A Comparison of Processed and Fresh Squeezed ‘Hamlin’ Orange Juice—Flavor Quality

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‘Hamlin’ orange juice was extracted using a commercial food service juicer ("fresh squeezed") followed or not with pasteurization and compared to pasteurized processed juice for quality attributes. There was much higher peel oil content (introduced from the flavedo), but lower insoluble solids and pectin content (introduced from albedo and segment membranes) in fresh squeezed juice compared to processed juice. Fresh-squeezed juice had less cloud loss in comparison with processed juice regardless of pasteurization. Titratable acidity (TA) was higher and the ratio of soluble solids to TA was lower in fresh squeezed juice. The fresh squeezed juices had higher concentrations of hexanal, octanal, 2-methylpropanol, hexanol, cis-3-hexenol, trans-2-hexenol, octanol, α-pinene, sabine, myrcene, limonene, methyl butanoate and ethyl butanoate, but lower concentrations of terpinen-4-ol and α-terpineol than processed juice. There were no differences between samples for preference or sweetness in sensory evaluations. However, the fresh juice had favorable attributes indicated by the higher sensory scores for freshness, mouthfeel and a lower score of cooked flavor, an unfavorable attribute associated with processing. The results indicate that extraction and finishing processes rather than pasteurization were major factors in influencing the orange juice flavor quality.

Fresh orange juice is generally perceived to be more wholesome than processed juice. Without processing and pasteurization, fresh juice may have flavor and nutrients that differ from pasteurized or processed juice.

The essence of orange flavor is a complex mixture of volatile compounds of which some 200 have been identified (Baldwin, 1993; Johnson et al., 1996). The most important volatiles are esters, aldehydes, and terpenes, followed by alcohols, ketones, and hydrocarbons (Plotto et al., 2004, 2008; Shaw, 1991). Esters, low molecular weight aldehydes and alcohols are water soluble and localized in the juice vesicles (Perez-Cacho and Rouseff, 2008; Shaw, 1991). After centrifugation, which reduces the insoluble solids, a large portion of esters and other water soluble compounds are lost (Jordan et al., 2001). Valencene occurs in juice oil instead of peel oil (Shaw, 1991). The major peel oil volatile components are limonene (>90%), α-pinene, sabine, myrcene, linalool, and decanal, introduced to orange juice by juice extraction (Coleman and Shaw, 1971; Pino et al., 1992; Verzera et al., 2004). The amount of peel oil, albedo and segment membranes can be influenced by different extraction methods (Carter et al., 1975). Thermal processing generally causes loss of esters and aldehydes, and formation of off-flavors from compounds such as α-terpineol (Perez-Cacho and Rouseff, 2008).

The objective of this research was to address changes in flavor and other quality factors caused by heat (pasteurization) and other industrial process compared to fresh squeezed juice from the same batch of fruit.

Materials and Methods

Source of fruit and processing methods. ‘Hamlin’ oranges were harvested from a commercial grove located in central Florida on 6 Jan. 2010. Fruit were stored at 5 °C until they were processed on 14 Jan. 2010. A total of 600 fruits were washed (Fruit Cleaner 395; JBT Food Tech., Lakeland, FL), then randomly divided into three equal groups for juicing treatments: fresh-squeezed (fresh), fresh squeezed with pasteurization (fresh/pasteurized), or processed (which included pasteurization).

For industrial processing, a commercial JBT 391 Extractor with Standard Industry Setting was used. Juice was passed through a pressure filtration finisher with screen size 0.51 mm and then pasteurized at 90 °C for 10 s (1.2 L·min⁻¹) using a pilot pasteurizer (UHT/HTST Lab 25EHV Hybrid; Microthermics, Inc., Raleigh, NC). Juices were cooled to 10 °C immediately after pasteurization, and further to 5 °C using an ice bath.

For fresh squeezed juice, fruit and all contact surfaces were sanitized with 100 ppm peroxyacetic acid (PAA). After draining, fruit were processed using a commercial food service juicer (Automatic Commercial Orange Juicer Citrus Squeezer; Orange, Glendale, CA). Pasteurization of fresh squeezed juice was performed using a Microthermics pilot pasteurizer at 90 °C for 10 s (1.2 L·min⁻¹).

