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Production System and Storage Temperature Influence Grapefruit Vitamin C, Limonoids, and Carotenoids

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Supporting Information

ABSTRACT: Concentrations of grapefruit (cv. 'Rio Red'; *Citrus paradisi* Macf.) bioactives grown under organic and conventional production systems were evaluated after storage at various temperatures. The first experiment was conducted in November 2008 and the second experiment was conducted in February 2011 using commercial production, processing, and packing procedures. The harvested grapefruits were stored at 23 °C (room temperature) or 9 °C for 4 weeks and analyzed for vitamin C, limonoids, and carotenoids at the end of each week using HPLC. Vitamin C levels were higher in organically grown grapefruits (41.8 mg/100 g) compared to conventionally grown grapefruits (39.2 mg/100 g) at 0 days after harvest in the first experiment. However, production system did not significantly affect vitamin C levels in the second experiment. During storage at room temperature, vitamin C degradation losses ranged from 0.5 to 7% for organically produced grapefruits and from 3 to 18% for conventional grapefruits in both experiments. In the first experiment at harvest, organically produced grapefruits had 77% higher ($p \leq 0.05$) nomilin than conventionally produced grapefruits, whereas grapefruits grown under the conventional production system had 2-fold higher lycopene levels compared to organic grapefruits. In the second experiment, both β-carotene and lycopene levels were significantly ($p \leq 0.05$) higher in conventionally produced grapefruits than in organic grapefruits. Overall, conventional production significantly increased grapefruit carotenoid levels in both experiments. In general, storage temperature (room temperature and 9 °C) had minimal effects on vitamin C degradation but significant effects on the degradation of carotenoids in the first experiment.

KEYWORDS: *grapefruit, organic, conventional, bioactives, HPLC*

INTRODUCTION

Grapefruit (*Citrus paradisi* Macf.) is one of the major commercial citrus crops grown in the subtropical regions of the United States including Florida, Texas, and California for the fresh market as well as processing. The red-colored varieties such as 'Rio Red' grown in Texas are particularly rich in bioactive compounds (health-promoting compounds) such as vitamin C, limonoids, and carotenoids.[1,2] Although genetics play a key role in determining the levels of bioactive compounds in fruits and vegetables, cultural practices such as fertilization and pesticide use and environmental factors such as temperature can also significantly affect bioactive concentrations. In recent years, the role of production systems, especially the effect of organic versus conventional practices, on the bioactive properties of fruits and vegetables has been a topic of intense public debate and research. Organic fruits and vegetables are perceived by most consumers to be safer than conventionally grown produce.[3] This perception has been partly responsible for the dramatic rise in sales of organic produce in the United States from $3.6 billion in 1997 to $21.1 billion in 2008.[4] Organic produce, according to U.S. regulations, is grown under conditions devoid of synthetic pesticides, growth hormones, antibiotics, chemical fertilizers, genetically modified organisms, and sewage sludge.[5] Organically grown produce generally attracts approximately 73−108% higher prices compared to the conventionally grown foods in the fresh food market.[6] Whereas organic agriculture has traditionally focused on risk reduction of chemical residues and heavy metals, recent studies have indicated that organic production practices such as fertilization may also influence the contents of health-promoting compounds.[7−9]

Unlike the synthetic fertilizers used in conventional production systems, most organic supplements have slow nutrient release properties. This slow availability of nutrients and the resulting changes in photoassimilate partitioning between various metabolic processes may lead to preferential accumulation of secondary metabolites that have bioactive properties.[10,11] Previous studies have suggested that nitrogen availability to plants may have an inverse relationship with vitamin C content and a positive influence on β-carotene levels,[12] but other studies also suggest that minimal synthetic chemical use in organic production may increase the nutrient quality of fruits and vegetables.[13,14] In addition to different production practices, several postharvest procedures including storage duration and storage temperature can have a significant influence on levels of bioactive compounds in fruits and vegetables.[15−17] These effects are expected to vary depending on the type of bioactive compound, plant species, organ, and

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Table 1. Farm Inputs in Organic and Conventional Grapefruit Orchards for Fertilization and Insect and Weed Control

input	rate of application during growing season/acre	no. of applications
	Organic Production System	
compost	N, 47.5 kg; P, 29.5 kg; K, 18 kg	1
compost spray formula	N, 9 kg	3
micronutrient spray	Ca, 1.3 kg; Mn, 1.3 kg; Mg, 2.2 kg; Zn, 0.9 kg	2
sulfur spray	5.4 kg	5
pest control	DesX (fatty acid), 5.6 L; Safe Tside (vegetable oil), 5.6 L	3
flood irrigation	15 cm	4
	Conventional Production System	
inorganic fertilizer	N-32, 87 L	1
herbicides	Krover IDF, 1.13 kg; simazine 90 DF, 0.9 kg; diuron 80 DF, 0.58 kg; glyphosate, spot application if necessary, Bucanneer Plus, 60 mL; Bronc Max, 60 mL	1
insect control	Vendex, 1.13 kg; Danitol, 0.5 L; Gem, 0.25 L	1
fertilizer spay	Foli Gro, booster fertilizer	2
fungicide	Kocide 3000, 1.8 kg	1
flood irrigation	12 cm	5

tissue. The effects of production system and storage temperature on grapefruit bioactives were poorly understood.

