Physicochemical and Sensory Properties of Cold Pressed Oils from Florida Hamlin and Valencia Oranges Affected by Huanglongbing

Brittany M. Xu, Charles A. Sims, Edgardo Etxeberria, and Renée M. Goodrich Schneider

Abstract: Cold pressed oils from huanglongbing (HLB) symptomatic (SY) and asymptomatic (AS) Hamlin and Valencia oranges were assessed for 2 y (2014 to 2015 and 2015 to 2016 seasons) with 2 harvest dates for each orange variety per year. Physicochemical properties (optical rotation, aldehyde content, ultraviolet [UV] absorbance, refractive index, and specific gravity) mandated by the United States Pharmacopeia (USP) for orange oil quality were assessed. Hamlin and Valencia oils showed minor differences in physicochemical properties based upon disease stage. However, all Hamlin oils had aldehyde contents below the USP minimum and Valencia oil from late season SY oranges had specific gravities above the USP maximum. Significant differences based on harvest year were seen for aldehyde content, refractive index, optical rotation, and UV absorbance. While none of these changes led to an oil being out of USP specifications, they indicate a need to monitor the quality of oil every year to ensure a consistent product. Flavor taste panels were performed both years by adding 0.035% oil samples to a uniform orange juice base. Aroma panels were done by smelling pure oil. There were no significant differences between SY and AS oils for flavor, although panelist race was a significant factor in several of the panels. There were significant differences between the aroma of SY and AS oils for both 2015 to 2016 Hamlin Early and Valencia Late samples. Overall, these results show HLB can have an effect on the aroma and USP mandated physicochemical properties of Florida orange oils, although flavor may be unaffected by this plant disease.

Keywords: citrus greening disease, citrus oil physicochemistry, citrus quality, cold pressed oil, huanglongbing

Practical Application: Research on the effect of huanglongbing on the physicochemical properties, taste, and aroma of orange oils has not previously been published. This work is important to those working in the flavor and fragrance industries, for whom cold pressed orange oil and/or its chemical fractions are important products used in the formulation of many beverages, flavors, and perfumes. This work is additionally important to orange juice manufacturers, for whom cold pressed orange oil is a significant byproduct.

Introduction

Huanglongbing (HLB), also known as citrus greening disease, is a phloem-limited bacterial disease of citrus that is pervasive throughout Florida. The presumed bacteria *Candidatus* Liberibacter asiaticus (Bove 2006) is vectored by the Asian citrus psyllid *Diaphorina citri* Kuwayama (da Graça 1991). There is no known cure for HLB once a tree is infected (Bove 2006). Before noticeable changes in fruit productivity occurs, there are noticeable anatomical and physiological changes in the fruit, leaves, and roots including root system decay and asymmetrical leaf chlorosis, commonly called yellow blotchy mottle (da Graça 1991). Symptomatic (SY) fruits are small, somewhat green in color, misshapen, and contain aborted seeds (da Graça 1991). More than $3.63 billion in revenue and over 6600 jobs were lost in Florida between 2006 and 2011 due to this disease (Hodges and Spreen 2012).

Cold pressed (CP) orange peel oil is a valuable commodity extracted from a byproduct that would otherwise become waste or animal feed (Kesterson and Braddock 1976). Hood (1916) found wide variations in the yield of oil from Florida oranges, reporting values of 0.11% to 0.58% of the total orange weight. Oil content reaches a maximum when the orange is fully mature; however, commercial quantities are present before the fruit is ready for harvest (Kesterson and others 1971). The most common method of extracting CP oil from oranges is by using a JBT (formerly FMC) in-line extraction system where the oil is extracted simultaneously with the juice (Braddock 1999). This method uses pressure and a shredding mechanism to extract oil from the peel. The JBT extractor separates oranges into 4 parts: an oil emulsion, peel, core, and juice. The extractor is composed of an upper and lower cup. A mechanism pushes through the lower cup to cut a hole in the fruit, through which the core will be removed. The upper cup presses down on the lower cup. Pressure causes the juice to squeeze out of the fruit, which is strained through the lower cup, along with some pulp. The pressure also causes the peel to break apart and the peel and core are caught in the strainer cup. As the peel is forced through the fingers of the cup, oil is extracted from the peel, which is washed with water to form an oil emulsion (Ringblom 2004).