After processing, all samples were cooled to 5 °C using an ice bath, and stored at 5 °C for 4 d. Samples, three replicates per treatment, were taken at day 0 and day 4 after juicing. Analyses were carried out immediately or after frozen storage at –20 °C or –80 °C.

Peel oil. Peel oil content was analyzed by a bromate titration method (Scott and Veldhuis, 1966). The sample (25 mL) and 2-propanol (25 mL) with a few boiling stones were distilled until the solvent ceased to reflux. After adding 10 mL of 4 N HCl with a drop of 0.1% methyl red indicator, peel oil content
was determined by titrating the distilled fraction with 0.0247 N bromide-bromate solution until the color disappeared.

**Insoluble solids content.** Juice samples were centrifuged at 27,000 g for 30 min. Supernatants were discarded and pellets were carefully resuspended with deionized water (equivalent amount as in original juice). The final pellets collected after centrifugation (27,000 g, for 30 min) were vacuum dried at 55 °C. The ratio of dry weight to original juice weight was used as the insoluble solids content.

**Analysis of galacturonic acid.** Each juice sample was adjusted to pH 2.4 with nitric acid. A 7.00-mL aliquot was heated for 5 min at 110 °C in a Focused Microwave™ Synthesis System (Discover model 908005, CEM Corp., Matthews, NC) using the manufacturer’s 10-mL glass reaction vessel. This extraction was cooled to room temperature then centrifuged for 30 min at 1000 g (Luzio, 2008). Each supernatant was mixed with 14 mL of cold anhydrous isopropyl alcohol (IPA), refrigerated for 60 min at 4 °C and centrifuged for 1 h at 3000 g. Supernatant was discarded and the pellet rewarshed once with IPA and then twice with 70% IPA and centrifuged at 3000 g for 1 h between each wash while discarding all supernatants. Pellets were dried for 16 h at 50 °C in the centrifuge tube under vacuum. A 14-mm diameter glass marble and 4 mL of deionized water was added to the dry pellet in the centrifuge tube and then shaken at ~150 rpm for 24 h. To an 800-µL aliquot of the rehydrated sample, 200 µL of 0.5 M sodium acetate buffer (pH 5.0) and 2 µL (8 units) of pectinase (Pectinex Ultra SP-L, P-2611, Sigma-Aldrich, St. Louis, MO) was added (Grohmann and Baldwin, 1992). Samples were then incubated at 37 °C for 24 h and then centrifuged for 5 min at 14,000 g. Determination of galacturonic acid was performed on the supernatants using a microtiter plate assay (Luzio, 2004). Supernatant (50 µL), deionized (DI) water (650 µL) and 3 mL of concentrated sulfuric acid (96.2%) containing 0.1% NaCl were added into a test tube and vortexed for 15 s. The mixture was immediately placed on an ice bath before transferring to wells of a microtiter plate, which had been preheated to 80 °C, and heated for 15 min at 80 °C. The plate was removed and cooled in water at room temperature for 15 min. The baseline reading of the plate was obtained at 450 nm (Power Wave 340 microtiter plate reader; Bio-Tek Industries, Highland Park, Winooski, VT). After adding 100 µL of 3.5-dimethylphenol solution (0.2% in glacial acetic acid), the plate was read again at 450 nm.

**Cloud loss analysis.** Juice samples were brought to 0.02% lithium azide and 4.35 g·L⁻¹ potassium metabisulfite, placed in glass bottles, and incubated at 30 °C. At selected times, duplicate samples (10 mL per sample) from each of three replicates per treatment were pipetted (after inverting the glass bottle three times) into 15-mL graduated, conical centrifuge tubes. The samples were centrifuged for 10 min at 360 g, and 1 mL supernatant of each sample was transferred to a cuvette and absorbance at 660 nm was recorded.

**Pectinmethylesterase activity assays.** Total and thermally tolerant pectin methylesterase (PME and TT-PME, respectively) activity in juice was determined titrimetrically with 0.5% citrus pectin (94% degree of esterification; DE, Sigma-Aldrich, St. Louis, MO, USA) with a Radiometer PHM290 pH-stat controller (assayed at pH 7.5, 200 mM NaCl, 30 °C, using 10 mM LiOH as the titrant). Raw juice pH was adjusted to 7.5 with LiOH prior to titration. TT-PME activity was estimated after the sample had been heated for 20 min in a 70 °C water bath, a treatment that inactivates the thermally labile PMEs.

