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Relationship between Juice and Flesh Calcium during Apple Fruit Development

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Additional index words. calcium electrode, plasma emission spectrometry, strain, *Malus domestica*

Abstract. **The ionic Ca content of expressed apple juice conceivably could be used to estimate the total calcium content of fruit flesh. To evaluate this method, samples of 2 strains of 'Delicious' apple (*Malus domestica* Borkh.) were analyzed at 2-to 3-week intervals, from 4 weeks after full-bloom until full-maturity. Ionic Ca in the juice (juice Ca) was analyzed with a selective electrode, total Ca in the flesh (flesh Ca) with a plasma emission spectrophotometer. The correlation coefficient between calcium concentration in flesh vs. juice was very low during the early stages of fruit development, but increased to +0.758 (significant at $P<0.01$) for samples collected 5, 3, and 0 weeks prior to fruit maturity. The correlation was generally significant at $P<0.01$ when all sampling dates were used ($r = +0.734$ for 'Miller Spur', +0.928 for 'Starking', and +0.831 for both strains). The calcium concentration in juice samples taken within 35 days of physiological fruit maturity paralleled the calcium concentration in the flesh on any given date, but was not a reliable predictor of flesh Ca concentration in fruit harvested 2 to 3 weeks thereafter.**

Apple xylem sap and juice expressed from apple and tomato leaves and fruit have been analyzed for water soluble Ca ion using ion-selective electrodes (1, 2, 7, 8). Himelrick and Ingle (7) reported the following soluble Ca^{++} as a percentage of the total Ca concentration in selected apple tissues: leaf, 15%; fruit peel, 20%; fruit flesh, 20%. Ionic Ca in the extracted sap of apple shoots represented about 50% of the total calcium (1). In mature 'Delicious' apple fruit, the correlation coefficient between ionic and total Ca was +0.91 for the flesh and +0.87 for the peel (7). A value of +0.69 was reported by Dewey et al. (4) in 'Jonathan' flesh. In each of these studies, individual fruit were halved longitudinally, one portion being used for determination of Ca^{++} in the juice, the other for total Ca in the juice and flesh.

Our purpose was to establish the time(s) at which the Ca^{++} content of the juice of developing apple fruits was a reliable indicator of the total Ca content of the flesh, and if preharvest analysis of Ca^{++} could be used to predict total fruit calcium content at harvest. If Ca status could be predicted early enough, one might be able to apply treatments to correct deficiencies and/or decide whether fruit should be harvested for immediate sale or for storage.

Six 16-year-old trees of both Miller Spur and Starking 'Delicious' bearing good crops of fruit were selected. Samples of fruit were collected from individual limbs on 9 dates starting at 2 weeks after full-bloom (3 June) until 2 weeks after the time of normal commercial harvest (22 Oct.) One limb on each tree was chosen at randon for harvest at each date, at which time 50 (20 after 1 July) fruit were removed for analysis. The fruit from each limb were maintained as one replicate.

The fruit in each replicate were separated into 2 subsamples by slicing each fruit longitudinally, one-half of each fruit being included in each subsample. Skin and core were removed, except where fruit portions were compared, and a porton of each subsample (10 g fresh weight) was oven-dried at 70°C to constant weight, ashed at 550° for 8 hr, and analyzed for total Ca. The ash was dissolved in 10 ml 10% HNO_3 containing $LiCl_2$ (5000 ppm), and the solution was filtered through analytical grade filter paper. Total Ca in the solutions was analyzed using a DC arc plasma emission spectrophotometer and expressed as ppm flesh fresh weight or as total Ca per fruit (= flesh Ca).

The remaining half of each fruit was ground, the juice extruded and centrifuged at 9150 × g for 12 min in a model 6001 Acme Supreme Juicerator (Acme Juicer Mfg. Co., Sierra Madre, Calif). The extruded juice was filtered through Whatman No. 42 paper, frozen and held at -18°C prior to further analysis. On thawing, the pH of the juice was adjusted to 6.0 with KOH and the ionic strength of the solution measured with a Beckman Solu-Bridge conductivity indicator. Ionic Ca (= juice Ca) was measured using an Orion 93-20 Ca-specific ion electrode and a 90-20 reference electrode with a Fisher Accumet 144 digital pH meter. The samples were agitated with a magnetic stirring bar during analysis; response time following equilibration of the instrument was 2 to 3 min.

A series of standard $CaCl_2$ solutions was prepared to give Ca^{++} concentrations of 1.25 to 75 ppm. Ionic strength (mmhos/cm^2) was adjusted with KCl to equal sample ionic strength and voltage was plotted against log ppm Ca^{++}.

