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Effect of Fruit Size and Huanglongbing Disease on Orange Juice Attributes

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This study examined the sensory impact of the combined factors of orange fruit size and huanglongbing (citrus greening) disease status on juice sweetness, orange flavor and overall acceptability. A sensory evaluation test was carried out in duplicate at the University of Florida campus with two untrained panels (n = 99 and n = 88) performing a consumer acceptance test on juice from two sizes (large and small, L/S) and two disease-affected statuses (healthy and greening-affected, GR–/GR+) oranges. Panelists rated the sensory characteristics (sweetness, orange flavor and overall acceptability) of four juice samples from healthy small (GRS–) and large (GRL–) oranges as well as greening-affected small (GRS+) and large (GRL+) oranges using the 9-point hedonic scale. Overall, the panelists rated the juice from "GRS–" the highest in all three sensory categories. Similarly, panelists rated the juice from "GRL+" oranges the lowest in all three sensory categories. Juice from "GRS+" oranges was found to be rated higher in all three sensory categories than juice from "GRL+" fruit. This may be relevant in large-scale commercial orange juice processing where juice from greening-affected small fruit may be more acceptable when blended with juice from healthy oranges.

Citrus greening, also known as "huanglongbing," is considered to be the most destructive disease to citrus species posing a significant threat to citrus production worldwide (Bove, 2006). Citrus greening is caused by a phloem-limited, gram negative bacteria named "*Candidatus* Liberibacter spp." and belongs to the α subdivision of Proteobacteria (Bove, 2006; Lin, 2010). Citrus greening has been known to spread by grafting with propagative material that is infected with the disease (Bove, 2006; Lin, 2010). However, citrus greening spreads naturally by psyllid vectors, *Diaphorina citri* and *Trioza erytreae*, which are of Asian and African origin respectively (Bastianel, 2005; Bove, 2006). Symptoms of infected trees are yellow shoots, blotchy mottled leaves, stunted growth and small-sized lop-sided fruits that are poor in color (green) and often inverted in coloration starting from the stem end (Bove, 2006). Consequently, infected trees are usually destroyed within 5 to 8 years coinciding with a manifestation and subsequent decline in productivity of the trees (Gottwald, 1989; Li, 2007).

As symptomatic fruit from infected trees is small, green, underdeveloped and misshapen, there is a high likelihood that it will not meet quality regulations in the fresh fruit market (Shokrollah, 2011; Sullivan, 2011). In addition, juice from symptomatic fruit has been shown to have a "bitter" or "sour" off flavor albeit there is a variation in intensity among varieties (Hamlin and Valencia) with the juice of 'Valencia' fruit having a less intense off-flavor (Plotto, 2010). In orange juice, sugars and acids play a significant role in the perception of the bitterness/sourness flavor (Plotto, 2010). An analysis of the chemical composition of symptomatic fruit has shown a lower sugar and higher acidity level compared with control (healthy) fruit (Dagulo, 2010). Symptomatic fruit also have an increased level of limonin which may be responsible for bitterness albeit not above average threshold levels of bitterness (Dagulo, 2010). Furthermore, it is well known that as fruit matures, sugars increase while acids decrease (Bain, 1958; Braddock, 1999). Small fruit tend to have a higher sugar concentration than larger fruit (Bartholomew and Sinclair, 1943). Similarly, limonin concentration decreases as the fruit matures (Fong, 1982). Since recent studies suggest that citrus greening imparts an off flavor in juices very similar to that of an immature fruit (Dagulo, 2010; Plotto, 2010), it would be interesting to further investigate this immature flavor in symptomatic fruit as it relates to fruit size. In particular the effects of fruit size and huanglongbing disease on orange juice acceptance attributes, specifically sweetness, orange flavor and overall acceptability are investigated in this study.