Vitamin C, limonoids, and carotenoids are the grapefruit bioactives that primarily contribute to the fruit's sensory attributes such as flavor and color and to its health-promoting properties.[18−20] Vitamin C occurs as both ascorbic acid (reduced form) and dehydroascorbic acid (oxidized form) in the fruit at the time of harvest and storage. Consumption of both forms is beneficial to human health due to their antiscorbutic properties.[21,22] Therefore, vitamin C analysis is critical, as the levels of ascorbic acid and dehydroascorbic acid continuously interchange during storage. Limonin and nomilin, the two major limonoid aglycons in grapefruit, have been demonstrated to have anticarcinogenic properties and are also responsible for grapefruit's bitterness.[23,24] Lycopene and β-carotene are two major antioxidant carotenoids that contribute to the grapefruit flesh color. The integrated studies including production systems, postharvest handling practices, and levels of bioactive compounds including limonoids in grapefruit have not been studied comprehensibly. The objectives of the current study were to investigate the influence of organic and conventional grapefruit production systems and simulate postharvest storage conditions on grapefruit health-promoting bioactive compounds.

MATERIALS AND METHODS

Chemicals. L-Ascorbic acid, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), and metaphosphoric acid (MPA) were purchased from Sigma Chemicals (St. Louis, MO, USA), orthophosphoric acid was obtained from EMD Chemicals (Gibbstown, NJ, USA), and dihydrogen ammonium phosphate was obtained from Acros Chemicals (Morris Plains, NJ, USA) for ascorbic acid analysis. Potassium chloride and nitric acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA). β-Carotene, lycopene, and *tert*-butyl methyl ether were purchased from Sigma Chemicals, HPLC grade methanol was purchased from Fisher Scientific, and sodium hydroxide was purchased from EMD Chemicals. Limonin and nomilin were isolated and identified according to our published procedure.[25,26]

Organic and Conventional Orchards. In both organic and conventional orchards, the grapefruit trees were planted in 1990. Organic grapefruits were harvested from the South Texas Organics (Mission, TX, USA), and conventional grapefruits were harvested from the Rio Queen Citrus Farms (Mission, TX, USA). The certified organic Rio Red grapefruit orchard, South Texas Organics (latitude 26° 29′ N, longitude 98° 38′ W, lat, elevation 60 m) is located 3 miles from the conventional grapefruit orchard, Rio Queen Citrus (latitude 26° 26′ N, longitude 98° 38′ W, lat, elevation 60 m). Rio Red grapefruits with uniform color (without patches of green and red), size 48 (10 cm in diameter), and maturity were selected from four quadrants of the trees. The first experiment (E1) was conducted in November 2008 and the second experiment (E2) in February 2010. The 7-day mean precipitation, temperature, potential evapotranspiration, solar radiation, relative humidity, and temperature (maximum and minimum) were obtained from the weather station located at Weslaco, TX (Figure S1in the Supporting Information). Due to the similarities in soil type (sandy loam), climate, and source of irrigation (Rio Grande River) in the two production systems (Table 1), fruits were compared for their nutrient quantity produced from organic and conventional management systems under common storage conditions.

Harvest, Storage, and Processing. Adjacent trees of five in a row (block) were randomly selected, and three such blocks were selected from each production system. The harvest was started around early morning and completed by noon, followed by washing, waxing (carnauba wax for conventional grapefruits and Decco Natur 550 wax for organic grapefruits), and packing of the fruits in the respective packing sheds. The whole process was completed on the same day and shipped to the Vegetable and Fruit Improvement Center (Texas A&M University, College Station, TX, USA) by overnight shipping. Furthermore, fruits were stored at 23 °C (room temperature) and 9 °C (cold storage) for 4-week storage studies. The relative humidity (RH) for cold and room temperature storage was maintained at 95 and 65%, respectively. Every week, weight loss and fruit decay were measured.

In this experiment, blocks were considered as replications for organic and conventional production systems. A set of 27 fruits (3 fruits × 3 samples × 3 blocks) were collected from room temperature and 9 °C storage of organic grapefruit lot (a total of 54 fruits) each week during storage. A similar procedure was followed for sample collection from conventional grapefruits during storage. A total of 108 fruits were processed during each week of storage for grapefruit bioactive analyses. The fruits were collected on 0th, 7th, 14th, 21st, and 28th days after harvest. Three fruits were peeled and blended using a Vita Prep blender (Cleveland, OH, USA) to prepare each individual grapefruit sample, and three samples were prepared from each block (Figure S2 in the Supporting Information).