**Analysis of sugars and acids.** Titratable acidity (TA) was determined by titrating to pH 8.1 with 0.1 N NaOH using an autotitrator (Mettler Toledo DL50, Columbus, OH). Soluble solids content (SSC) was determined using a refractometer (Atago PR-101, Tokyo, Japan).

For analysis of individual sugars and acids, approximately 40 g of juice was extracted using 70 mL 80% ethanol solution. The mixture was boiled for 15 min, cooled and filtered (Whatman #4 filter paper). The filtered solution was brought to 100 mL with 80% ethanol. Ten milliliters of the filtered solution were then filtered through a C-18 Sep-Pak (Waters/Millipore) followed by a 0.45-µm Millipore filter (Baldwin et al., 1991).

Individual sugar analysis was performed by high performance liquid chromatography (HPLC) with a refractive index detector (Perkin Elmer, Norwalk, CT) equipped with a Waters Sugar Pak column (Baldwin et al., 2004; 1991; 1998). The mobile phase was 10⁻² M ethylenediaminetetraacetic acid disodium calcium salt (CaEDTA) (0.5 mL·min⁻¹ flow rate at 90 °C). To better represent the sweetening power of individual sucrose, glucose and fructose concentrations were converted to sucrose equivalents (Koehler and Kays, 1991) by multiplying their concentrations by 1.0, 0.74, and 1.73, respectively (Baldwin et al., 1998; Maul et al., 2000)

Organic acids were analyzed using an Alttech OA 1000 Pre-vail organic acid column (Alttech Corp., Flemington, NJ) with a flow rate of 0.2 mL·min⁻¹ at 35 °C and a mobile phase of 0.01 N H₂SO₄. The injection volume was 20 µL using a Perkin Elmer Series 200 autosampler, a Spectra System P4000 pump and a Spectra System UV 6000 LP detector (Shimadzu) was used for the analysis.

**Analysis of aroma compounds.** Three milliliters of juice were transferred to a 10-mL crimp-capped vial with headspace gas replaced by argon, rapidly frozen in liquid nitrogen then stored at −80 °C. Frozen samples were thawed under running tap water and injected onto an Agilent 6890 (Agilent Technologies) GC using a Gerstel multipurpose autosampler equipped with Stabilwax and HP-5 low bleed columns. The flow rate was split equally to the two columns at 17 mL·min⁻¹ at 40 °C with an increase in temperature at 6 °C·min⁻¹ up to 180 °C, where the temperature was held constant for an additional 5.8 min. The GC peaks for the aroma volatile compounds were quantified using standard curves as determined by enrichment of deodorized orange juice by known concentrations of authentic volatile compound standards. Some samples were also analyzed using Solid Phase Micro Extraction (SPME) fibers with mass spectroscopy (MS).

For electronic nose (e-nose) analysis, a FOX 4000 system (Alpha MOS, Toulouse, France) was used, fitted with 18 metal-oxide gas sensors, some with coated surfaces (Bai et al., 2004). The electrical output from the sensors was measured at 0.5-s intervals. Samples (3 mL of juice in a 10-mL vial) were incubated in an agitator at 500 rpm and 40 °C for 2 min before the headspace sample (500 µL) was taken from the vial and injected into the e-nose. The carrier gas was pure air with a flow rate of 150 mL·min⁻¹. The e-nose data acquisition program was a 2-min sampling time followed by an 18-min delay between samples for sensor recovery.

**Sensory analysis.** On the day after processing the fresh juice (3 d after the commercial process), and after 4 d in storage at 5 °C, juice was served to 20+ laboratory staff panelists. Samples were presented as 60 mL in 120-mL cups (SOLO, Urbana, IL) at 14 ± 1 °C on a tray in a random order. Panelists were asked to taste and rank the samples, first by increasing order of preference, then for the following attributes (increasing intensity): sweetness, sourness, “freshness”, peel oil flavor, cooked flavor,
mouthfeel/body (explained as low mouthfeel/body = watery; high mouthfeel/body = pulpy). Reference standards were presented to clarify the definition of attributes: for sweetness, 8% sucrose, for sourness, 0.25% citric acid, for peel oil, 0.03 ppm orange oil in Minute Maid™ from concentrate (no pulp) orange juice, for “cooked flavor,” Minute Maid™ from concentrate orange juice was heated to 70 °C and allowed to cool down. Tasting took place in isolated booths under red lighting. Data collection was performed using Compusense 5.0 software (Compusense Inc., Guelph, ON, Canada).