The increase in fruit weight was rapid until 15 weeks after bloom, then slowed (Fig. 1). Flesh Ca per fruit (Fig. 1) increased for the first 15 weeks and then stablized. The concentration declined rapidly through July and early August, then more slowly until harvest (Fig. 2).

Juice Ca per fruit (Fig. 1) rose with fruit weight and flesh Ca until 15 weeks after full-bloom, and then leveled off as the fruit ma-

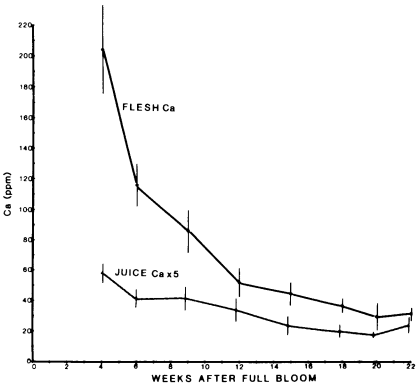

Fig. 2. Concentrations of flesh and juice Ca in 'Delicious' apple fruit in 1980. Each point is the mean for 2 strains, and vertical bars indicate SD. Optimum maturity was attained at 20 weeks.

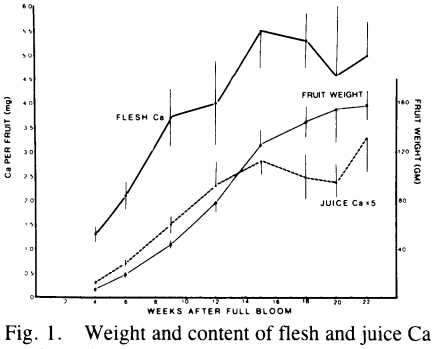

Fig. 1. Weight and content of flesh and juice Ca of 'Delicious' apple fruit in 1980. Each point is the mean for 2 strains, and vertical bars indicate SD. Optimum maturity was attained at 20 weeks.

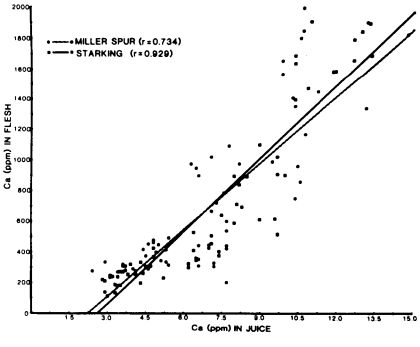

Fig. 3. Relationship between Ca concentration in flesh vs. juice of 2 strains of 'Delicious' apple fruit sampled between 4 and 22 weeks after full bloom. Each point represents one tree.

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Table 1. Correlation coefficients (r) for concentrations of Ca in juice vs. flesh of 'Delicious' apple fruits. Pooled data for Starkrimson and Miller Spur strains.

	Sampling date							
	17 June	1 July	22 July	12 Aug.	3 Sept.	22 Sept.	7 Oct.	22 Oct.
	Week after full-bloom							
	4	6	9	12	15	18	20	22
	Juice vs. flesh Ca within sampling dates							
r	0.324	.0379	0.529	0.566	0.721	0.783	0.774	0.173
Significance	NS	NS	NS	NS	**	**	**	NS
	Juice Ca on date sampled vs. flesh Ca at harvest on 7 Oct							
r	0.012	0.120	0.221	0.297	0.487	0.110		
Significance	NS	NS	NS	NS	NS	NS		

[z],**Significant at $P < 0.01$.

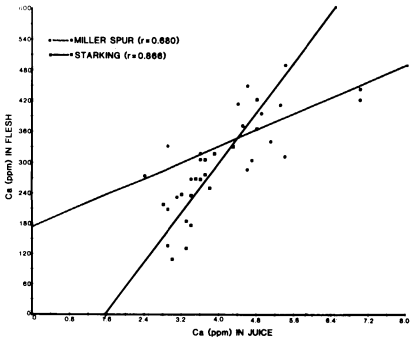

Fig. 4. Relationship between Ca concentration in flesh vs. juice of 2 strains of 'Delicious' apple fruit sampled between 15 and 20 weeks after full bloom. Each point represents one tree.

tured in early October (Fig. 1). The concentration of juice Ca slowly declined until the fruit became mature (Fig. 2). Overmature fruit harvested after 22 weeks (22 Oct.) contained somewhat more juice calcium than did mature fruit. The concentrations of both juice and flesh Ca were lower in Starking than in Miller Spur in most samples (data not shown).

Mean values for the concentration of juice Ca were consistently correlated with those for flesh Ca during the entire period examined ($r = +0.831$; $P<0.01$). Data analysis indicated no improvement in correlation by partitioning into linear, quadratic, and cubic components. When data for individual strains were analyzed separately (Fig. 3), the r values were +0.734 and +0.928 for Miller Spur and Starking, respectively, both significant at $P <0.01$. Differences between strains were not significant at $P <0.05$.

