplasts of plant cells is biologically active and this protoplasmic fraction, which is present in vital cell structures such as membranes, is almost certainly of importance in the development of bitter pit. It is possible that the failure to find a better parameter than the total Ca content for predicting the occurrence of bitter pit could be that too much Ca was extracted by the weakest extraction solution, especially from the fruits, and the specific Ca fraction was not detected.

The correlation coefficients for the relationships between Ca, calculated from six determinations, and the incidence of bitter pit were very high and highly significant for the fruits, but for leaves they were much lower and failed to reach significance at P=0·05 (Table III).

TABLE III

Correlation coefficients for the relationships of several variables with the occurrence of bitter pit.

Variables	Correlation coefficient	
	leaves	fruits
Ca-total	–0·34	–0·77**
Ca-HCl	–0·41	–0·78**
Ca-HAc	–0·35	–0·84**
Ca-NH_4Cl	–0·32	–0·84**
Ca-H_2O	–0·30	–0·81**
Ca-C_2H_5OH	–0·27	
Ca-NaCl		–0·83**
K/Ca in equiv.	0·61*	
(K+Mg)/Ca in equiv.	0·54*	0·92**
K-total	0·65**	0·73**
Mg-total	–0·26	0·62*

*, **correlations significant at P=<5% and P=<1% respectively.

The correlation coefficients decreased when the percentages of Ca in the different fractions from fruits or leaves were subtracted from each other and related to bitter pit occurrence.

Very high correlation coefficients of 0·92 were found for the relationships between the ratios K/Ca-total and (K+Mg)/Ca-total (in equivalents) in fruits and the incidence of bitter pit. These coefficients, which are much higher than those normally reported in the literature (van der Boon *et al.*, 1968; Bünemann and Lüdders, 1975; van der Boon, in press), may be due to the fact that

TABLE I

Ranges and mean concentrations (mg/100 g DM) of total K, Mg, Ca and Ca (% of total calcium) in different fractions from Cox's Orange Pippin apples at harvest and incidence of bitter pit after storage for 10 weeks at 4°C followed by 2 weeks at 20°C.

	Total (mg/100 g)			% extracted of total Ca by					% bitter pit
	K	Mg	Ca	H_2O	2M NaCl	2M NH_4Cl	2M HAc	2M HCl	
Minimum	720	36	20	54·3	59·1	64·1	68·3	92·6	2·8
Maximum	1130	43	33	65·6	84·4	86·8	80·2	100·0	69·9
Mean	869	39	27	57·8	73·4	77·0	72·6	95·5	25·3

TABLE II

Ranges and mean concentrations (% DM) of total K, Mg, Ca and Ca (% of total calcium) in different fractions from Cox's Orange Pippin leaves at harvest and incidence of bitter pit in fruits after storage for 10 weeks at 4°C followed by 2 weeks at 20°C.

	% total			% extracted of total Ca by					% bitter pit
	K	Mg	Ca	50% C_2H_5OH	H_2O	2M NH_4Cl	2M HAc	2M HCl	
Minimum	0·85	0·11	0·99	7·1	13·7	27·1	23·7	94·2	2·8
Maximum	1·82	0·32	2·02	11·6	24·4	39·0	34·0	100	69·9
Mean	1·33	0·21	1·50	9·3	18·1	33·6	29·2	97·1	25·3

measurements were restricted to apples of the same size range (diam.=6·85–7·15 cm).

It should be noted that adding the percentage of fruits with breakdown but without bitter pit (1–8%) to the percentage of bitter pit did not alter the results in any way.

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