Materials and Methods

Fruit and juice material. The sweet orange cultivar Valencia [*Citrus sinensis* (L.) Osbeck] was selected for this study. All 'Valencia' fruit was harvested on 6 Apr. 2010 at the Orange Hammock Grove located in Felda, FL. The 'Valencia' fruit supplied for the study consisted of fruit from greening-affected trees as well as fruit from non-greening-affected trees. In 2008, symptomatic trees were confirmed for citrus greening by polymerase chain reaction (PCR) which amplifies 16S ribosomal DNA from affected trees (Hocquellet, 1999; Jagoueix, 1996; Li, 2006). Following harvest, the fruit was sent to the University of Florida Citrus Research and

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Education Center (CREC) pilot plant located in Lake Alfred, where they were conveyed on a brush bed and pre-washed. Alkaline soap was applied at a rate of 1 gal per 14 gal onto the brush bed within 45–60 s of washing. The fruit was then rinsed with water for 90 seconds and then dried at 140 °F on a conveyor belt. The fruit was then passed to the color vision (CVS) grading system for sizing. Oranges vary in fruit size according to cultivar and fruit load but most often measure between 2.5 to 4 inches in diameter (Okoye, 2011). Lerin and others (1998) using a commercial extractor to size their fruit grouped "small" oranges as having a diameter of less than 2.25 inches. In this study, the "small" fruit size was less than 2.25 inches in average diameter and was based on the sizes used by the CREC extractor. "Large" fruit size was determined as greater than 2.25 inches in average diameter. Both "large" and "small" fruit were pooled from samples of fruit that were greater and less than 2.25 inches respectively.

After sizing, the fruit samples were then gently hand reamed to obtain the juice. Four juice treatments were obtained from the fruit samples; "GRL–" (healthy large fruit), "GRS–" (healthy small fruit), "GRL+" (greening-affected large fruit) and "GRS+" (greening-affected small fruit). Each juice treatment was 1.5 gal and was stored in 0.5-gal high-density polyethylene (HDPE) bottles. In total, there were 6 gal in volume and 12 HDPE bottles. All juice treatments obtained were "freshly squeezed" and unpasteurized. "Freshly squeezed" juice has a very limited shelf life (<20 d at 1 °C) and can deteriorate rapidly due to microbial growth, enzymatic activity resulting in loss of cloudiness and oxidation (Wenzel, 1955; Braddock, 1999). Freezing "freshly squeezed" juice gives it a longer shelf life than refrigeration (Lee, 1999). As such, the juice treatments were then immediately frozen at –20 °C until ready for use. One gallon per treatment was then taken out of the freezer and placed in a refrigerator operating at 4 °C to let thaw thawed for 72 h. Thawed juice bottles were then gently agitated for 30 seconds before being transferred into serving cups to ensure consistency in juice samples.

Sensory evaluation. Two sensory evaluations were carried out using untrained panelists, 99 and 88 tasters in Experiments 1 and 2, respectively. Prior to each sensory evaluation, thawed and agitated juice was served (about 15 mL) in 30 mL plastic cups coded with random numbers at 20 °C along with crackers and still water to cleanse the palate. Laboratory staff carried out a hedonic test on the four treatments: "GRL–", "GRS–", "GRL+" and "GRS+", using a 9-point hedonic scale [(1 = dislike extremely; 5 = neither like or dislike; 9 = like extremely)] (Jones et al., 1955). Panelists were asked to use the scale to rate the following orange juice acceptance attributes: sweetness, orange flavor and overall acceptability. Each panelist was served a set of four treatments at the same time presented in a randomized order. Sensory evaluation was conducted in two separate trials within 30 d for consistency and reproducibility of results.

Juice analysis. A portion of juice samples set aside for analysis of soluble solids content (SSC) and titratable acidity (TA). SSC measurements were taken with the aid of a hand-held refractometer (Fisher Scientific, Catalog number 13-946-21, Range 0% to 32% °Brix). TA measurements were taken by titrating the juice samples against 0.1 N NaOH to an endpoint pH of 8.2 detected using a pH electrode (Fisher Scientific, Pittsburgh, PA). SSC to TA ratios were also calculated based on corrected (acidity and temperature) readings. Average BrimA scores were calculated using this formula (BrimA = SSC – k TA, where k is constant and = 3 (Obenland 2009). K values can range from 2 to 10 but the best values for prediction range between 3, 4, and 5 (Jasayena, 2008;Obenland, 2009). All measurements were performed in duplicates.