Another common extractor is the Brown oil extractor, a reamer-type extractor in which the oil is recovered before juice extraction. In this procedure, the peel is punctured with sharp needles and subsequently washed with water to create an oil emulsion. Downstream, oranges are cut in half, then reamers penetrate fruit, squeezing juice through one outlet and rag material through

JFDS-2017-0016 Submitted 1/4/2017, Accepted 6/20/2017. *Authors are with Dept. of Food Science and Human Nutrition, Univ. of Florida, 359 FSHN Bldg, 572 Newell Drive, Gainesville, FL, 32611, U.S.A. Direct inquiries to author Goodrich-Schneider (E-mail: goodrich@ufl.edu).*

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another. The end products of both extraction methods are very similar, but may vary slightly in physicochemical properties (Kesterson and others 1971).

CP orange oil is used in beverages, including juices and juice concentrates. In particular, orange oil is often added back to the concentrated product from which it is derived, or to other citrus juices (Ringblom 2004). Orange oil gives juice fruity, green, and sweet top notes, and is generally added to concentrate at a 0.01% volume-to-volume ratio (Ringblom 2004). CP orange oil can additionally be utilized in soft drinks, beverages, cookies, desserts, confections, and chewing gum (Braddock 1999).

Many studies were performed on the physicochemical properties of orange oils in the 1930s to 1970s (Nelson and Mottern 1934; Foote and Gelpi 1943; Bartholomew and Sinclair 1946; Kesterson and Hendrickson 1953; Hendrickson and others 1969; Kesterson and others 1971; Wolford and others 1971). These studies showed that factors including fruit maturity at harvest (Hendrickson and others 1969), extraction method (Kesterson and others 1971), relative volume of aqueous phase during extraction (Kesterson and Hendrickson 1953, 1967), and storage time after harvest (Kesterson and Hendrickson 1953, Kesterson and others 1971) are easily controlled factors that affect the physicochemical properties of orange oil. Other factors such as soil moisture (Kesterson and Hendrickson 1953, 1971) are more difficult to control and can cause year-to-year variation in orange oil quality parameters.

The aroma of orange oil has been previously studied by gas chromatography-olfactometry (GCO) studies (Hognadottir and Rousseff 2003; Rega and others 2003; Qiao and others 2008). The primary purpose of these studies was to determine the chemical composition of various orange oils. However, direct flavor and aroma panels for orange oils have not been commonly performed as there has not been a historical need to directly compare 2 types of orange oil by flavor or aroma as commercial product is uniform if sold under the United States Pharmacopeia (USP) authority. However, with the onset of HLB, and considering the substantial changes in fruit physiology (da Graça 1991), it is important to determine whether significant differences exist between the taste and aroma of oils derived from SY and asymptomatic (AS) fruits.

Although there are no published studies on the effect HLB has on the flavor or aroma of CP orange oils, there have been several studies that analyze the effect HLB has on these characteristics in orange juice (Baldwin and others 2010; Dagulo and others 2010; Plotto and others 2010). In a sensory panel analyzing fruit from the 2007 harvest year, experienced panelists found flavor differences between SY and control Hamlin juices in a "difference from control" test (Plotto and others 2010). In their study, some panelists described the SY fruit as being fermented, sweeter, overripe, sour, or bitter. The same panel also found juice from SY Midsweet fruit to be different from the control, and participants also described the SY juice fruit as being fermented, sweeter, and overripe (Plotto and others 2010). Compared to the 2007 season, somewhat different results in juice properties were seen for the 2008 season. Similar to the 2007 season, juice from SY Hamlin fruit showed significant differences compared to juice from control fruit in a "difference from control" test (Plotto and others 2010). Panelists found SY Hamlin juice to be bitter, sour, grapefruit-like, peppery, and metallic. A trained panel described the same SY juice as fatty, fermented, salty, or musty/earthy. Overall, SY Hamlin juice had more off-flavors than Valencia or Midsweet juice, and was most commonly described as bitter, sour, or fermented.

