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Water-soluble Cation Contents of 'Jonathan' Apple Fruit Pulp as an Estimation of Total Content

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Abstract. **A method was developed to determine the total Ca, Mg, and K content of apple (*Malus domestica* Borkh.) pulp based on an assay in the water-extractable fraction. Water-soluble and total levels of each of the three elements in the fruit correlated significantly ($P < 0.001$) at harvest. The method is accurate (CV = 4.1%, 4.1%, and 2.5% for Ca, Mg, and K, respectively) and time-saving. The total level of minerals determined by the method proposed correlated well ($P < 0.001$) with that obtained by acid digestion of freeze-dried apple pulp.**

The importance of minerals in the development of various apple storage disorders has been recognized (4, 12, 13, 19), but their effects are evidently complex and dependent on certain endogenous (11, 12) and ecological (2, 12) factors. Consequently, the nature of the correlation between some of the disorders and the mineral content is not yet clear. Calcium deficiency often has been correlated with internal breakdown (3, 7, 12), but not always (10). This disorder has been reported to be both positively (4) and negatively (19) correlated with or unaffected by K (3, 11). Bitter pit has generally been correlated with the Ca content of fruit (5, 8, 17) but correlations with either K alone (4, 18) or with K:Ca and K+Mg:Ca (4, 8) ratios have sometimes been better. To predict potential storage disorders, various formulae based on the total concentrations of N, P, K, Ca, and Mg have been proposed (3, 15, 16, 18). For commercial application, any of these formulae would require a rapid and efficient method for mineral analysis suitable for use on a large scale.

In reports related to the mineral content of plant tissues, it is common to determine the total concentrations of minerals. This determination requires a multistep and time-consuming procedure, involving either ash preparation or acid digestion of dried powdered plant tissue. This study strengthens the possibility, suggested also by others (9, 17), of assessing total Ca by measuring its level in the water-soluble fraction, which is easily obtained by centrifugation of a fruit homogenate. It also justifies the application of this approach to assess the total Mg and K in apple fruit.

Materials and Methods

Six commercial 'Jonathan' orchards in different regions of Israel were selected to represent fruit with various degrees of susceptibility to senescent breakdown. Ten fruit were sampled for mineral analysis from each of seven tagged trees in each orchard. Samples were collected 10 to 14 days prior to, and again during, commercial harvest.

Stem-to-calyx crescents were sliced from the 10 fruit, peeled, and cored. A 20-g sample was homogenized for 1 min in 40 ml of deionized water to avoid gelling of the homogenate. The homogenate then was centrifuged at 30,000× *g* for 10 min to pelletize the cell wall, mitochondria, and a large part of the cell membrane. The supernatant obtained was filtered through Miracloth and the pellet was resuspended in 20 ml of distilled water and centrifuged again as described above. The volume of the combined supernatants was measured, and an aliquot was taken for determination of water-soluble Ca, Mg, and K. The concentrations of insoluble minerals were obtained by measuring the amounts remaining in the pellet. The pellet was freeze-dried and weighed and 100 mg was digested overnight in 4% H_2SO_4. Wet digestion was completed within 1 hr on a hot plate with repeated additions of H_2O_2. After appropriate dilution with distilled water, the mineral content of the fraction was determined. The total Ca, Mg, and K concentrations in the tissue were calculated from the contents in the water-soluble and -insoluble fractions and expressed as mg/100 g fresh weight. To establish the reproducibility of the method, 10 replicates of the same pulp (pooled from several apples) were processed and the CV was calculated for the extraction and assay of each of the minerals.

The total levels of the investigated minerals also were determined in a freeze-dried sample from the same fruit by the acid digestion method described for the pellet; the values obtained by the two methods were compared. Potassium was quantified with a flame photometer (Corning 4100) and Ca (in the presence of 1% La_2O_3) and Mg by atomic absorption.

The number of replicates in each experiment is specified in the figure legends.

Results and Discussion

The percentages of water-soluble Ca, Mg, and K of the total mineral levels found on both sampling dates did not generally differ significantly for the six orchards (Table 1). The largest variation was for Ca (8%) and the smallest was for K (2%). This result was in spite of the fact that a) there were significant differences among orchards in total mineral levels (Table 2), and b) the levels of Ca and Mg diminished and that of K remained unchanged from the preharvest to the harvest assessment. The latter finding is in agreement with previous reports on changes in mineral levels during fruit maturation (2). The uniform percentage of water-soluble Ca, extracted at harvest from the pulp of 'Jonathan' apples grown in various regions of Israel, is in close agreement with the 54% to 65% of total Ca extracted in water from the pulp of 'Cox's Orange Pippin' apples from 16 different orchards in the Netherlands (17). The

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Table 1. Water-soluble Ca, Mg, and K in 'Jonathan' apples from different locations.