Statistical evaluation. Statistical analysis was performed using Compusense Five sensory software. Means and standard deviations were calculated for each treatment. Data from the hedonic test were analyzed using analysis of variance (ANOVA). Tukey-Kramer's honest significant difference (HSD) method at $\alpha = 0.05$ was used to determine significant differences among the means.

Results and Discussion

Juice analysis. Table 1 summarizes treatments and their coding for this experiment as well as average SSC, TA, SSC/TA ratios and BrimA measurements.

Sensory analysis—Orange flavor. Table 2 shows treatments and means for Experiments 1 and 2, respectively, outlining those with significant differences in orange flavor. ANOVA results show a statistical significance ($P < 0.00001$) among treatments by panelist.

In Experiment 1, "GRS–" juice had the highest mean (6.37) in orange flavor and was significantly preferred in terms of orange flavor. However, there was no significant difference between "GRS+" and "GRL–" juice in terms of orange flavor. "GRL+" juice had the lowest mean (4.90) in orange flavor. In Experiment 2, "GRS–" juice was significantly different from "GRL–" and "GRL+" juice. Similarly, "GRS+" juice was significantly different from "GRL+" juice in terms of orange flavor. As was observed in Experiment 1, GRL+ had the lowest rankings in orange flavor in Experiment 2. Although this study did not examine juice volatiles, it is well known that orange flavor is a complex combination of over 20–34 volatile compounds including esters (1%), aldehydes (1%), ketones (1%), hydrocarbons (75% to 98%) and alcohols (1% to 5%) (Jia, 1999; Nisperos-Carriedo and Shaw, 1990; Selli, 2004). Limonene is a major peel oil constituent and is the volatile compound produced in the highest amount (Jia, 1999). However, it is not the most important contributor in flavor with respect to quality because of its high sensory threshold (Jia, 1999). The degradation of limonene into α-terpineol gives off flavors (Nisperos-Carriedo and Shaw 1990). The major contributors to

Table 1. Average soluble solids content (SSC), titratable acidity (TA), SSC/TA ratio and BrimA index of juice treatments.[z]

Treatment	SSC (°Brix)	TA (%)	SSC/TA	Brim A
GRS–	12.320.85	1.350.29	9.12	8.25
GRL–	11.120.07	1.110.31	10.01	7.76
GRS+	12.100.42	0.940.06	12.87	9.26
GRL+	11.250.98	1.190.39	9.45	7.65

[z]Values are presented as means of two trials.

Table 2. Mean panelist ratings of orange flavor in Experiments 1 and 2.[z]

Sample	Experiment 1 (n = 99)	Experiment 2 (n = 88)
GRS–	6.41.523 a	6.31.544 a
GRL–	5.61.721 b	5.41.761 bc
GRS+	5.81.726 b	5.91.774 ab
GRL+	4.91.764 c	5.21.816 c

[z]According to Tukey (HSD) multiple comparison of orange flavor by treatments. Means with the same letters show no significance at α = 0.05.

orange flavor include acetaldehyde, citral, ethyl butyrate, limonene, octanal, decanal, and α-pinene (Ahmed, 1978). In this study, SSC and intensities of orange flavor followed a parallel trend. In other words, the treatments such as "GRS–" and "GRS+" juice with the higher SSC were rated comparatively higher in orange flavor than "GRL–" and "GRL+" juices. This is in agreement with research that shows the enhancement of flavor with the addition of sugars. Hewson and others (2007) reported that the addition of sugars (fructose and glucose) to a simple mix of citrus aroma volatiles (limonene and citral) enhanced the citrus flavor as rated by panelists. Pfeiffer and others (2006) also found that the perception of strawberry flavor (strawberry aroma volatiles) was enhanced with the addition of sugar (sucrose) and acids (citric, malic, lactic and phosphoric acids). Pfeiffer and others (2006) reported the strawberry flavor perception was stronger when both acids and sugars were added to the media. Another possible explanation for the results could be the high presence of pulp in the "GRL–" and "GRS+" juices. Several panelists commented on the pulpiness of these juices, and it has been reported by Rega (2004) that pulp enhances the orange flavor of juices. This is because pulp contains large amounts of aroma compounds including terpenes and acetaldehyde (Rega, 2004). Nevertheless, it is clear that orange juice is a complex media and as such further research with a trained panel is essential to understand the effects of pulp and SSC levels on orange flavor perception in healthy and greening-affected orange juices.