Statistical analysis. SAS Version 9.1 (SAS Institute, Cary, NC) was used for analysis of instrumental analytical data except e-nose data which were analyzed with discriminant factor analysis (DFA) using the manufacturer’s statistical program (AlphaSOFT). Each quality attribute with three replicates was analyzed using analysis of variance (ANOVA). The treatment means were separated at the 0.05 significance levels by least squares means test (LSD). For sensory panel, data were analyzed using the Friedman test for non-parametric data using Compusense 5.0.

Results and Discussion

Basic juice quality

1. Peel oil content. The processed juice had 0.0202% peel oil in ‘Hamlin’ (Fig. 1A). However, the amounts were 0.0908% and 0.0844% in fresh squeezed and fresh/pasteurized juices, respectively (Fig. 1A), over 4-fold higher than the processed juice. For the fresh juice extraction system, fruit were cut in half and the halved fruit were pressed onto the automatic self-reversing reamer, and seeds and segment membranes were screened by a strainer. Although the peel appeared nearly undisturbed, a significant amount of peel oil was pressed into the juice. On the contrary, the industrial extractor is designed to separate most of the oil from juice. Generally, levels of 0.010% to 0.025% peel oil are preferred in most citrus juices (Kimball et al., 2004). The peel oil content in fresh juice from ‘Valencia’ reached about 0.15% and separated into an oil layer after centrifugation (unpublished data).

2. Insoluble solids and pectins. The processed juice had 0.52% insoluble solids content, about 30% higher than fresh squeezed juices, and pasteurization did not affect insoluble solids content (Fig. 2). Total pectin, determined as galacturonic acid content, was 1.7 ng·g⁻¹ in commercial juice, but only 0.71–0.93 ng·g⁻¹ in the fresh juices, about half that of the processed juice (Fig. 1B). Pectin content is important in the orange juice matrix system and juice cloud stability (Croak and Corredig, 2006). Fig. 2 shows a schematic representation of the hydration condition of the insoluble solids after centrifugation and again after wash.

Fig. 1. Effect of processing method and storage time on ‘Hamlin’ orange juice quality. Pasteurization condition was 90 °C for 10 s for both processed and fresh/pasteurized juice. Vertical bars with the different letters are significantly different at $P = 0.05$ using Duncan’s multiple range test.
Particles in the fresh juice absorbed much more liquid, which was reduced by thermal pasteurization in the fresh/pasteurized juice, although still greater than for processed juice. Further research is needed to understand how processing and thermal pasteurization modify the backbone structure of the particles, and thus alter hydration characteristics.

The insoluble solids of the juice pellets were shown in a concurrent study (Bai et al., 2010) to contain far higher insoluble solids-bound, bitter tasting limonoid aglycones than in juice supernatants. The taste properties of these solid-bound compounds are uncertain, but it is possible that by preferentially binding these compounds to the insoluble solids, the bitterness associated with these compounds would be diminished in the soluble portions of the juice, i.e., juice serum.

3. **Juice cloud.** In orange juice, loss of cloud leads to a decrease in consumer acceptability. The cloud particles impart the characteristic color turbidity and mouthfeel to orange juice, and affect flavor. Cloud is composed of a complex mixture of proteins, pectin, lipids, hemicellulose, cellulose and other minor components (Baker and Cameron, 1999; Klavons et al., 1991). The juice cloud experiment showed that fresh squeezed juice had more stable cloud than the processed juice (Fig. 3), possibly because of the lower pectin content (Fig. 2), and lower activities of PME in the fresh juice (Fig. 4). Cameron et al. (1997) showed that peel extracts caused the most rapid cloud destabilization, and suggested that cloud stability of lightly or unpasteurized orange juice may be extended if care is taken to prevent peel tissue or

![Graph](image1.png)

**Fig. 2.** Pellets as percent of juice weight (% w/w) from different juices. Juice samples were centrifuged at 27,000 g, for 30 min. Pellets were washed with water and centrifuged again, then vacuum dried at 55 °C. Weight at each stage was measured and presented as wet, after wash, and dry. Values followed by the different letters in the same column are significantly different at $P = 0.05$ using Duncan’s multiple range test.