Juice and Soil Mineral Analysis. The grapefruit juice and soil samples were analyzed at the soil, water, and forage testing lab (College Station, TX, USA). The nitrite nitrogen was extracted from grapefruit juice using 1 N potassium chloride (KCl) solution on a reciprocal shaker for 30 min followed by nitrite to nitrate reduction through a cadmium column in a colorimetric apparatus (FIA Lab Instruments Inc., Bellevue, WA, USA). Furthermore, the nitrate nitrogen of the sample was quantified in soil and grapefruit.[27] Other

Table 2. Sensory Evaluation of Grapefruit Grown under Organic and Conventional Production Systems[a]

color	roughness	appearance acceptability	sweetness	sourness	tartness	flavor acceptability
Organic Production System						
3.76 ± 1.8 b	5.16 ± 1.7	4.35 ± 1.9	4.86 ± 2.2	5.57 ± 1.9	4.84 ± 1.9	5.60 ± 2.0
Conventional Production System						
5.26 ± 1.9 a	4.78 ± 2.2	4.64 ± 1.8	5.44 ± 2.2	5.08 ± 2.2	5.15 ± 2.4	5.37 ± 2.4

[a]The values are expressed as the mean ± SD (n = 41) on a scale of 9. Letters a and b indicate significant differences at $p \leq 0.05$. Values without letters indicate no significant difference between the treatments.

grapefruit juice minerals were quantified by inductive coupled plasma−atomic emission spectroscopy (ICP-AES) (Spectro Genesis, Deutschland, Germany). After digesting the juice samples in concentrated nitric acid, they were allowed to stay overnight at room temperature.[28] The digested samples were heated to 125 °C for 4 h. After cooling and sample dilution, the intensity of the ion response was measured in ICP-AES. For other soil minerals, the extractions were conducted using Mehlich III reagent and analyzed in an ICP.[29]

Sensory Analysis. Fruits were evaluated on the basis of a previously established protocol for color, roughness, and overall appearance.[2] Grapefruits were cut into four quarters and used for flavor evaluation. A 41-member untrained sensory panel evaluated the grapefruits. Additionally, a 9 cm hedonic scale was constructed, similar to that of a published report.[30] The unstructured hedonic scale was anchored at 0, 3, 6, and 9 cm, respectively, but numbers were not provided on the scale to prevent the panelist from selecting a specific number on the scale. The panelists were given clear verbal instructions and also evaluation sheets before being allowed to enter the booth and provided bottled water and unsalted crackers to remove residual flavor between evaluations. In E1, Rio Red grapefruits stored for 4 weeks were evaluated for sensory attributes such as sweetness, sourness, tartness, and overall acceptability. The panelists were asked to place a vertical line across the hedonic scale to indicate the intensity of each attribute. Furthermore, quantitation was performed by measuring the distance between 0 and the vertical line.

Titratable Acidity (TA) and Total Soluble Solids (TSS). The TA of the fruits was analyzed using a DL 22 Food and Beverage analyzer (Mettler Toledo, Columbus, OH, USA). Grapefruit juice (5 g) was taken and mixed with 45 mL of nanopure water and titrated against 0.1 N NaOH. The TSS were analyzed using a hand refractometer (American Optical Corp., South Bridge, MA, USA).

Analysis of Bioactive Compounds. *Vitamin C Analysis.* Sample preparation and analysis of vitamin C followed the same procedure as the previously reported method.[31] The grapefruit samples were analyzed using an HPLC (Thermo Finnigan, Austin, TX, USA), equipped with a PDA detector (UV6000 LP) coupled with a quaternary pump system (P4000) and an autosampler (AS3000). Rio Red grapefruit juice samples (0.75 mL) were mixed with 0.75 mL of 3% metaphosphoric acid, vortexed for 5 s, and centrifuged at 10000 rpm for 10 min. The supernatant was passed through a 0.45 μm acrodisc syringe filter. A 300 μL aliquot of the filtered sample was mixed with 300 μL of 10 mM tris(2-carboxyethyl)phosphine hydrochloride to reduce sample dehydroascorbic acid to ascorbic acid, and the resulting solution was analyzed for vitamin C (total ascorbic acid). The peak separation was carried out in a C-18, Spherisorb column (150 mm × 4.6 mm i.d. and 3 μm particle size) using an isocratic mobile phase of 10 mM ammonium dihydrogen phosphate buffer with a flow rate of 1 mL/min. Each sample was analyzed twice in the HPLC with a 5 μL injection volume. The total ascorbic acid peak was detected at 254 nm, and the data were analyzed using Chromquest 4.0. Standard ascorbic acid concentrations of 5, 2.5, 1.25, 0.625, 0.3125, 0.156, and 0.078 μg were injected into the HPLC to calculate the regression equation. The final ascorbic acid levels were expressed in milligrams per 100 g of grapefruit juice.

Limonoid Analysis. Sample preparation for limonoid analysis was modified from a previously reported method.[32] Rio Red grapefruit juice (10 g) was extracted with 20 mL of ethyl acetate on a shaker for 12 h. The organic fraction of the mixture was separated, and the residual juice was re-extracted with 10 mL of ethyl acetate for 2 h. Both of the organic fractions were combined and evaporated to dryness. The dried extract was then reconstituted in 5 mL of DMSO. One milliliter of the resultant extract was passed through a 0.45 μm acrodisc syringe filter into an amber glass vial, and 10 μL was injected into the HPLC.