Sweetness. Similar to orange flavor, ANOVA results show statistical significance ($P < 0.00001$) among treatment means for sweetness ratings. Table 3 shows treatments and means for Experiments 1 and 2, respectively, outlining those with significant differences as pertaining to sweetness.

Sweetness is determined by the concentration of predominant sugars in the fruit, with fructose ranking as sweeter than sucrose (Kader, 2008). Soluble solids are often used as an indicator of sweetness (Kader, 2008). While there is some correlation as sugars are a component of soluble solids in addition to phenols, acids, pectins and anthocynanins, the relationship between sugars and soluble solids is not necessarily definitive (Kader, 2008). In addition, the interaction between sugars and acids has been shown to influence the perception of sweetness in the food matrix. Stampanoni (1993) reported that increased citric acid can suppress the perceived sweetness in sherbets. The SSC/TA ratio has thus been widely regarded by the citrus industry as an important parameter in assessing the flavor quality, particularly the balance of sweetness and sourness in orange juice (Kimball, 1991). However, there are difficulties in utilizing SSC/TA ratio as a parameter in assessing sweetness, as different juices with different levels of SSC and TA can lead to different flavor perceptions with the same ratio (Obenland, 2009). A recently developed alternative index has been proposed by Jordan (2001) known as the BrimA which proposes that TA is subtracted from SSC after multiplying by some constant that differs by fruit type (Jayasena, 2008; Obenland, 2009). The rationale behind this parameter is the acknowledgement that acidity is more sensitive on the tongue than sweetness and thus must be better accounted for by the addition of a constant (Jayasena, 2008; Obenland, 2009).

Table 3. Mean panelist ratings of sweetness in Experiments 1 and 2.[z]

Sample	Experiment 1 (n = 99)	Experiment 2 (n = 88)
GRS–	6.31.698 a	6.11.487 a
GRL–	5.81.643 ab	5.71.799 ab
GRS+	5.61.851 b	5.41.910 b
GRL+	4.61.712 c	5.21.810 b

[z]According to Tukey (HSD) multiple comparison of sweetness by treatments. Means with the same letters show no significance at $\alpha = 0.05$.

In Experiment 1, sweetness rating in "GRS–" juice was significantly different from sweetness rating in "GRS+" and "GRL+" juice. In addition, "GRL–" and "GRS–" juice were significantly different from "GRL+" juice. In Experiment 2, "GRS–" juice was significantly different from "GRS+" and "GRL+" juice. In both experiments, greening juice (both large and small fruit) had the lowest ratings in sweetness. In Table 1, "GRL–" and "GRS+" juice have slightly higher SSC/TA ratios than "GRS–" and "GRL+" juice. This would suggest that "GRL–" juice is sweeter than "GRS–" juice which is contrary to sensory results. In addition, average BrimA values which were developed to be a better predictor of sweetness compared with SSC/TA ratios show that when disease-status is kept constant when comparing juices, juice from small fruit has a higher average BrimA score than juice from large fruit. The average BrimA scores largely reflect the soluble solids content in the juices with smaller fruits having the higher soluble solid contents compared with large fruits. The juice used for this study came from a late 'Valencia' harvest (04–09–2010) and it has been reported by Plotto (2008) that greening-affected juices from late harvests tend to be sweeter than healthy juice.