Juice made from SY fruit has less esters, aldehydes, and total sesquiterpenes than juice extracted from HLB unaffected fruit (Dagulo and others 2010). SY juice contained increased levels of alcohols and terpenes. Overall, these changes are comparable to juice made from immature fruit, which has a less favorable aroma profile than juice made from mature unaffected fruit (Dagulo and others 2010). Juice made from SY and control Hamlin oranges were shown to have significantly different aromas in difference from control tests (Plotto and others 2010). There were no significant differences for Midsweet and Valencia juices.

Chemical and sensory differences between juice made with SY compared with healthy oranges in previous studies could be explained by differences in volatiles from the peel getting in the juice stream. Therefore, the objective of this study was to measure the physicochemical properties and assess the flavor and aroma of CP oils derived from HLB SY and AS Hamlin and Valencia fruit. To achieve these goals, the USP mandated physicochemical properties that include optical rotation, refractive index, aldehyde content, specific gravity, and ultraviolet (UV) absorbance were measured. Oranges were harvested at 2 different times during each year (early and late harvest) over 2 y to determine the effect of maturity and harvest year in addition to HLB disease stage. The harvest years were 2014 to 2015 and 2015 to 2016. No previous studies have reported the effects of HLB on CP orange oil USP physicochemical properties, flavor, or aroma.

Materials and Methods

Harvesting and orange trees

Florida grown Hamlin and Valencia oranges were harvested twice per year each for 2 y. Fruits were harvested from established groves at the Univ. of Florida Citrus Research and Education Center in Lake Alfred, Florida. For both Hamlin and Valencia oranges, fruits were harvested early and late each year in the respective variety's season. The maturity and disease stage of the fruit was determined by a trained horticulturist using established protocols (Achor and others 2010). The time of maturity varied slightly year to year based on flowering time. Oranges were harvested for the 2014 to 2015 and 2015 to 2016 seasons. Early Hamlin oranges were harvested on December 2, 2014 and November 15, 2015. Late Hamlin oranges were harvested on January 27, 2015 and January 14, 2016. Early Valencia oranges were harvested February 3, 2015 and February 11, 2016. Late Valencia oranges were harvested March 24, 2015 and March 31, 2016.

Approximately 30 trees per variety per year were harvested. The trees harvested were in adjacent rows ($n = 2$ to 5). Approximately 300 kg each of SY and AS oranges were collected during each harvest. SY oranges were noted to be mature but with characteristic outward greening symptoms including small fruit with reversed ripening with the stylar end remaining green. While some trees contained both SY and AS fruits, many trees contained nearly 100% SY fruit. These trees commonly displayed yellow blotchy mottle on the leaves and overall sparse foliage. AS fruits appeared outwardly healthy. AS fruits were overall larger and did not display the reversed ripening common with HLB SY fruit. There are no longer significant quantities of HLB unaffected fruit in the state of Florida, as HLB infection rate at the grove level is near 100% (Browning 2015). Given these circumstances, only HLB AS and SY fruits were utilized for this study.