To determine when measurement of juice Ca concentration provided the most reliable estimate of total Ca concentration, correlation coefficients were calculated for each sampling date, using individual tree samples as replicates. Values of r were significant ($P <0.01$) only at 15, 18, and 20 weeks after full-bloom (Table 1) with the highest r values at 18 and 20 weeks. When the data for each strain were analyzed separately, correlation coefficents for juice vs. flesh Ca at 15, 18, and 20 weeks were +0.680 for Miller Spur and +0.866 for Starking, both significant at $P <0.05$. Correlation coefficients between juice Ca at various dates prior to harvest vs. flesh Ca at maturity or optimum harvest date (7 Oct.) were nonsignificant at $P <0.05$ (Table 1).

The correlation between flesh Ca vs. juice Ca in whole fruit, including core and seeds, was nonsignificant ($r = +0.020$). The concentration of total Ca in the flesh of the calyx half of the fruit was consistently lower than that in the entire fruit at 15, 18, and 22 weeks after full-bloom (Table 2). In contrast, the juice Ca content was almost always higher in the calyx half than in the flesh of the entire fruit.

Juice Ca was highly correlated with flesh Ca in fruit sampled 15, 18, and 20 weeks after full-bloom (3 Sept. to 7 Oct.), but not before or after this period. Juice Ca is, therefore, a reliable indicator of flesh Ca in the same fruit provided samples are taken sometime during the 5 weeks prior to the optimum harvest date. Once fruits become overmature, however, juice Ca is an unreliable indicator of flesh Ca.

The poor correlations observed between Ca content of juice samples taken prior to harvest and flesh samples taken at optimum maturity was unexpected, considering the good correlations between these values within sampling dates. On any one date, however, data for flesh vs. juice Ca were derived from paired halves of the same fruit, whereas pairing between sampling dates was impossible. Furthermore, different limbs were sampled on different dates. Therefore, the poor correlations may reflect fruit-to-fruit and limb-to-limb variability in Ca content. Analysis of individual fruit, rather than pooled samples from each limb, would have increased the number of observations and therefore might have increased the r value, but this would have been too laborious for routine measurement.

Bitter pit was evident 18 weeks after full-bloom in fruit from the Miller Spur 'Delicious' tree with the lowest juice Ca^{++} concentration (2.4 ppm 15 weeks after full-bloom), and increased during storage (data not shown). Flesh Ca, in relation to that in fruit from other trees, was very low in these fruit at early stages of fruit development, but juice Ca was not. Therefore, measurement of flesh Ca in fruitlets sampled during the cell division stage might provide a basis for corrective action.

Reasonably good correlations between flesh Ca at harvest and prior to harvest were obtained when fruit were sampled 3 to 5 weeks prior to harvest (data not shown), supporting previous reports (10, 14).

Faust et al. (5) found a slightly lower concentration of Ca in the apical (calyx) half than in the basal (stem) half of 'York Imperial' fruit, and Lewis (9) reported a significant downward trend in Ca concentration from the basal to the apical half of apple fruit of 9 cultivars. Our data for 'Delicious' (Table 2) indicate that flesh Ca concentration was consistently lower in the apical half than in whole fruit, particularly at 5 weeks before harvest. However, ionic Ca concentration was higher in juice extracted from the flesh of the apical half than in that from the flesh of the whole fruit in 5 out of 6 comparisons (Table 2), tree 4 being the exception. This apparent contradiction may reflect the changing status and the movement of Ca within the fruit. For example, Bradfield (1) demonstrated that about 50% of the Ca in the xylem sap of the apple tree moves in the ionic form, the remainder being complexed with organic acids and other compounds. Movement of ionic Ca into, out of, and within the fruit has not been studied.

Although Terblanche et al. (12, 13) found consistently higher levels of both ionic and total Ca in sound 'Golden Delicious' fruit than in those exhibiting bitter pit, the discrepancy we have observed between the distribution of juice vs. flesh Ca suggests that the ratio of ionic to total Ca may differ in different parts of the same fruit. Sampling procedures should therefore be designed to avoid differences caused by distribution within the fruit and/or tree, as opposed to those induced by treatment or cultivar.

The significance of correlation coefficients varied with time of sampling and strain, coefficients being consistently higher for Starking than for Miller Spur. Varying levels of chelating agents such as pectin, oxalic, phytic, malic, and citric acids (2, 3, 6, 8,

Table 2. Juice and flesh Ca concentrations (ppm fresh weight) in the flesh of the calyx half vs. the entire fruit from individual trees of Miller Spur 'Delicious' harvested 15, 18, and 22 weeks after full-bloom.