Separation of limonoids was performed using a Finnigan Surveyor Plus HPLC system (Austin, TX, USA). The HPLC system was equipped with a PDA Surveyor Plus detector coupled with a quaternary LC Pump Plus system, a Surveyor Plus autosampler (25 μL sample loop with valco fittings), and a C-18, PFP, kinetex column (100 mm × 4.6 mm i.d. and 2.6 μm particle size) (Torrance, CA, USA). Chromatographic separations were performed with a gradient mobile phase consisting of 3 mM phosphoric acid (A) and acetonitrile (B). Limonoids were eluted with the following solvent gradient: starting with 80% A; 0.1−7.00 min, gradient reached 75% A; 7−12.00 min, isocratic of 75% A; 12−16.00 min, gradient reached 70% A; 16−25.00 min, gradient reached 50% A; 25−30.00 min, gradient reached 40% A; the method had 5 min of equilibration at the end of the run. The sample injection volume for the analysis is 10 μL. Limonoids were detected at 210 nm, and the data were processed using Chromquest 5.0 software. The identity of the limonin and nomilin in the samples was obtained by matching with the retention times of pure standards. Each sample was analyzed three times by HPLC. The limonoid concentrations were expressed as micrograms per gram of grapefruit juice.

Carotenoid Analysis. The carotenoid analysis method was modified from a previously published paper.[33] Grapefruit juice samples (5 g) were extracted with 20 mL of chloroform.[34] The extractions were conducted in orange light to prevent any possible carotenoid degradation. The moisture from the samples was removed by adding sodium carbonate to the extracts. The samples devoid of moisture were used for the HPLC analysis. A 10 μL sample volume was injected into the HPLC for carotenoid analysis. Carotenoid separations were carried out on an YMC C-30 column (Milford, MA, USA). The elution of carotenoids occurred with the following mobile phase gradient constituting methanol (A) and *tert*-butyl ether (B). The carotenoids were eluted as follows: 0.1−10 min, 85% A; 10−18 min, 20% A; at 18−25 min the gradient combination reached 100% B. The column was equilibrated for 5 min with 85% A before successive injections and detected at 465 nm wavelength with the aid of a tungsten lamp (PDA detector). The samples in the autosampler were maintained at 6 °C throughout the analysis. The two carotenoids present in grapefruit were identified as β-carotene (retention time = 9.1 min) and lycopene (retention time = 15.5 min) by comparison with the standard carotenoids. The calibration curves for the standard β-carotene and lycopene were prepared by injecting six serial dilutions ranging from 0.3 to 0.007 μg/10 μL injection volume. Furthermore, each sample was analyzed three times in HPLC, and the carotenoid levels were expressed as micrograms per gram.

Data Analysis. Data were processed and analyzed using the statistical software program SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). A general linear model was used to analyze the variations of grapefruit bioactives between production systems and two storage temperatures for 4 weeks. The means and standard errors obtained from the outputs after the analysisof variance had been performed are presented. A split−split plot design including production system as the main plot factor and storage temperature as subplot factor 1 and storage time as subplot factor 2 were used in the analysis. In this experiment, blocks were used as replications and the treatment means

Table 3. Titratable Acidity and Total Soluble Solids of Organic and Conventional Grapefruit[a]

	first experiment				second experiment			
	room temperature		9 °C		room temperature		9 °C	
week	organic	conventional	organic	conventional	organic	conventional	organic	conventional
Titratable Acidity (Expressed in Grams per Liter (FW))								
0	9.57 ± 1.19	10.67 ± 0.75	9.57 ± 1.19	10.67 ± 0.75	9.22 ± 1.10	10.23 ± 0.71	9.22 ± 1.10	10.23 ± 0.71
1	10.19 ± 0.60	10.05 ± 0.61	10.99 ± 0.20	9.62 ± 0.09	11.27 ± 1.01	10.89 ± 0.47	10.68 ± 1.16	10.87 ± 1.52
2	11.51 ± 1.80	10.50 ± 0.87	11.42 ± 1.68	9.48 ± 0.62	11.22 ± 1.66	10.55 ± 0.99	10.75 ± 1.60	11.07 ± 1.75
3	10.31 ± 1.64	10.03 ± 0.15	9.67 ± 0.81	9.93 ± 0.55	12.46 ± 2.06	10.89 ± 2.19	10.53 ± 0.51	10.91 ± 2.13
4	11.81 ± 1.70	10.88 ± 0.72	10.72 ± 1.16	10.54 ± 0.49	11.65 ± 0.99	10.70 ± 0.47	10.47 ± 1.24	
Total Soluble Solids (Expressed in Degrees Brix)								
0	9.60 ± 0.40	10.10 ± 0.40	9.60 ± 0.40	10.10 ± 0.40	10.71 ± 0.30	11.56 ± 0.61	10.71 ± 0.27	11.56 ± 0.61
1	10.30 ± 0.20	10.30 ± 0.20	9.80 ± 0.60	10.50 ± 0.60	10.76 ± 0.50	11.73 ± 0.49	11.00 ± 0.56	11.56 ± 0.74
2	10.50 ± 0.60	10.60 ± 0.40	10.20 ± 0.70	10.30 ± 0.40	10.70 ± 0.50	11.82 ± 0.58	10.90 ± 0.43	11.53 ± 0.46
3	10.00 ± 0.60	10.50 ± 0.40	9.70 ± 0.30	10.40 ± 0.40	11.41 ± 0.50	11.53 ± 0.45	11.30 ± 0.58	11.92 ± 0.47
4	10.10 ± 0.60	10.40 ± 0.30	9.70 ± 0.40	9.90 ± 0.40	11.18 ± 0.20	11.40 ± 0.65	10.78 ± 0.82	11.42 ± 0.44

