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Mineral Composition of 'Marsh' Grapefruit Peel during Maturation

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Abstract. **Potassium, Ca, Mg, Na, and Zn were determined in 'Marsh' grapefruit peel during 2 growing seasons from September through May. Peel mineral concentrations, measured as mg·g^{-1} fresh peel weight, were: K (2.79–3.52), Ca (0.55–0.96), Mg (0.18–0.28), Na (0.003–0.034), and Zn (0.001–0.002). Freezing temperatures resulted in higher peel concentrations for K but lower concentrations for Ca and Mg.**

Extensive studies have delineated the requirements of specific minerals for citrus growth (1, 2, 4, 5, 7, 8), but limited studies (3, 9) have been conducted on mineral variation during fruit maturation. We conducted a study on the variation of peel Na, K, Mg, Ca, and Zn contents during fruit maturation. These 5 minerals are essential elements known to be critical for growth and membrane integrity of multicellular plants (6).

'Marsh' grapefruit (*Citrus paradisi* Macf.) from mature trees on rough lemon (*C. jambhiri* Lush.) rootstock were harvested from field research plots receiving normal horticultural care. Each harvest involved a random sampling of 8 fruit per tree from a total of 5 trees. Each tree was considered a replication. Fresh peels (includes flavedo and albedo) from fruit of the same tree were combined and ground to a fine texture in a Waring blender. About 300 g, weighed to the nearest 1 g, were placed in a one-liter glass beaker and placed in a drying oven at 195°C for 18–26 hr to remove all water and to char the sample partially before weighing. Exactly 2.00 g of the charred peel was weighed, placed in a platinum crucible, and dry ashed in a muffle furnace at 500° to 550° overnight. Any dark material was broken up and dissolved with 1–2 ml of HNO_3 and reashed in the muffle furnace. The fine white ash was dissolved in 2 ml of the concentrated HNO_3 and diluted volumetrically to 100 ml with distilled water.

All elemental analyses were performed with a Perkin-Elmer 503 atomic absorption spectrophotometer capable of operating in either the atomic absorption or flame emission mode. Flame emission analyses for potassium and sodium were conducted without the use of elemental lamps. Concentrations were determined directly from the amount of radiant energy at 769.9 annd 589.0 nm, respectively. Magnesium, Ca and Zn concentrations were determined via atomic absorption at 285.2, 422.7, and 213.9 nm, respectively. Instrumental parameters used for each element were those recommended by the manufacturer.

Minerals were monitored in 'Marsh' grapefruit peel between 1 Sept. and 3 May, and were determined as milligrams mineral per gram fresh weight of peel (Table 1). Monthly sampling dates were not the same for each of the 2 years but were kept as close as practical. During the 1981–82 season, Central Florida experienced a hard freeze (12 Jan. 1982); temperatures plummeted to between -5° and -8°C in citrus groves. Damage to a fruit immediately following a freeze is not always noticeable. One week after the freeze (19 Jan.), we inspected grapefruit for freeze damage but could detect none. Two weeks after the freeze (26 Jan.), however, freeze damage was noted by drying of juice sacs, separation of juice sacs within a carpel, and a general ricey appearance (10).

The 2nd season (1982–83) was free of any freezing temperatures, and mineral change during maturation was considered normal for this growing period. Table 1 contrasts not only 2 different seasons (1981–82 vs. 1982–83), but also shows the effects of freezing temperatures on grapefruit peel mineral composition.

Potassium is the most abundant mineral in grapefruit peel. During the 1981–82 season, K was found within a range of 2.8–3.5 mg·g^{-1}. Between 31 Dec. and 1 Mar. (includes the freeze period), noticeable fluctuations were evident; samples varied as much as 700 ug·g^{-1} peel between sampling periods. In contrast, peel analyzed during the 1982–83 season showed K concentrations within a relatively confined region of 2.9–3.3 mg·g^{-1}: wide fluctuations in values were absent between 31 Dec. and 1 Mar. The K concentration on 1 Feb. 1983 was significantly lower than determined on 1 Feb. 1982 and strongly indicates that the peel accumulated potassium 3 weeks after the freeze. Potassium values determined for harvests after 1 Feb. were similar for both the 1981–82 and 1982–83 seasons.