	Element content (% of total)					
	Preharvest[z]			Harvest[y]		
Orchard	Ca	Mg	K	Ca	Mg	K
A	63	80	92	61	79	94
B	61	79	92	63	79	95
C	58	77	92	55	76	94
D	61	77	92	56	76	94
E	56	77	91	59	78	93
F	62	80	90	62	80	95
LSD (5%)	5.8	3.6	2.3	7.0	4.4	2.2

[z]Ten to 14 days prior to start of harvest.
[y]During the commercial harvest.

ionic Ca extracted by water from the pulp of 'Delicious' apples was 50% to 56% of total Ca (9). Perring (13) reports only 40% to 46% of the Ca extracted from English 'Cox's Orange Pippin' apples as being water-soluble, but his results are for stored fruit. Perring's lower values could be the result of Ca migration (2) or immobilization (14) during storage.

Ferguson et al. (6) warned that aqueous extracts might be of little value in studying Ca fractionation because of the differing extractabilities in different plant tissues (apple fruit and leaf, kiwifruit). However, our results regarding Ca extractability in water are similar to those of van Lune and van Goor (17), in spite of differences in cultivar, location, and state of tissue used for Ca extraction by each investigator (fresh and freeze-dried powder, respectively).

Little attention has been paid, so far, to the distribution of Mg and K between the water-soluble and non-soluble fraction in apple fruit, particularly in fruit prior to storage. For 'Cox's Orange Pippin' apples after storage, Perring and Plocharski (14) reported that 6% to 9% of K and 30% to 35% of Mg were found in the water-soluble fraction, resembling the value ranges we found. Aqueous extraction of K was more efficient than extraction with 75% HCl, following which 18% to 21% remained insoluble (14). High percentages of water-extractable K have been found for other plant tissues. Bar-Akiva (1) reported that at least 85% of total K was determined in aqueous extracts from leaves of several citrus cultivars.

The information available in the literature of water-extractable minerals has not been used thus far to estimate total levels of minerals in plant tissue. The correlations between total and soluble Ca, Mg, and K (Fig. 1) suggest that mineral determination in water extracts to assess their total content in apple tissue at harvest is justifiable. The variability within each orchard in the measurements of both water-soluble and total minerals was small, and the two parameters correlated at a high level of significance; for all three minerals the level of significance was $P = 0.001$ or higher at both sampling dates (similar levels of significance were obtained in preharvest sampling). There was, however, a difference in the coefficients of correlation for the three, the highest for K and the lowest for Ca in this year. In two other years, even higher correlations were obtained for Ca: 0.874 and 0.989. The method described is reproducible and precise: the coefficients of variance for a single determination were 4.1%, 4.1%, and 2.9% for water-soluble Ca, Mg, and K, respectively.

The determination of the total contents of all three elements in the pulp by the proposed method correlates very well with determination by the acid digestion method, $P < 0.001$ (Fig. 2). Bar-Akiva (1) found a good correlation ($P = 0.01$) between water-soluble and total K (determined by Kjeldahl digestion) in citrus leaves. Determination by the acid digestion method had the disadvantage of increased coefficients of variance for a single determination: 6.3%, 11.9%, and 5.5%, for Ca, Mg, and K, respectively. Again, the best correlation was obtained for Mg, followed by K, and then Ca.

The application of the suggested method appears justified, since a) in apple pulp most of the total mineral content is extractable in water; b) it might be of importance to measure the Ca content of fruit as close to harvest as possible, considering the fact that its concentration declines during maturation at or shortly before harvest (2); c) in 'Cox's Orange Pippin' water-soluble Ca at harvest correlated with bitter pit as did total Ca (17); and d) the method is simple, time-saving and efficient, and results are reproducible.

Comparison of sampling methods for predicting poststorage senescent breakdown of 'McIntosh' apple fruit from preharvest mineral composition revealed that predictions based on total mineral level did not differ significantly from observed breakdown, whereas those based on equations indicated significantly more breakdown than was later observed (19). However, the pre-storage level of total and water extractable Ca in 'Cox's Orange Pippin' fruit were highly correlated with bitter pit occurrence (17). Unfortunately, in our experiments the physiological disorders that developed during storage of the fruit, and which are generally related to Ca content, were either very slight

Table 2. Total Ca, Mg, and K in 'Jonathan' apples, calculated as the sum of water-soluble and insoluble fractions.