![Graph](image2.png)

**Fig. 3.** Cloud loss in fresh and processed, with and without pasteurization, ‘Hamlin’ orange juice at 30 °C.

![Graph](image3.png)

**Fig. 4.** Total PME and TT-PME activity in commercial and fresh squeezed ‘Hamlin’ orange juice.
peel juice from entering the juice. Pasteurization did improve cloud stability in the processed juice (Fig. 3) due to inactivation of PME (Fig. 4).

4. Sugars and Acids. SSC was similar in all treatments (Fig. 1C), but the fresh and fresh/pasteurized juices had higher TA contents which caused a lower SSC/TA ratio (Fig. 1D and 1E). The processed juice had the highest individual sugar content and sucrose equivalence value, followed by fresh/pasteurized juice, and fresh squeezed juice had the lowest (Fig. 1F–I). A similar pattern was also shown for individual acids (Fig. 1J–K). Higher TA content in the fresh juices over the total acid content of citric and malic acids may be caused by peel oil associated compounds.

Aroma Analysis. Preliminary volatile analysis by SPME showed that the peel oil in the fresh squeezed juice overloaded the GC column. However, using direct headspace injection onto a GC column showed differences for aroma volatiles to be mostly between the processed and the fresh juices with little effect due to pasteurization (Fig. 5). For aldehydes, hexanal and octanal were higher in the fresh juices, while acetaldehyde was slightly higher in the processed juice. For alcohols, 2-methylpropanol, hexanol, cis-3-hexenol, trans-2-hexenol and octanol were higher in the fresh juice, while α-terpineol was higher in the processed juice. Most terpenes were higher in the fresh juices including α-pinene, sabinene, myrcene and limonene. However, valencene was higher in the processed juice. Esters were higher in fresh than processed juice including methyl butanoate and ethyl butanoate (Fig. 5).

Studies of the distribution of aroma compounds between pulp and serum in different fruit juices showed that hydrocarbons (mono and sesquiterpene hydrocarbons) were associated with

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**Fig. 5. Aroma volatiles from ‘Hamlin’ orange juice headspace analysis. Pasteurization condition was 90 °C for 10 s for both processed and fresh/pasteurized juice. Vertical bars with different letters are significantly different ($P = 0.05$) using Duncan’s multiple range test.**
pulp and that oxygenated compounds (i.e., ethyl butanoate and octanal) were mainly contained in the serum (Brat, 2003; Radford et al., 1974).

A compound which is derived from albedo and segment membranes, and often a contributor of significant impact to flavor quality is \( \alpha \)-terpineol. \( \alpha \)-Terpineol is formed by oxidative degradation of limonene and is well known for its contribution to the off-flavor of orange juice when its concentration exceeds 2 \( \mu g \cdot g^{-1} \) (Tatum et al., 1975). Jordan et al. (2001) found that \( \alpha \)-terpineol is associated with pulp, indicating that a decrease in the pulp content would be beneficial in maintaining flavor quality of stored orange juice. In this research, \( \alpha \)-terpineol was 1.24 \( \mu g \cdot mL^{-1} \) in the processed juice, more than 50% higher than in the fresh juice, and may cause off-flavor by acting synergistically with other compounds.

The major component of peel oil is limonene which contributes very little to the juice aroma, but is a diluent for other flavors (Kimball et al., 2004). Fellers (1980) reported that a level of peel oil in excess of about 150 \( \mu g \cdot mL^{-1} \) limonene added an undesirable flavor to orange juice, and a peel oil level above 0.02% could contribute to bitter flavor and a burning sensation in the mouth. In this research, the fresh juice contained more limonene than the processed juice, although the content was higher than 250 \( \mu g \cdot mL^{-1} \) in all treatments. Other volatile compounds associated with peel oil include myrcene, \( \alpha \)-pinene, octanal, nonanal, decanal, sinensal, and linalool (Shaw, 1991). Arctander (1969) reported myrcene to have an “almost citrusy” aroma and a “sweet-balsamic-herbaceous” taste at levels below 10 ppm, but at higher concentrations noted “pungency” and “bitterness.” Moshonas and Shaw (1994) showed different volatile profiles of mechanically extracted and hand squeezed orange juices caused mainly by peel oil content. Our results agreed with this conclusion.