Calcium is an important element associated with pectin and the 2nd most abundant in the peel. Calcium concentration was noticeably constant (0.78–0.85 mg·g^{-1}) during the 1st part of the 1981–82 growing period but showed a marked decrease (0.81–0.56 mg·g^{-1}) after the freeze (values for 1 Feb. and 1 Mar.). In contrast, Ca values determined during Feb. and 1 Mar. of the 1982–83 season showed no noticeable change; however, they were significantly higher when compared to the 1981–82 season. One interesting observation of peel Ca is that after the freeze of 12 Jan. 1982 its concentration decreased; but, by 1 Apr. peel Ca had recovered to levels typical of the nonfrozen fruit of the 1982–83 season.

The divalent mineral, Mg, is found in citrus peel at a concentration about 1/4 the level

Table 1. Variation of mineral contents of 'Marsh' grapefruit peel as related to maturation and freezing temperatures (mg·g^{-1} fresh weight).

	Potassium		Calcium		Magnesium	
Month/day	1981–82	1982–83	1981–82	1982–83	1981–82	1982–83
1 Sept.	3.04 ± 0.27	---	0.82 ± 0.03	---	0.28 ± 0.04	---
1 Oct.	2.99 ± 0.22	---	0.85 ± 0.04	---	0.27 ± 0.05	---
2 Nov. 5 Nov.	3.14 ± 0.19	3.30 ± 0.20	0.82 ± 0.06	0.96 ± 0.09	0.25 ± 0.01	0.23 ± 0.03
30 Nov. 1 Dec.	3.03 ± 0.07	2.79 ± 0.31	0.80 ± 0.02	0.90 ± 0.10	0.23 ± 0.02	0.25 ± 0.03
31 Dec. 4 Jan.	2.78 ± 0.18	2.97 ± 0.08	0.78 ± 0.06	0.83 ± 0.05	0.21 ± 0.03	0.22 ± 0.03
19 Jan.	3.52 ± 0.34	---	0.84 ± 0.09	---	0.22 ± 0.03	---
26 Jan. 24 Jan.	3.29 ± 0.13	3.16 ± 0.15	0.81 ± 0.09	0.79 ± 0.05	0.23 ± 0.03	0.21 ± 0.02
1 Feb.	3.47 ± 0.22	3.10 ± 0.12*	0.56 ± 0.07	0.80 ± 0.05*	0.18 ± 0.02	0.22 ± 0.02*
1 Mar.	3.08 ± 0.28	2.94 ± 0.14	0.55 ± 0.05	0.72 ± 0.03*	0.19 ± 0.03	0.20 ± 0.01
1 Apr.	3.14 ± 0.19	2.93 ± 0.15	0.64 ± 0.06	0.68 ± 0.04	0.20 ± 0.02	0.19 ± 0.02
3 May 2 May	3.33 ± 0.67	3.13 ± 0.22	0.76 ± 0.15	0.70 ± 0.05	0.22 ± 0.04	0.19 ± 0.01

*Significantly different from previous year's results at the 1% level.

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of Ca and is an important mineral associated with membrane structure. The concentration of Mg was slightly higher during the 1st part of the 1981–82 sampling period (0.25–0.28 $mg \cdot g^{-1}$; September-November) when contrasted to the latter period (0.18–0.23 $mg \cdot g^{-1}$; December-May). When Mg values of the 1981–82 season were compared to the 1982–83 season values, the only significant difference was noted with the 1 Feb. sampling date; the concentration on that date was elevated in the 1982–83 season.

Because Na was found at very low levels in peel, its concentration range was not reported in Table 1. Over the 9-month maturation period, Na fluctuated between 0.003 and 0.034 $mg \cdot g^{-1}$ fresh peel weight. It was difficult to assess any impact of freezing on peel Na because of its low levels (about 1/100 to 1/1000 the level of K).