CP oil extraction

After harvest, oranges were stored in a cold room at 4 °C until processing. Processing of the raw oranges into CP oil was performed in the Pilot Plant at the Citrus Research and Education

Center in Lake Alfred, Fla. according to typical industry practices (Braddock 1999). Prior to processing, the oranges were sanitized with Fruit Cleaner 395 (JBT FoodTech, Lakeland, Fla., U.S.A.) and surface dried. Juice and an oil emulsion were extracted simultaneously with a commercial JBT extractor (JBT FoodTech). The oil emulsion was collected in 190-L tanks and put into cold storage at 4 °C for approximately 40 h. The crude emulsion was brought to room temperature over the course of 3 h before being refined into CP orange oil.

A DeLaval table top centrifuge (DeLaval, Tumba, Sweden) specifically for oil was used to produce the CP oil. The centrifuge is equipped with a Dayton motor (Dayton Motor Co., Dayton, Tex., U.S.A.) that operates at 1000 x *g*. Raw material from the topmost oily portion of the emulsion was very slowly fed into the centrifuge by hand. Due to the large quantity of emulsion (approximately 110 L) produced, only the top part of the emulsion that contains the most oil was fed through the centrifuge. Oil was aliquoted into vials and stored at –20 °C until use.

USP orange oil quality tests

The USP (2010) mandates several tests for determining the quality of CP Florida orange oils for commercial sale. These tests are aldehyde content, refractive index, UV absorbance, optical rotation, and specific gravity. A detailed description of each test performed can be found in the "Procedures for Analysis of Citrus Products" laboratory manual released by JBT (JBT FoodTech 2011).

Aldehyde content. This test was performed in triplicate, using the USP Aldehyde method (JBT FoodTech 2011). In this method, 5 g of oil was weighed into an Erlenmeyer flask. Fifty milliliters of hydroxylamine hydrochloride solution was added to the flask, which was stoppered and swirled. The flask was allowed to stand at ambient temperature for 30 min with occasional swirling before being titrated with 0.5 normal alcoholic KOH to pH 3.4.

Refractive index. An Abbe Mark III automatic refractometer (range 1.3106 to 1.7000 ± 0.0001) (Ametek Reichert Technologies, Depew, N.Y., U.S.A.) was used. The refractive index was measured in triplicate. Refractive index must be between 1.472 and 1.474.

UV absorbance. UV absorbance has a USP minimum of 0.240, and was calculated by the following method, performed in triplicate. In a 100 mL volumetric flask, 250 mg of oil was mixed with 98% isopropanol up to the 100 mL mark and mixed thoroughly. A DU® 730 Life Science UV/VIS spectrophotometer (Beckman Coulter, Brea, Calif., U.S.A.) was used to measure absorbance. The machine is set to 1 nm automatic scanning from 260 to 400 nm. Isopropanol was read as a blank before the sample is read.

To determine final wavelength, the absorbances are plotted against wavelength graphically. A base-line tangent linking the points of minimum absorbance (A and B) was 1st drawn. Next, the point of maximum absorbance (C) was located, and a vertical line was drawn from it to the *x*-axis, so that it intersects line AB at a new point D. The difference in absorbance between points C and D is the UV absorbance of the oil, on the basis of a 250 mg sample.

Optical rotation. This test was measured in triplicate using an Anton Paar automatic MCP 150 polarimeter (Anton Paar, Graz, Austria) with automatic temperature adjustment. The quartz sample cell was injected with approximately 5 mL of the orange oil. A 50 mm cell was used, so the obtained optical rotation values were multiplied by 2 for a final reading. Optical rotation needs to in the range of 94 to 99 to be within USP specifications.

Specific gravity. Specific gravity must be between 0.842 and 0.846 and was measured in quintuplicate. A 10-mL Pyrex pycnometer was used to determine specific gravity. First, the pycnometer was tared on an analytical balance. The pycnometer was filled with distilled water at 20 °C, wiped to remove any excess moisture, and weighed accurately in quintuplicate. The average mass of the water was used in the final calculation. The same procedure was repeated in quintuplicate for each oil sample. Each oil measurement could then be divided by the average water mass to calculate the specific gravity of the oil.