[a]The values are expressed as the mean ± SD.

Table 4. Changes in the Vitamin C[a] Contents of Organic and Conventionally Produced Grapefruit Juice during 4 Weeks of Storage at Room Temperature and 9 °C from the First Experiment (E1) and Second Experiment (E2)

	2008		2010	
storage (weeks)	organic (mg/100 g)	conventional (mg/100 g)	organic (mg/100 g)	conventional (mg/100 g)
Room Temperature				
0	41.85 ± 0.07 a	39.25 ± 0.07 b	40.98 ± 1.74	44.59 ± 1.71
1	26.87 ± 0.22 a	26.06 ± 0.22 b	61.99 ± 0.33 a	64.80 ± 0.33 b
2	47.39 ± 0.04 a	43.77 ± 0.04 b	39.20 ± 1.30	41.63 ± 1.23
3	36.96 ± 0.05 a	37.24 ± 0.05 b	36.03 ± 0.94	36.86 ± 0.54
4	38.88 ± 0.10 a	38.09 ± 0.10 b	41.14 ± 1.33 a	36.62 ± 1.33 b
Cold Storage (9 °C)				
0	41.85 ± 0.07 a	39.25 ± 0.07 b	40.98 ± 1.74	44.59 ± 1.71
1	25.74 ± 0.10 a	26.30 ± 0.10 b	60.62 ± 0.50 b	63.44 ± 0.47 a
2	45.90 ± 0.05 a	44.20 ± 0.05 b	39.87 ± 0.93	39.31 ± 0.87
3	37.62 ± 0.05 a	36.48 ± 0.04 b	35.07 ± 1.25	37.98 ± 1.19
4	39.52 ± 0.04 a	37.39 ± 0.04 b	38.56 ± 0.62 b	33.63 ± 0.65 a

[a]Values are expressed as the mean ± SD; $n = 9$ per each treatment and reported on fresh weight basis. Letters a and b indicate significant differences at $p \leq 0.05$ between organic and conventional production systems. Values without letters indicate no significant difference between the treatments.

Table 5. Grapefruit Micronutrients from Organic and Conventional Production Systems and Soil Nutrient Analysis of Organic and Conventional Grapefruit Orchards

expt	production system	NO_3-N[a]	P	K	Ca	Mg	S	Na	Zn	Fe	Cu	Mn
Juice Mineral Analysis of Organic and Conventional Grapefruit (Part per Milliion)												
1	organic	0.08	61.62	1979.78	324.27	86.00	NA	352.78	0.58	3.99	0.60	0.70
	conventional	0.10	48.72	1977.13	274.83	83.95	NA	280.19	0.67	14.70	0.67	0.67
2	organic	0.10	243.92	3146.92	472.80	110.98	NA	53.16	0.60	10.93	0.27	0.93
	conventional	0.11	208.14	2428.37	276.78	75.33	NA	44.96	0.26	4.32	0.23	0.20
Soil Micronutrient Analysis of Organic and Conventional Grapefruit Orchards (Parts per Million)												
1	organic	8.00	108.00	335.00	2012.00	410.00	28.00	NA				
	conventional	20.00	61.00	310.00	5568.00	463.00	32.00					
2	organic	3.00	87.00	283.00	4016.00	399.00	20.00					
	conventional	2.00	67.00	610.00	7866.00	785.00	29.00					

[a]Values are expressed in percentage for juice mineral analysis.

were separated by Tukey's test at a significance level of $p \leq 0.05$. The compositional variations that occurred during harvest and storage were expressed on a fresh weight basis to have a better representation of actual concentrations experienced by the consumer.