	Total element content (mg/100g fresh wt)					
	Preharvest[z]			Harvest[y]		
Orchard	Ca	Mg	K	Ca	Mg	K
A	3.68 b[x]	4.88 a	100.78 a	3.51 a	4.39 a	99.23 a
B	3.62 bc	4.33 b	92.29 b	3.27 ab	3.80 b	83.70 bc
C	3.37 bc	3.61 c	64.80 e	3.46 a	3.23 c	63.30 c
D	4.44 a	4.25 b	87.94 c	3.65 a	4.18 ab	95.96 a
E	3.24 bc	3.75 c	77.43 d	2.96 b	3.31 c	76.31 b
F	3.19 c	3.48 c	80.34 d	2.93 b	3.10 c	83.14 b
Mean	3.59	4.05	83.9	3.30	3.67	83.6
Max. SE	0.28	0.29	0.36	0.28	0.29	0.35

[z]Ten to 14 days prior to the beginning of harvest.
[y]At the end of the commercial harvest.
[x]Means in each column separated by Duncan's multiple range test, $P = 5\%$.

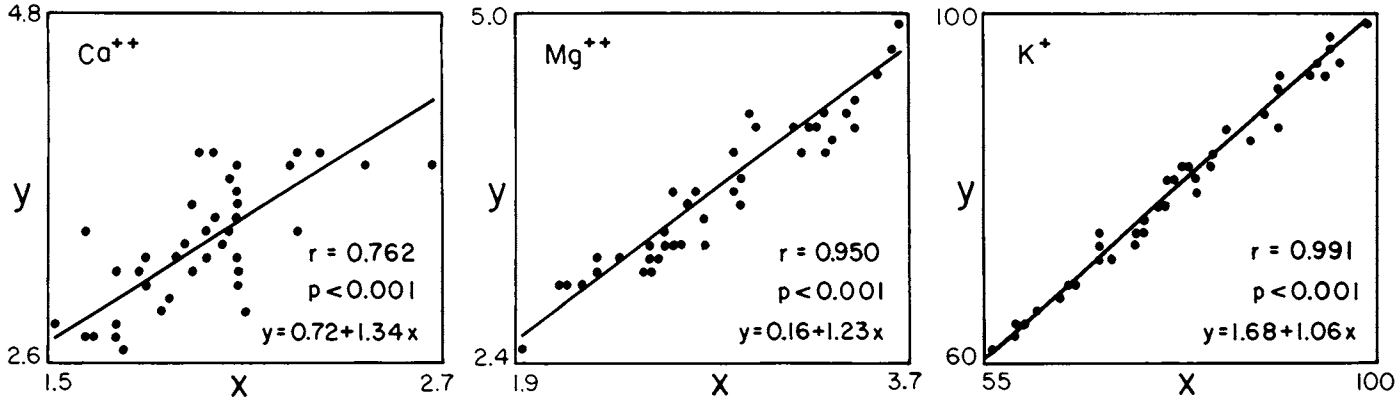

Fig. 1. Correlation between total and water-soluble Ca, Mg, and K in 'Jonathan' apples at harvest. x axis: water-soluble level of Ca, Mg, or K (mg/100 g fresh weight). y axis: total mineral (Ca, Mg, or K) content, i.e., the sum of the water-soluble and water-insoluble fractions (mg/100 g fresh weight). df = 40.

