

Functional Components in Citrus: Alteration by Mineral Elements

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Abstract

Grapefruit functional compounds such as carotenoids, flavanones, limonin 17- β -D glucopyranoside (LG), and vitamin C have been shown to have potential human health benefits including cancer and cardiovascular disease prevention. This study was conducted to determine different levels of potassium (K) on functional compounds. Field and laboratory studies were conducted at the University of Florida, Citrus Research and Education Center (CRES) in Lake Alfred, and Texas A&M University-Kingsville Citrus Center (TAMUK-CC) in Weslaco, respectively. 'Ray Ruby' grapefruits on Carrizo citrange rootstock were harvested from a nitrogen (N) and K rate effect study conducted in CREC and shipped to TAMUK-CC. Fifteen fruits each from three replications per treatment were stored. Reversed phase high performance liquid chromatography was used to quantify the functional compounds. The highest levels of N/K rate significantly decreased naringin concentration compared to that of fruits sampled from the tree which received no supplemental N or K. Although there were no significant differences in levels of beta carotene and lycopene due to different levels of K applied, total carotenoids levels were significantly higher in control fruits compared to that in the fruits from 186 kg K/ha treatment. Higher levels of K application decreased ascorbic acid while increased dehydroascorbic acid concentration as compared to those in the fruits from N/K unamended treatment. Total vitamin C concentration was not significantly influenced by different levels of K. Grapefruits sampled from the trees which received 93 kg K/ha had significantly lower LG concentrations compared to that of the fruits in the N/K unamended treatment. Since this study was conducted with variable rate of both N and K, further studies with variable rates of single elements are necessary to understand the effects of each element on the phytonutrient contents in the fruits.

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Introduction

Grapefruit (*Citrus paradisi* Macf.) contain various functional components including flavonones (naringin, narirutin), carotenoids, limonoids (limonin, nomilin, limonoid glucosides), folic acid, aurapatene, and vitamin C. The bitter compounds in grapefruit are naringin (7- β -neohesperidoside of naringenin), limonin, and nomilin. However, narirutin (naringenin 7- β -rutinoside) and limonoid glucosides are tasteless (Fong et al., 1990).

Certain functional components may not be considered essential by conventional measures, but are increasingly recognized for their beneficial effects on health promotion and disease prevention. Market research data from many sources showed that consumers in the U.S., Japan, and Western Europe have already accepted the concept of biogenic diets containing biologically active functional components and are willing to try diets to prevent chronic diseases (Shukla, 1992). In recent years, many epidemiological and case control studies have shown that citrus flavonoids have potential biological activities which include antioxidant, anticancer, antiviral, anti-inflammatory activities, effects on capillarity, cholesterol lowering ability, and an ability to inhibit human platelet aggregation (Benavente-Garcia et al., 1997; Guthrie et al., 2000; Middleton and Kandaswami, 1994). Bronner and Beecher (1995) reported that average intake of flavonones from citrus juices by Americans is estimated to be about 23 mg per day while the average daily intake of flavonoids is 1 g per day (Kuhnau, 1976).

The limonoids exist in citrus fruit primarily in the form of glucosides and are not bitter (Fong et al., 1990). Recent studies have shown that limonoids and their corresponding glucosides are inhibitors of chemical carcinogenesis in laboratory animals (Lam et al., 1994; Miller, et al., 1989, 2000) and in human breast cancer cells (Guthrie et al., 2000; Tian et al., 2001) as well as reduce cholesterol (Kurowska et al., 2000).

Vitamin C is an essential nutrient for humans and citrus fruit as well as their processed products are the most commonly consumed sources (Kefford, 1973). Investigators have postulated a role of vitamin C in the prevention of cancer, heart disease, and in the augmentation of immune function such as in the prevention of common cold (Hemila, 1992). It is clearly established that the pigment lycopene is most

sensitive to low levels of K. Evidences have shown increased lycopene concentration in tomato with increasing K concentration (Trudel and Ozbun, 1971), and higher levels of carotene in cucumber leaves supplied with higher K (Lamarani et al., 1996).