RESULTS AND DISCUSSION

Sensory Evaluation and Weight Loss. For sensory evaluation, taste parameters such as sweetness, sourness, tartness, and overall acceptability were evaluated, and no significant differences ($p \leq 0.05$) were observed between the organic and conventional grapefruits (Table 2). The sensory evaluation results were consistent with TA and TSS (Table 3) levels in the organic and conventional grapefruits. Furthermore, the overall appearance of the organic and conventional grapefruits was not significantly different ($p \leq 0.05$), with the

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Table 6. Concentrations of Limonoids from Organic and Conventional Grapefruit during 4 Weeks of Storage at Room Temperature (23 °C) and 9 °C Reported on a Fresh Weight Basis[a]

	limonin (μg/g)					nomilin (μg/g)				
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	0 weeks	1 week	2 weeks	3 weeks	4 weeks
First Experiment										
conventional, room temperature	89.91 ± 3.04 a	71.71 ± 3.04 b	71.182 ± 3.04 b	72.53 ± 3.24 b	72.91 ± 3.04 b	18.65 ± 0.92 a	13.33 ± 0.92 bc	11.42 ± 0.94 bc	14.79 ± 0.98 ab	0.974 ± 0.92 c
organic, room temperature	94.82 ± 3.30 a	90.07 ± 3.30 a	75.25 ± 3.30 b	87.25 ± 3.30 ab	82.07 ± 3.30 ab	31.78 ± 1.41 a	23.19 ± 1.41 b	15.84 ± 1.41 c	9.36 ± 1.41 d	14.57 ± 1.41 cd
conventional, 9 °C	89.91 ± 3.60 a	77.51 ± 3.60 ab	72.23 ± 3.60 bc	55.80 ± 5.00 c	71.87 ± 3.60 bc	18.65 ± 0.82 b	13.18 ± 0.82 c	10.65 ± 0.82 cd	25.19 ± 1.15 a	7.52 ± 0.82 d
organic, 9 °C	94.82 ± 3.22 a	85.45 ± 3.22 ab	80.84 ± 3.22 b	82.47 ± 3.22 ab	80.58 ± 3.22 b	31.78 ± 1.60 a	20.55 ± 1.60 bc	22.38 ± 1.70 b	10.12 ± 1.65 d	14.96 ± 1.60 cd
Second Experiment										
conventional, room temperature	42.92 ± 1.98 b	25.48 ± 2.89 c	49.64 ± 2.80 ab	24.29 ± 2.89 c	57.48 ± 3.94 a	3.85 ± 0.82 ab	2.21 ± 0.80 b	6.72 ± 0.77 a	3.60 ± 0.80 ab	3.96 ± 0.77 ab
organic, room temperature	40.87 ± 2.09 a	26.85 ± 2.09 b	41.46 ± 2.09 a	21.57 ± 2.53 b	38.02 ± 2.24 a	3.35 ± 0.48 bc	2.06 ± 0.48 c	5.18 ± 0.48 ab	3.91 ± 0.61 ab	5.85 ± 0.51 a
conventional, 9 °C	42.92 ± 1.98 b	27.07 ± 1.85 b	44.54 ± 1.85 b	29.22 ± 1.91 b	62.64 ± 2.14 a	3.77 ± 0.23 b	2.65 ± 0.22 c	6.23 ± 0.22 b	4.71 ± 0.23 a	1.69 ± 0.22 d
organic, 9 °C	40.87 ± 1.78 a	23.39 ± 1.78 b	45.52 ± 1.78 a	26.81 ± 1.78 b	42.33 ± 1.83 a	3.35 ± 0.58 bc	2.16 ± 0.58 c	5.57 ± 0.58 b	4.95 ± 0.64 b	8.98 ± 0.76 a

[a]The mean ± SD of limonin and nomilin that resulted from the analysis of nine individual samples. Letters a–d depict significant differences ($p \leq 0.05$) between limonin and nomilin during grapefruit storage.

fruit color being an exception. Conventional grapefruits had a brighter red colored flesh compared to organic grapefruits.

The percentage of moisture lost during storage was slightly higher in E2 compared to E1. Furthermore, the moisture lost during room temperature storage was higher than that lost in cold storage due to lower (65%) relative humidity at room temperature. From Table S1 (Supporting Information), the moisture losses in storage for organic grapefruits in E2 were 4.2% at 9 °C and 16.2% at room temperature. The moisture losses for conventional grapefruits at 9 °C and room temperature in E2 were 2.5% and 11.1% respectively. Furthermore, the percentage of fruit decay in E2 (13.5%) was greater than in E1 (8.1%). Unlike organic grapefruits, conventional grapefruits were coated with carnauba (shiny) wax, which probably reduced moisture losses during storage.