Sensory analysis

Sensory panels were performed at the Univ. of Florida Food Science and Human Nutrition Sensory Lab. This lab consists of 2 sensory panel rooms, each of which is equipped with 10 private booths. Data were collected electronically using Compusense 5 (Compusense, Guelph, Canada). Sensory analysis was performed to evaluate both the flavor and aroma of the CP orange oils. For the flavor analysis, the CP oil was experimentally added to both a model solution and to not from concentrate orange juice. For the aroma panels, a small amount of unaltered orange oil was smelled by panelists.

Flavor panels. The model solution was made using a base of deionized water, containing 5.0% sucrose, 0.5% citric acid (Alpha Chemicals, Stoughton, Mass., U.S.A.), 1.0% 190 proof food grade ethanol (Decon Labs, King of Prussia, Pa., U.S.A.), and 0.035% CP orange oil. The sucrose and citric acid were added to the water in large plastic pitchers and shaken until the solutes were dissolved. The orange oil was added to the ethanol, which was then added to the water mixture. One model solution was made with SY oil, and another was made with AS oil. Model solutions were made the day before the sensory panel. They were refrigerated (4 °C) overnight and then allowed to come to room temperature 2 h before the start of the panel.

Each orange juice test solution was made using not-from-concentrate orange juice (WalMart, Bentonville, Ark., U.S.A.). Ethanol at 1.0% final concentration by volume and orange oil at 0.035% final concentration were mixed and then added to the orange juice. One juice sample was made with SY oil, and another was made with AS oil. The juice samples were prepared the day before the taste panel and were kept refrigerated (4 °C) at all times.

On each test day, 2 sets of samples (model solution and orange juice test solution) were presented to 75 panelists. The panelists were screened prior to testing to ensure they had consumed orange juice in the past month. The model solution was served at room temperature while the orange juice test solution was served at approximately 4 °C. Both samples were shaken vigorously before being poured into 118 mL (4 oz) cups to ensure the oil was well mixed into the sample. The model solution was presented prior to the orange juice test solution to minimize taste fatigue. The panelists were instructed to cleanse their palate with crackers and deionized water prior to the 1st sample and between the 2 samples. Panelists were given both verbal and written instructions on how to evaluate samples for a triangle test and were instructed to taste their samples from left to right.

A triangle test format, where 2 samples are the same and 1 is different, was used to determine if panelists could differentiate between samples made with SY oil and those made with AS oil. A triangle test is a forced choice test where panelists must choose which sample they believe is different from the other 2 samples.

Each oil type was assigned 2 random numbers. All 6 orders of presentation were presented approximately an equal number of times. Order of presentation was randomized to avoid any bias based on sample presentation order. On each day, 75 panelists performed a triangle test for the model solutions; the same panelists then immediately completed a 2nd triangle test for the orange juice test solutions.

Panelists who identified the correct odd sample were asked to choose which sample they preferred by identifying the random number on the cup they liked best. Panelists who identified the correct odd sample were also given the opportunity to describe what they believed the differences were between the 2 samples, although this was optional.

For both the 2014 to 2015 and 2015 to 2016 seasons, Early Hamlin, Late Hamlin, Early Valencia, and Late Valencia samples were tested.

Aroma panels. Aroma panels were utilized to determine if panelists could differentiate between the aroma of HLB SY and AS oils. These tests were also triangle tests: panelists were presented with 3 samples; 2 were the same and 1 was different. Panelists attempted to identify the different sample by aroma alone.

To prepare the samples, 8 drops (170 μL) of either SY or AS oil were added to a cotton round (Swisspers, Gastonia, N.C., U.S.A.), which had been placed in the bottom of 50-mL plastic centrifuge bottles free from odors. The bottles were prepared approximately 1 h prior to the start of the panel to ensure freshness. The bottles were capped when not in use and panelists were given instructions to only uncap 1 bottle at a time while sniffing.