There is considerable interest in understanding the relationship between the management of crop production inputs and the crop product quality including the components that contribute to human health benefits. These relationships are extremely important in an effort to cater to the needs of rapidly increasing health conscience consumers. Studies have begun to evaluate the effects of different fertilization rates on fruit production and fruit quality of grapefruits. This study was conducted to understand whether functional components of grapefruit are influenced by different levels of N and K.

Materials and Methods

Field experiment. The fruits used in this study were from a large field experiment to evaluate the long term effects of application of variable rates of N or K on fruit production and fruit quality in sandy soils in Central Florida. 'Ray Ruby' grapefruit trees on Carrizo citrange rootstock were used. The plot unit comprised five trees within a row chosen in alternate rows. The N and K sources used in this experiment were granular ammonium nitrate and muriate of potassium. A commercial dry granular fertilizer spreader calibrated to apply different per hectare rates was used. Most of the fertilizer was delivered under the canopy within the wetting area under the tree micro sprinklers. Fruits were sampled from middle three trees to minimize the border effects. The treatments included: 112, 168, 224, and 280 kg N/ha rates as well as no supplemental N application treatment. The N:K₂O ratio in the fertilizer was 1:1. Therefore, with the above rates of N, K rates also varied at 93, 140, 186, and 233 kg K/ha.

Functional component analysis. Fifteen fruits were sampled per plot from the above five fertilizer rate treatments with three replications. These fruits were used for analysis of lycopene, beta carotene, naringin, narirutin, vitamin C, ascorbic acid, dehydroascorbic acid, and LG concentrations.

Standards. Standard naringin, ascorbic acid and dehydroascorbic acid were purchased from Sigma Chemical Co. St. Louis, Missouri. Limonin 17- β -D-glucopyranoside was supplied by Shin Hasegawa of the USDA-ARS, Albany, CA. Narirutin was supplied by John Manthey, USDA-ARS, Winter Haven, FL.

Sample preparation. Fruits (commercial size 40) were washed, and flavedo and albedo were removed with a potato peeler. The remaining parts (juice sacs and pulp) were blended for 3 min and filtered with qualitative 415 filter paper. Filtered samples were collected in 50 mL tube and stored in -80°C until analyzed for limonoids, flavonones, and vitamin C.

Chromatographic conditions. A high performance liquid chromatography (HPLC) consisted of a Perkin-Elmer binary LC pump 250, an LC-600

autosampler, a UV/VIS Spectrometric detector LC295, and PE 1020 Integrator program was used for limonoid and flavonones. The same HPLC system with an HP 3394 integrator was used for measuring total vitamin C.

Carotenoids. Sample preparation procedures described by Sadler et al. (1990) and Rouseff et al., (1992) were used. Approximately 12 g of pulp sample was weighed in a 125-mL brown Erlenmeyer flask. A 100 mL solvent with hexane: ethanol: acetone (50:25:25) was added to the flask and agitated for 10 min on a magnetic stirrer at 8 speed, 15 mL water was added followed by another 5 min agitation. Solvent layers were allowed to separate into distinct polar and non polar layers. The top hexane layer of 2 mL was taken and dried with liquid N and heated at 30°C for 5 min. Samples were dissolved in 100 μL tetrahydrofuran (THF), diluted to 2 mL with methanol : THF (75:25), and filtered through 0.45 μL filter prior to injection on to the HPLC system.