Vitamin C Analysis. In E1, organic grapefruits showed significantly higher levels of vitamin C over conventional grapefruits at 0 days after harvest (Table 4). However, the vitamin C levels ranged from 25.74 to 61.99 mg/100 g in organic grapefruits and from 26.06 to 64.80 mg/100 g in conventional grapefruit in both experiments. It seems that vitamin C levels at harvest had an inverse relationship to the levels of nitrate nitrogen (NO_3-N) content of the respective soils (Table 5). Lower levels of NO_3-N in organic grapefruit orchard soils could have caused higher grapefruit vitamin C content. Additionally, the lower vitamin C levels in conventional grapefruits could be due to the dilution effect, a secondary response to increased vegetative growth because of excess plant-available soil nitrogen.[11,35] Although P, K, Ca, Mg, and Na were higher in organic grapefruits in the current study, only nitrate nitrogen has been linked to vitamin C levels in previous studies.[11]

In E2, vitamin C levels were not significantly different at 0 days after harvest in organic and conventionally grown grapefruits. These vitamin C levels (25.74–64.80 mg/100 g) are in accordance with a previously reported study.[31] Additionally, higher levels of ascorbic acid levels were shown in organically produced grapefruits in a previous study.[14]

In E1, the vitamin C lost during grapefruit storage (both organic and conventional) at room temperature was significantly higher than at 9 °C. However, a similar pattern was not observed in E2. The vitamin C degradation was minimal in both organic and conventional grapefruits during storage at room temperature and 9 °C (Table 4). Variability in vitamin C degradation and accumulation is a very common phenomenon observed in fruits and vegetables during storage.[17,36−38] In plants, vitamin C is the first line of defense against oxidative stress that occurs due to increased respiration in storage.[35] Therefore, in E1, vitamin C decreased immediately in the first week of storage. Although the first week of storage showed vitamin C degradation, vitamin C levels returned to original concentrations by the second week of storage. Normally fruits tend to maintain their vitamin C levels during storage by denovo biosynthesis.[39,40] However, prolonged storage periods would decrease vitamin C content due to excessive free radical accumulation as a result of increased respiration. In E2, the vitamin C levels increased immediately in the first week of storage, but their levels reached their original concentrations (concentrations at the time of harvest) in the second week. Generally, the fruits harvested in February had softer tissue (thinner cell walls) than those harvested in November,[41] which could have contributed to more glucose 6-phosphate, a major substrate for vitamin C biosynthesis.[42] In a previous study, cell

Table 7. Storage Variations[a] in β-Carotene and Lycopene from Grapefruit Harvested from Organic and Conventional Production Systems Reported on a Fresh Weight Basis

	β-carotene (μg/g)				lycopene (μg/g)			
	room temperature		9 °C		room temperature		9 °C	
storage (weeks)	conventional	organic	conventional	organic	conventional	organic	conventional	organic
First Experiment								
0	3.57 ± 0.05 a	2.61 ± 0.05 b	3.57 ± 0.05 a	2.61 ± 0.05 b	25.87 ± 0.23 a	12.52 ± 0.22 b	25.87 ± 0.23 a	12.52 ± 0.22 b
1	5.05 ± 0.15	5.27 ± 0.12	4.69 ± 0.03 a	2.68 ± 0.02 b	23.07 ± 0.83 a	26.13 ± 0.67 b	25.51 ± 0.28 a	16.69 ± 0.25 b
2	3.82 ± 0.13 a	3.49 ± 0.15 b	3.47 ± 0.06 a	4.74 ± 0.11 b	18.00 ± 0.50 a	11.46 ± 0.52 b	19.96 ± 0.08 a	15.46 ± 0.08 b
3	4.90 ± 0.05 a	3.50 ± 0.05 b	3.61 ± 0.02 a	1.94 ± 0.02 b	15.63 ± 0.20 a	9.63 ± 0.20 b	15.57 ± 0.26 a	8.80 ± 0.26 b
4	4.51 ± 0.15 a	2.89 ± 0.16 b	2.00 ± 0.08 a	1.23 ± 0.11 b	11.17 ± 0.21 a	6.15 ± 0.23 b	8.08 ± 0.23 a	4.35 ± 0.23 b
Second Experiment								
0	4.23 ± 0.04 a	2.5 ± 0.04 b	4.23 ± 0.04 a	2.50 ± 0.04 b	11.64 ± 0.18 a	7.54 ± 0.19 b	11.64 ± 0.18 a	7.54 ± 0.19 b
1	2.69 ± 0.08	2.90 ± 0.08	3.71 ± 0.03 a	2.57 ± 0.02 b	6.27 ± 0.24 a	8.73 ± 0.22 b	8.92 ± 0.27	8.55 ± 0.27
2	4.25 ± 0.07 a	3.80 ± 0.08 b	4.58 ± 0.05 a	3.73 ± 0.05 b	11.78 ± 0.28 a	10.32 ± 0.34 b	12.16 ± 0.19	12.61 ± 0.19
3	5.97 ± 0.05 a	6.32 ± 0.05 b	4.17 ± 0.02 a	4.63 ± 0.02 b	15.49 ± 0.24 a	17.31 ± 0.23 b	11.31 ± 0.15 a	16.34 ± 0.15 b
4	4.31 ± 0.04 a	2.40 ± 0.04 b	2.43 ± 0.02	2.53 ± 0.03	12.40 ± 0.06 a	4.52 ± 0.07 b	8.22 ± 0.06 a	5.85 ± 0.09 b

[a]Values are expressed as the mean ± SD; $n = 9$ per each treatment. Letters a and b indicate significant differences at $p \leq 0.05$ between organic and conventional production systems. Values without letters indicate there is no significant difference between the treatments.

wall softening was found to be related to apoplastic ascorbic acid concentrations.[42] In some fruits, including strawberries, vitamin C biosynthesis occurs as the fruit cell wall degrades during ripening.[43] It seems that in E2, harvest time could have contributed to an immediate increase in ascorbic acid in the first week of storage.