On each test day, 75 panelists were presented with 2 sets of triangle samples. There was a mandatory 90-s rest period between the 2 sample sets to aid in avoiding fatigue. Panelists were given both verbal and written instructions on how to evaluate samples for a triangle test and were instructed to smell their samples from left to right.

Panelists who identified the correct odd sample were asked to choose which sample they preferred by identifying the random number on the bottle they liked best. Panelists who identified the correct odd sample were also given the opportunity to describe what they believed the differences were between the 2 samples, although this was optional.

Aroma panels were conducted for the 2015 to 2016 samples on 2 d. On each day, 2 triangle tests were performed by the same set of 75 panelists. On the 1st day, panelists were presented with Hamlin Early and Hamlin Late samples. On the 2nd day, panelists were presented with Valencia Early and Valencia Late samples.

Statistical analysis

t-Tests were performed to determine if AS samples differed from SY samples. For the USP physicochemical tests, *t*-tests were also used to determine differences based on harvest time (early or late) or harvest year. For the sensory panels, chi-squared tests were used to determine the effect of race, gender, and orange juice consumption on panelist perception. A critical number table (Meilgaard and others 2007) was used to determine significant differences for triangle tests. Significance was set at $\alpha \leq 0.05$ for all tests.

Results and Discussion

USP physicochemical tests

Aldehyde content, optical rotation, specific gravity, refractive index, and UV absorbance were assessed for all samples. The aldehyde content of Florida orange oils must be 1.2% to 2.5%, measured as decanal (USP 2010). Results showed that all Hamlin samples were below the USP minimum (1.2%) for aldehyde content. The range of mean aldehyde content for Hamlin samples was 0.52% to 0.75%. Additionally, late season SY Valencia samples for both the 2014 to 2015 and 2015 to 2016 seasons had specific gravities above the USP maximum (0.846). For both years, the mean specific gravity for late season SY Valencia oil was 0.848.

Significant differences between AS and SY samples were observed for aldehyde content and specific gravity (Table 1), but not for UV absorption, refractive index, or optical rotation. For aldehyde content, AS samples of Hamlin Late 2015 to 2016 and Valencia Early and Late 2015 to 2016 had significantly higher mean aldehyde content than SY samples. Early 2015 to 2016 Hamlin AS oil had a higher mean specific gravity that SY oil. For both harvest years, Valencia Late SY oil had significantly higher mean specific gravity than AS oil. As previously noted, for both harvest years Valencia Late SY oil exceeded the USP maximum for specific gravity.

Differences in aldehyde content, specific gravity (Table 1), refractive index, and UV absorbance (Table 2) were detected for different harvest times (early or late in the harvest season). Early AS Hamlin (2014 to 2015 and 2015 to 2016), SY Hamlin 2015 to 2016, and AS Valencia 2015 to 2016 samples had higher mean aldehyde content than late season samples (Table 1). Late SY Hamlin 2015 to 2016 and Late SY Valencia samples for both harvest years had significantly higher specific gravities than did Early samples, in the case of Valencia due to the Late SY samples having specific gravity values above the USP maximum (Table 1). Early Hamlin SY 2014 to 2015 and Early Hamlin AS 2015 to 2016 had significantly higher refractive indices than Late season samples, but there does not appear to be an overall trend based on harvest time (Table 2). Early SY Hamlin 2015 to 2016, AS Valencia 2015 to 2016, and SY Valencia 2015 to 2016 had higher mean UV absorbance than Late samples. Late AS Valencia oil 2014 to 2015 had higher mean UV absorbance than did Early (Table 2).

Refractive index and UV absorbance both had significant differences based on the year the samples were harvested (Table 2). For all samples except Hamlin Early AS, the 2014 to 2015 season mean refractive index was higher than in 2015 to 2016. For UV absorbance, all Hamlin samples had significantly higher mean UV absorbance in the 2015 to 2016 season. Conversely, all Valencia samples had significantly higher UV absorbance in the 2014 to 2015 season.