Flavonoid analysis. Samples were analyzed for flavonone content by reverse phase HPLC with some modifications of the Berhow (2000) method. An aliquot of juice sample was diluted with dimethylsulfoxide (1:1), centrifuged and filtered through 0.45- μm nylon filters (Alltech Associates, Deerfield, IL.) and 20 ml of this solution was injected into HPLC system. The same HPLC system as explained in the LG section was used and a Adsorbosil C-18 column (250 X 4.6 μm , 5mm) was eluted with a linear gradient starting with 10% acetonitrile, 5 mM phosphoric acid and ending at 26% acetonitrile in acetonitrile 5 mM phosphoric acid for 36 min. The narirutin and naringin peak were detected at 280 nm with retention times of 25 and 27 min, respectively. The flavonones were identified by confirmation of their respective spectra, authentic standard and retention times.

Limonoid analysis. Juice sample prepared as described in sample preparation section was filtered through qualitative P8 filter paper. The LG was analyzed with little modification from the previous procedure (Ozaki et al., 1991). A 10 mL of juice was passed through a C-18 Sep-Pak (Waters Associates, Milford, MA.), LG was retained on the column, rinsed with water, eluted with methanol and determined by HPLC using a Waters Spherisorb ODS2 C-18 column (250 X 4.6 μm , 5mm) along with a guard column. One mL of juice was diluted with 3 mL of 80% ethanol. The mobile phase consisted of linear gradient starting with 10% acetonitrile, 3 mM phosphoric acid and ending at 26% acetonitrile in 3 mM phosphoric acid for 60 min. The flow rate was 1 mL/min. Detector was set to 210 nm and LG peak was identified at 21 min.

Vitamin C. Modified procedure of Zapata and Dufour, (1992) was used to separate dehydroascorbic acid, ascorbic acid. Twenty μL of the solution was injected into HPLC system. A Waters Bondapak C-18 column (30 X 0.4 cm) with a guard column was used and the flow rate was 1.8 mL/min. Mobile phase

was methanol:water (5:95 v/v) with 0.01 M ammonium phosphoric acid. Dehydroascorbic acid and ascorbic acid were detected at 348 and 261 nm, respectively.

External standards were used to quantify the compounds. Peak areas were normalized to the external standard and the standard curve was fitted by linear regression (peak area vs. concentration in $\mu\text{g/g}$). Total flavonone concentration was calculated by combining naringin and narirutin concentration. The long term variability of LG and both flavonones in the laboratory was low (coefficient of variation <4% and 6%, respectively).

Statistical analysis. Data were analyzed as a completely randomized design using GLM procedures (SAS Institute Inc., Cary, NC). Fisher protected LSD was employed for all mean separation analysis.

Results and Discussion

Potassium rates in the range of 0 to 233 kg/ha/year did not significantly influence lycopene and beta carotenoids. However, concentrations of total carotenoids were significantly greater in the fruits from no supplemental K treatment as compared to that of the fruits from trees which received 186 kg K/ha/year (**Figure 1**). Lycopene concentrations increased with increasing rate of K in Tomato (Trudel and Ozbun, 1971), and in cucumber leaves (Lamarani et al., 1996).

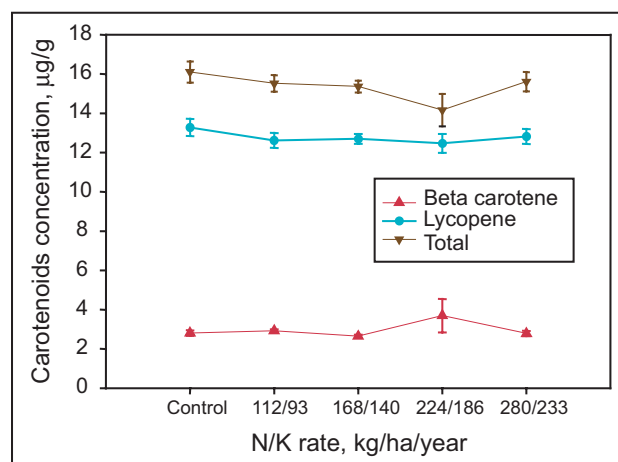


Figure 1. Effect of K and N on beta carotene, lycopene, and total carotenoids.