Zinc, also not reported in Table 1, showed no significant variation over the 1981–82 and 1982–83 maturation periods. Its concentration (0.001–0.002 $mg \cdot g^{-1}$ fresh peel weight) in peel was negligible.

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Endogenous Plant Growth Substances in Developing Fruit of *Prunus cerasus* XIII. Relationship between Conjugated and Free Abscisic Acid in Pericarp

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Abstract. **The presence of abscisic acid (ABA) in both conjugated and free forms was established by thin-layer and gas-liquid chromatography and by combined gas chromatography-mass spectrometry. The concentration of the free acid was greater than that of conjugated ABA early in fruit development (Stages I and II); however, the conjugated form was more prevalent than the free acid during Stage III. The highest concentration of both the free acid and the conjugated ABA was found during periods of most active fruit enlargement and low concentrations during the retarded phase of pericarp growth. The conjugated form represented a significant and, during Stage III, the major portion of the total ABA present.**

Abscisic acid (ABA) occurs naturally in fruits of numerous species (3, 4, 6, 7, 8, 12, 15). In addition, a water-soluble conjugate, abscisyl-β-D-glucopyranoside, has been isolated from fruit of *Lupinus luteus* (6) and since has been found in other fruits (8, 10, 13). Of the total ABA found in fruit of *Rosa arvensis*, 20% was in the form of the glucosyl abscisate (8). Plant tissues readily convert exogenously supplied ABA to the glycosyl abscisate (8, 16), and there is indication that this conjugate may be hydrolyzed (8). Any assessment of the role of ABA in fruit growth should take into consideration not only the free acid, but also the conjugated fraction. We reported (4), based on biological activity, that inhibitor levels in sour cherry fruit were directly related to growth rate of the fruit. We now report on changes in levels of a hydrolyzable ABA conjugate during fruit development and discuss its relationship to ABA.

Sour cherry (*Prunus cerasus* L.) fruit were collected from 7-year-old trees at weekly intervals from one week after anthesis until fruit maturity. The fruit were frozen immediately in dry ice, transferred to the laboratory, and lyophylized. A fruit growth curve was constructed based on pericarp dry weight. Fruit from collections made on 21 and 28, 35 and 42, and 56 and 70 days after anthesis, selected as being in Stage I, II, and III of fruit development, respectively, were used for analysis. The pericarp (exo-, meso-, and endo-) tissue was removed, ground to pass a 20-mesh screen, and stored at -25°C until extraction.

Procedures employed in extraction, fractionation, thin-layer chromatography (TLC), and wheat coleoptile bioassay of ABA have been described previously (4, 13). The aqueous fraction of the methanol extract, after partitioning against diethyl ether at pH 2.5, was adjusted to pH 11 with NaOH and hydrolyzed for 30 min at 60°C. The solution was cooled, the pH adjusted to 3.0, and the released inhibitor extracted and purified as previously described.

The ABA in the acid-ether fraction and that released on hydrolysis was methylated with diazomethane following TLC and subjected to gas-liquid chromatography (Packard 7300) using a U-column (183 cm × 2 mm) packed with 3% SE 30 on silanized Gas Chrome Q (60-80 mesh). The injector block and detector temperature was 250°C, the column was isothermal at 170°, and the carrier gas (N_2) flow was 42 $ml \cdot min^{-1}$. To quantify ABA, the peak heights were compared with those of standard quantities of both the *cis-trans* and *trans-trans* isomers of MeABA. Conclusive identification of the inhibitor (both the free acid and that released on hydrolysis) as ABA was obtained by combined gas chromatography-mass spectrometry (GC-MS). Procedures employed and equipment used were identical to those described earlier (4).

Two zones of growth inhibition, as indexed by the wheat coleoptile assay, were found after hydrolysis of the aqueous phase and partial purification by TLC. We previously identified (13) *p*-coumaric acid as the inhibitor running at Rf 0.6–0.9. The inhibitory substance running at Rf 0.0–0.2 cochromatographed with synthetic ABA and, after further purification on TLC (benzene:ethyl acetate:formic acid, 70:30:1), was identified by GC-MS as ABA. The inhibitory substance in the acid-ether fraction (free acid)

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