Aldehyde content is an important indicator of quality for orange oil; higher aldehyde content generally means a better quality oil (Kesterson and others 1971). Thus, Hamlin samples that failed the aldehyde test in this study were deemed of a lesser quality than that of the Valencia samples. Historically, Hamlin oil has been generally considered to be of lower quality than Valencia oil in general given its lower aldehyde contents (Kesterson and others 1971).

For commercial purposes, Florida orange oil has a minimum acceptable UV absorbance but not a maximum value. This characteristic, as well as optical rotation, refractive index, and specific gravity can be used to indicate whether an oil is potentially adulterated. However, these values being higher or lower than average do not necessarily mean the oil is of a higher or lower quality. This is different from aldehyde content, where higher content generally indicates higher quality.

A previous study on orange juice (Dagulo and others 2010) showed that SY juice had only 48% of the aldehyde content of control juice unaffected by HLB. This study found that aldehyde

Table 1–Differences for the United States Pharmacopeia tests between asymptomatic, symptomatic, early, and late cold pressed orange oils.

Sample		Percent aldehyde content					Specific gravity			
		AS[a] Mean ($n = 3$)	SY[b] Mean ($n = 3$)	*P*-value			AS Mean ($n = 5$)	SY Mean ($n = 5$)	*P*-value	
Hamlin 2014 to 2015	Early	0.65	0.60	AS compared with SY Early	0.531	Early	0.843	0.844	AS compared with SY Early	0.326
				AS compared with SY Late	0.667				AS compared with SY Late	0.195
	Late	0.52	0.55	Early compared with Late AS	0.0047[c]	Late	0.844	0.846	Early compared with Late AS	0.456
				Early compared with Late SY	0.185				Early compared with Late SY	0.160
		AS Mean ($n = 3$)	SY Mean ($n = 3$)	*P*-value			AS Mean ($n = 5$)	SY Mean ($n = 5$)	*P*-value	
Hamlin 2015 to 2016	Early	0.75	0.63	AS compared with SY Early	0.379	Early	0.846	0.843	AS compared with SY Early	0.0022[c]
				AS compared with SY Late	0.0052[c]				AS compared with SY Late	0.402
	Late	0.74	0.51	Early compared with Late AS	0.0052[c]	Late	0.844	0.845	Early compared with Late AS	0.166
				Early compared with Late SY	0.0091[c]				Early compared with Late SY	0.0023[c]
		AS Mean ($n = 3$)	SY Mean ($n = 3$)	*P*-value			AS Mean ($n = 5$)	SY Mean ($n = 5$)	*P*-value	
Valencia 2014 to 2015	Early	1.49	1.62	AS compared with SY Early	0.161	Early	0.844	0.844	AS compared with SY Early	0.710
				AS compared with SY Late	0.585				AS compared with SY Late	0.011[c]
	Late	1.45	1.33	Early compared with Late AS	0.379	Late	0.845	0.848	Early compared with Late AS	0.578
				Early compared with Late SY	0.069				Early compared with Late SY	0.008[c]
		AS Mean ($n = 3$)	SY Mean ($n = 3$)	*P*-value			AS Mean ($n = 5$)	SY Mean ($n = 5$)	*P*-value	
Valencia 2015 to 2016	Early	2.07	1.61	AS compared with SY Early	0.0016[c]	Early	0.844	0.844	AS compared with SY Early	0.455
				AS compared with SY Late	0.0183[c]				AS compared with SY Late	0.00019[c]
	Late	1.81	1.54	Early compared with Late AS	0.0330[c]	Late	0.844	0.848	Early compared with Late AS	0.792
				Early compared with Late SY	0.177				Early compared with Late SY	<0.0001[c]

[a]AS = Huanglongbing asymptomatic samples.
[b]SY = Huanglongbing symptomatic samples.
[c]Significant differences observed at $\alpha \leq 0.05$ using a *t*-test.