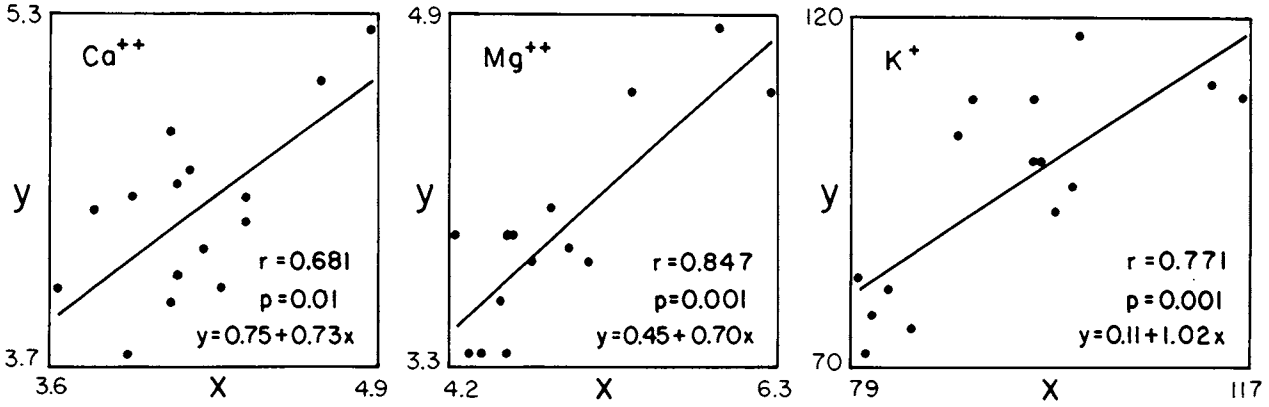

Fig. 2. Correlation between the total Ca, Mg, and K contents of 'Jonathan' apple pulp tissue determined by acid digestion and that calculated from the assay of the water-soluble and water-insoluble fractions. x axis: total mineral (Ca, Mg, or K) content determined by acid digestion (mg/100 g fresh weight). y axis: total mineral (Ca, Mg, or K) content, i.e., the sum of water-soluble and the water-insoluble fractions (mg/100 g fresh weight. df = 13.

(bitter bit) or did not correlate with the total Ca level of the fruit at harvest (senescent breakdown). Therefore, no comparison could be drawn between the correlation of disorders to water-soluble Ca vs. total Ca.

In conclusion, we believe that the information presented should encourage a change in the approach to the method of mineral determination in plant tissue. The total Ca, Mg, and K contents can be measured easily in the water-soluble fraction, a method that has many advantages over that commonly used for total mineral determination.

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Evaluation of DRIS for Judging the Nutritional Status of Hazelnuts

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Abstract. **The Diagnosis and Recommendation Integrated System (DRIS), which uses nutrient element concentration ratios as indicators of nutrient deficiency, was used to evaluate current sufficiency ranges for hazelnut trees. Reference values that were derived from published and unpublished field data were used to calculate DRIS indices for N, P, K, Ca, Mg, Mn, Fe, Cu, B, and Zn. A nutritional imbalance index (NII) was computed as the sum of DRIS indices irrespective of sign, and a threshold NII value (mean NII + 1 SD), above which severe imbalances are expected, was established. DRIS diagnoses were then compared with the sufficiency range approach to determine if relative deficiencies or excesses associated with severely imbalanced trees would have been routinely detected in 624 mineral analyses of hazelnut leaves. A previously published field trial was also reevaluated. DRIS diagnosis generally agreed with the diagnoses made by the sufficiency range method, especially if sufficiency ranges for some elements were made more narrow. However, some nutrients were never identified by DRIS as a major relative deficiency or excess in any of the trees judged severely imbalanced, based on the sum of DRIS indices. Nitrogen and Mg deficiencies were not detected unless lower NII thresholds were used. Unfortunately, lowering NII thresholds enough to detect N and Mg deficiencies identified some high-yielding trees as severely imbalanced. DRIS will not detect all deficiencies or excesses. Therefore, DRIS is best viewed as a supplement to sufficiency range diagnoses that provides additional information when severe imbalances are detected.**

The Diagnosis and Recommendation Integrated System (DRIS) is a diagnostic approach that uses nutrient concentration ratios rather than concentrations themselves to interpret tissue analyses (2). Many reports suggest that DRIS can provide a better indication of nutritional status than conventional sufficiency range approaches (1, 3–6, 10). A diagnosis is based on values of a given element relative to other important elements, rather than a rigidly defined sufficiency range. This approach minimizes the results of a general dilution or concentration and can better evaluate possible nutritional interactions (5, 9). Early work on rubber (1) and more recent studies on 'Valencia' oranges (6) and 'Royal Anne' sweet cherries (9), suggest that DRIS can be used successfully to diagnose nutritional disorders on perennial trees. The principal advantage is that DRIS provides a measure of nutritional balance rather than evaluating only a single deficiency or excess at a time. Details of use of DRIS are presented elsewhere (2–5, 11).

Hazelnuts are an important crop in Oregon, but their limited distribution has resulted in few studies of mineral nutrition in relation to productivity. Leaf analysis is used now to make fertilizer recommendations, but sufficiency ranges could be refined. For example, although field trials have established an optimum sufficiency range for N (2.2% to 2.4%), there is not a strong relationship between leaf N concentrations and nut yield. Furthermore, N treatments also alter the concentrations of other mineral elements in leaves, complicating interpretation. It is possible that induced imbalances involving N, P, and Mn have adversely affected trees and weakened the relationship between leaf N concentration and yield. Our goals were to develop DRIS norms for hazelnuts, evaluate the possibility of N-induced imbalances, and determine if DRIS evaluations could provide an approach to independently evaluate current sufficiency ranges.

Materials and Methods

Published and unpublished data used in this study for DRIS norm development consisted of leaf mineral composition (N, P, K, Ca, Mg, Mn, Fe, Cu, B, Zn) and corresponding yield for

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