Nevertheless, there is a clear contrast between sensory results and juice analysis emphasizing the complexity of flavors and sweetness perception. It is difficult to explain the discordance of the test. As noted earlier, several of the panelists commented on the pulpiness of the "GRL–" and "GRS+" juices. However, there is no clear evidence that pulpiness influences the sweetness of juice (Rega, 2004). One possible explanation of the discordance of results is the possibility of greening-affected juice aroma suppressing the perception of sweetness. A few comments left by panelists acknowledged they perceived the aroma of greening-affected juice before tasting it. It has been well documented that the effect of aromas on flavor is dependent and often times influencing the intensity of tasted flavors. Frank and Byram (1988) observed that the perception of sweetness increased by adding strawberry odor to beaten cream albeit they did not observe this phenomenon with peanut butter aroma (Lawrence, 2009). In contrast, Stevenson (1999) observed that angelica oil and cedryl acetate can suppress the perception of sweetness in a sucrose solution. Greening-affected juice aroma varies according to fruit maturity and cultivar variability. However, Plotto (2008) reports that late season 'Valencia' juice has a slight difference in aroma when comparing greening-affected juice to healthy juice and this is attributed to the higher oil content in the former. In addition, it has also been reported that greening-affected juice has a higher amount of alcohols and terpenes compared with healthy juice (Dagulo, 2010). The sensory panel results tend to lead one to suspect the plausibility of sweetness suppression of greening-affected juice by odorized volatiles. However, in this study, no volatile aroma analyses were carried out to either support or refute this theory. Further testing of juice from different harvest seasons is strongly recommended to clear up this inconsistency.

Overall acceptability. Similar to orange flavor, ANOVA results show statistical significance ($P < 0.00001$) among treatment means. Table 4 show treatments and means for Experiments 1 and 2, respectively, outlining those with significant differences as pertaining to overall acceptability.

Table 4. Mean panelist ratings of overall acceptability in Experiments 1 and 2.[z]

Sample	Experiment 1 (n = 99)	Experiment 2 (n = 88)
GRS–	6.31.666 a	6.11.646 a
GRL–	5.61.733 b	5.31.915 bc
GRS+	5.81.722 ab	5.71.786 ab
GRL+	4.61.793 c	4.91.778 c

[z]According to Tukey (HSD) multiple comparison of overall acceptability flavor by treatments. Means with the same letters show no significance.

In Experiment 1, "GRS–" juice was significantly different from "GRL–" and "GRL+". In addition, "GRS+" and "GRL–" juice were significantly different from "GRL+" juice. In Experiment 2, "GRS–" juice was significantly different from "GRL–" and "GRL+" fruit. Similarly, "GRS+" juice was significantly different from "GRL+" fruit. Although freshly squeezed juice is usually ranked highly in terms of overall acceptability (Luckow 2003; Min 2003; Walkling-Ribeiro 2008), in this study "GRS+" juice was preferred by panelists to "GRL–" juice which suggests that small fruits are preferred to large fruits in terms of overall acceptability irrespective of the greening disease status.

Conclusions

In summary, the objective of this study was to investigate the effects of fruit size and disease status (citrus greening) on orange flavor, sweetness and overall acceptability of juice. Two sensory evaluation experiments were carried out for reproducibility.

Albeit there was discordance between the juice analysis and sensory results, it is pertinent to emphasize that, "GRS–" juice was the highest rated treatment by panelists in all categories (orange flavor, sweetness and overall acceptability) in both experiments. Similarly, "GRL+" juice was the lowest rated treatment in all categories and in both experiments. In all sensory categories, there were no significant differences between "GRL–" juice and "GRS+" juice with the later ranking higher than the former in every category except sweetness. This suggests that there is a potential for greening-affected ("GRS+") juice to be utilized in the citrus industry via blending. In addition, small fruits (healthy and greening-affected) ranked higher in all categories (except sweetness) than large fruits. Therefore, the factor of size (a common factor in fruit grading) may be a useful determinant in selecting greening-affected fruits with highest potential for juice extraction and blending with healthy juice.

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