In E2, grapefruit orchards continuously experienced cooler temperatures (60 °F) over a period of 3 months before commencement of harvest (Figure S1, Supporting Information). The cold weather could have contributed to the higher vitamin C levels.[35] Overall, the results of this study demonstrated that vitamin C levels in fruits and vegetables are highly influenced by various factors including production system, storage, and time of harvest.

Limonoid Analysis. Limonoids are antifeedants that are primarily produced by plants as a response to pests and diseases. Although organic grapefruits had quality attributes (TSS, TA, and taste) similar to those of conventional grapefruits, they showed higher levels of total limonoids in juice at 0 days after harvest. Nomilin but not limonin levels were significantly higher in organic grapefruit compared to conventional grapefruits in E1 (Table 6). Plants exposed to biotic stress could have increased the levels of phytoalexins, and this could have increased the levels of limonoids in organic grapefruit.[10] The levels of these compounds (21.57−94.82 μg/g of limonin and 1.69−31.78 μg/g of nomilin) in grapefruits are in accordance with the reported levels[44] and demonstrate that biosynthesis of limonin and nomilin in grapefruits is a complementary but not a continuous process.[45]

In plants, nomilin (limonoid aglycon substrate) is synthesized in the stem tissues and translocated to fruits.[46,47] The nomilin accumulated in fruits is used for the biosynthesis of other limonoid aglycons including limonin. This is probably the main cause for lower concentrations of nomilin compared to limonin in all grapefruits after harvest (Table 6). Although there are no major differences observed between different storage temperatures, the concentrations of limonin and nomilin decreased significantly during storage in the fruits from both production systems. Additionally, after harvest, the accumulation of nomilin in fruit tissues would be halted, but the remaining nomilin would be continuously used for biosynthesis of limonoid aglycons.[46,47]

In E2, there were no major differences in organic and conventional grapefruit limonoid concentrations at harvest. The colder temperatures that prevailed prior to the second harvest might have decreased the biotic stress on the plant, which could have led to the moderate levels of limonoids in organic and conventional grapefruits. Furthermore, E2 showed lower levels of limonin and nomilin due to possible glucosidation of limonoid alycons.[48]

Carotenoid Analysis. Grapefruits grown under a conventional system had higher β-carotene and lycopene levels than organic grapefruits in both experiments at 0 days after harvest (Table 7). In E1, lycopene was >2-fold higher in conventional grapefruits compared to organic grapefruits. The carotenoid levels (1.23−4.51 μg/g of β-carotene and 4.35−26.13 μg/g lycopene) are in agreement with a previously published study.[14] In a carrot study, the variations in carotenoid content due to different production systems demonstrated that higher plant-available nitrogen in conventional production significantly increased β-carotene levels.[12]

Degradation losses of carotenoids were greater during storage in both organic (57.5%) and conventional grapefruits (53%) in E1. It seems that in E1, carotenoids were slightly stable at room temperature compared to 9 °C. Furthermore, similar degradation losses of carotenoids were not observed in E2 for conventional grapefruit at room temperature, but carotenoids in organic grapefruits were more stable. Previous studies have demonstrated that harvest time[14,49] and storage conditions had a tremendous influence on citrus carotenoids.[50] However, temperature did not show a greater effect on the β-carotene and lycopene contents of stored grapefruits.

In the current study, carotenoid levels were greatly influenced by harvest time in both E1 and E2. Another study showed that harvest time had significantly influenced lycopene biosynthesis in citrus fruits.[51] β-Carotene is converted to violaxanthin, which occurs downstream in carotenoid biosynthesis, as the harvest time progressed in Satsuma mandarin.[20]

In conclusion, vitamin C and nomilin levels were higher in organic grapefruits compared to conventional grapefruits in E1. Vitamin C levels are inversely related to soil nitrate nitrogen

content in the two production systems. The vitamin C loss during storage was minimal in both organic and conventional grapefruits. However, lycopene and β-carotene levels were higher in conventional grapefruits compared to organic in both E1 and E2. Carotenoid levels were generally higher in E1 than in E2; this may be due to the effect of harvest time. Lycopene and β-carotene levels were higher in conventional than in organic grapefruits in both experiments. It is likely that cooler temperatures at the time of harvest might have caused the variation of carotenoids in E1 and E2. The current research encompasses information on several parameters including productions systems, time of harvest, and storage conditions, which influence grapefruit bioactive compounds. However, the underlying mechanisms that cause these variations due to plant nutrition, environmental factors, biotic and abiotic stress, plant growth, and their interactions need to be addressed in further studies.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Weather data; scheme for harvesting, storing, and sampling grapefruit; and weight loss and fruit decay data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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