Weeks after full bloom	Tree No.	Juice Ca		Flesh Ca	
		Whole fruit	Calyx	Whole fruit	Calyx
15	2	6.02	7.03	48.9	42.0
	3	6.48	7.20	42.9	39.5
	4	6.38	6.12	43.0	36.9
	5[z]	3.30	4.28	25.4	16.3
18	5[z]	4.64	5.59	38.2	35.1
22	5[z]	3.80	5.30	36.8	23.2

[z]Bitter pit appeared on some fruit on this tree at 18 weeks after full-bloom, but only unaffected fruit were analyzed.

11) in the tissue may have caused this variability. Cooke (2) demonstrated that addition of pectin to synthetic apple juice depressed the ionic Ca to about the same level as that found in natural juice (15.7% of recovered total Ca.) Other soluble components of apple fruit tissue which could have interfered with analysis of Ca^{++} include oxalic acid (6), which may comprise 0.2 to 0.6 micromoles per grams of dry weight (15), phytic acid (11, 12) which occurs mainly in storage organs (6), and other organic acids occurring in the vacuole, such as malic and citric acids (8). Juice pH was consistently low during the early fruit growth period; additonal KOH was required to adjust the pH to 6.0 and may have affected the readings.

High fruit Ca at harvest is associated with improved flesh firmness and reduced bitter pit development during storage. However, preharvest measurement of ionic Ca in fruit does not seem to be a practical means for determining whether the fruit should be sold or stored for later sale.

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Effects of a Vacuum Infusion of a Partially Purified β-Galactosidase Inhibitor on Apple Quality

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Abstract. **An ethyl acetate extract of ground apples (*Malus domestica* Borkh.) was concentrated and purified for a specific β-galactosidase inhibitory function. Vacuum infusion of either H_2O or the extract restricted initial C_2H_4 evolution from apples but had no effect on fruit soluble solids and titratable acid levels in apples held at 20°C. Infusion of the extract did not affect the mean CO_2 evolution from 'McIntosh' apples over a 5-day period but reduced the rate of CO_2 evolution over time. Vacuum infusion of the extract containing the β-galactosidase inhibitor resulted in retention of fruit firmness in 'McIntosh' and 'Gravenstein' apples held at 20°.**

Apple firmness, which depends upon cellular wall matrix integrity and cohesion, is disrupted by enzymatic (1, 2, 4) or possibly by chemical (5) processes during ripening and storage. Galactose residues account for the largest proportion of monomeric carbohydrates released from cell wall polysaccharides (4). β-galactosidase was found to degrade β-1, 4-galactan (1) and to release galactose residues from cellular preparations, although the release of galactose residues was not dependent upon increased β-galactosidase activity (2). Bartley (2) concluded however, that hydrolysis of galactan was responsible for the observed loss of fruit firmness.

A previous study (3) indicated the presence of an inhibitor fraction that could inhibit reversibly β-galactosidase activity of acetone powder preparations. This inhibitory fraction may retard in vivo the release of galactose residues implicated in apple softening (2). This study investigated the effect of a concentrated, partially purified apple extract with β-galactosidase inhibitory properties on apple softening at 20°C.

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Extract preparation and purification. A 400-kg lot of 'McIntosh' apples, held in CA storage (5% CO_2 + 3% O_2, 3.3°C) for 8 months plus another 2 months in air at 0°, were ground in an Apex 314 comminuting mill (Apex Construction Ltd., London, England) equipped with a 0.562 mm screen using a 1:1 ratio intermediate speed and the sharp side of the blades. Batches of 25-kg each of ground apples were extracted twice (10 min each, with continuous agitation) with equal volumes (1:1 v/v) of purified ethyl acetate (Anachemia Ltd., Mississauga, Ontario). The ethyl acetate supernatant was decanted, concentrated by evaporation (30°, 0.1 atmosphere) to a combined volume of about 1 liter, and filtered to remove insoluble material. The addition of 5 volumes of hexane (reagent grade) to the concentrate resulted in the separation of a semi-solid gummy precipitate. This precipitate was collected, resuspended in 5 liters of distilled water, and filtered. The aqueous solution was re-extracted with ethyl acetate, and this extract was evaporated to dryness. The residue was dissolved in 1 liter of distilled water. These preparations contained chlorogenic acid, catechins, and quercetin glycosides. Over 75% of the inhibitor activity of the initial ethyl acetate extract was contained in the final preparation.

Assay of β-galactosidase inhibition. Fresh solutions of β-galactosidase were prepared by extracting acetone powders of 'McIntosh' apples with sodium phosphate buffer (0.005 M, pH 7.0) (3). β-galactosidase activity in