Low molecular weight esters, alcohols and aldehydes are water soluble and most likely dissolved in serum (Perez-Cacho and Rouseff, 2008; Shaw, 1991). Thermal pasteurization and processing usually cause a loss of these compounds (Perez-Cacho and Rouseff, 2008; Shaw, 1991). Therefore, the fresh juice had high concentration in both of the peel oil related and water soluble volatiles (Fig. 5). Radford et al. (1974) showed that hydrocarbons are almost exclusively associated with the pulp, whereas oxygenated compounds are more closely associated with the serum. However, Jordan et al. (2001) reported that the reduction of pulp in the orange juice results in a significant reduction of many aldehydes, terpenic hydrocarbons and alcohols.

The carbon-6 aldehydes and alcohols, including hexanal, cis-3-hexenol and trans-2-hexenol, were 40% to 500% higher in the fresh juices than in the processed juice, indicating a dramatic loss of these components in the industrial extraction and finishing (Fig. 5).

Many reports showed that in orange juice thermal pasteurization generated off-flavors, such as \( \alpha \)-terpineol (Berlinet et al., 2007; Tatum et al., 1975), 4-vinyl guaiacol (Bazemore et al., 1999; Tatum et al., 1975), furaneol (Tatum et al., 1975), neral and geranial (Berlinet et al., 2007). Nisperos-Carriedo and Shaw (1990) and Moshonas and Shaw (1997) showed a decrease in the amount of aldehydes and esters in pasteurized juices. Bazemore and Rouseff (1999) found that 2-methyl propanoic acid, 3-ethoxy-1-propanol, nonanal, carvone and two other unknown components disappeared in excessively heated juice. Conversely, Baxter et al. (2005) reported that in 'Navel' orange juice, thermal treatment did not influence the key aroma compounds. Our data also showed very limited impact caused by pasteurization in the fresh juice; pasteurization decreased methanol at both day 0 and 4, hexanal and ethyl acetate at day 0, and decanal at day 4, but increased 2-methylpropanol at day 4 (Fig. 5).

Discriminant factor analysis (DFA) of e-nose data separated the commercially processed juice from other samples in the first DFA variable, which had 89.8 of the discriminatory power.
(Fig. 6) based on juice volatile content. Comparably, DFA also separated unpasteurized fresh squeezed juice from its pasteurized counterpart, but by a much smaller distance. There were also small distances between day 0 and day 4 samples (Fig. 6). These results confirmed the GC analysis that showed that the main differences in volatiles were between commercial juice and fresh squeezed juices regardless of pasteurization.

**Sensory evaluation.** As shown in Table 1, there was no difference between samples for preference, sweetness, or sourness on day 1 and day 4. On day 1, fresh and fresh/pasteurized samples had higher ratings for freshness and lower ratings for “cooked flavor”, in comparison with processed samples. Peel oil rating was higher in the fresh juice than in processed juice on day 1 and for fresh and fresh/pasteurized on day 4. Although not significant, processed juice was perceived to have less sourness, corresponding to the lower TA in that juice. That juice was also perceived by some panelists to have some off-flavor.

### Conclusions

The processed juice was markedly different from the fresh squeezed juice in flavor and quality. The fresh juices had much higher peel oil content, but lower insoluble solids and pectin content in comparison with the processed juice. Fresh-squeezed juices were stable in terms of cloud loss likely due to reduced pH and lower SSC/TA ratio was lower in fresh squeezed juice. There were higher concentrations of hexanal, octanal, 2-methylpropanol, hexanol, cis-3-hexenol, trans-2-hexenol, octanol, α-pinene, sabinene, myrcene, methyl butanoate and ethyl butanoate in the fresh juices over the processed juice, many of them were associated with high peel oil. Panel rated higher freshness to the fresh juice, but higher “cooked flavor” to the processed juice.

### Literature Cited


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**Table 1. Effect of processing method on sensory quality of ‘Hamlin’ orange juice**

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Means followed by a different letter were significantly different by the Friedman analysis of ranks and the Tuckey HSD test (5% significance level).