In general, naringin decreased with an increase in K rates (**Figure 2**). Fruits from the highest K rate treatment 233 kg/ha/year had the lowest naringin concentration. Indeed, naringin, narirutin, and total flavonone concentrations were high in the fruits of the trees which received no fertilization.

It is evident that although total vitamin C concentrations were not significantly affected by K treatments, K applied at 93 kg/ha/year significantly increased dehydroascorbic acid and decreased ascorbic acid and LC concentrations in the fruits compared to those in the fruits from unfertilized trees (**Figures 3 and 4**). This agrees with the previous con-

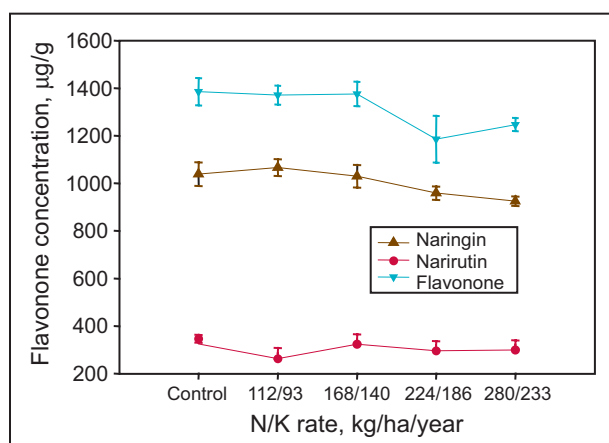


Figure 2. Effect of K and N effect on naringin, narirutin, and total flavonone concentrations.

clusion that optimum supplies of K increases the vitamin C content of citrus fruit (Hearn, 1993; Smith, 1966; Jones et al., 1973; Nagy, 1980). Because carbohydrate metabolism is strongly influenced by K availability, optimum K nutrition of citrus trees has a beneficial effect on the vitamin C content of citrus fruits (Embleton and Jones, 1966; Smith and Rasmussen, 1960).

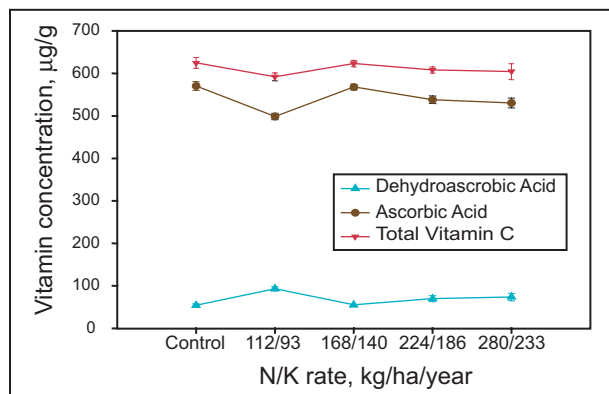


Figure 3. Effect of K and N on dehydroascorbic acid, ascorbic acid and total vitamin C concentrations.

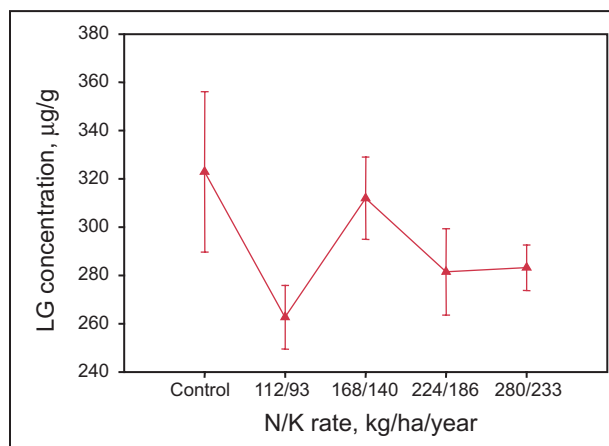


Figure 4. Effect of K and N on limonin 17-b-D glucopyranoside concentrations.

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