Table 2–Differences for the United States Pharmacopeia tests between 2014 to 2015, 2015 to 2016, early, and late cold pressed orange oils.

Sample		Ultraviolet absorbance				Refractive index				
		Year 1[a] Mean ($n = 3$)	Year 2[b] Mean ($n = 3$)	*P*-value			Year 1 Mean ($n = 3$)	Year 2 Mean ($n = 3$)	*P*-value	
Hamlin asymptomatic	Early	0.261	0.345	Early compared with Late Year 1	0.569	Early	1.4724	1.4725	Early compared with Late Year 1	0.116
				Early compared with Late Year 2	0.069				Early compared with Late Year 2	<0.0001[c]
	Late	0.269	0.314	Year 1 compared with Year 2 Early	0.00033[c]	Late	1.4727	1.4718	Year 1 compared with Year 2 Early	0.469
				Year 1 compared with Year 2 Late	0.00094[c]				Year 1 compared with Year 2 Late	<0.0001[c]
		Year 1 Mean ($n = 3$)	Year 2 Mean ($n = 3$)	*P*-value			Year 1 Mean ($n = 3$)	Year 2 Mean ($n = 3$)	*P*-value	
Hamlin symptomatic	Early	0.264	0.368	Early compared with Late Year 1	0.652	Early	1.4727	1.4718	Early compared with Late Year 1	0.0022[c]
				Early compared with Late Year 2	0.00040[c]				Early compared with Late Year 2	1.00
	Late	0.273	0.306	Year 1 compared with Year 2 Early	0.00030[c]	Late	1.4725	1.4718	Year 1 compared with Year 2 Early	<0.0001[c]
				Year 1 compared with Year 2 Late	0.026[c]				Year 1 compared with Year 2 Late	<0.0001[c]
		Year 1 Mean ($n = 3$)	Year 2 Mean ($n = 3$)	*P*-value			Year 1 Mean ($n = 3$)	Year 2 Mean ($n = 3$)	*P*-value	
Valencia asymptomatic	Early	0.378	0.294	Early compared with Late Year 1	<0.0001[c]	Early	1.4727	1.4725	Early compared with Late Year 1	1.00
				Early compared with Late Year 2	<0.0001[c]				Early compared with Late Year 2	1.00
	Late	0.480	0.271	Year 1 compared with Year 2 Early	<0.0001[c]	Late	1.4727	1.4725	Year 1 compared with Year 2 Early	<0.0001[c]
				Year 1 compared with Year 2 Late	<0.0001[c]				Year 1 compared with Year 2 Late	<0.0001[c]
		Year 1 Mean ($n = 3$)	Year 2 Mean ($n = 3$)	*P*-value			Year 1 Mean ($n = 3$)	Year 2 Mean ($n = 3$)	*P*-value	
Valencia symptomatic	Early	0.394	0.292	Early compared with Late Year 1	0.455	Early	1.4727	1.4725	Early compared with Late Year 1	1.00
				Early compared with Late Year 2	0.0012[c]				Early compared with Late Year 2	1.00
	Late	0.403	0.255	Year 1 compared with Year 2 Early	0.00032[c]	Late	1.4727	1.4725	Year 1 compared with Year 2 Early	<0.0001[c]
				Year 1 compared with Year 2 Late	<0.0001[c]				Year 1 compared with Year 2 Late	<0.0001[c]

[a]Year 1 = 2014 to 2015 samples.
[b]Year 2 = 2015 to 2016 samples.
[c]Significant differences observed at $\alpha \leq 0.05$ using a *t*-test.

Sensory & Food Quality

Table 3–Panelist preference for AS or SY Valencia oil in taste panels.

	Valencia early				Valencia late			
	MS 2014 to 2015 $n = 22$	MS 2015 to 2016 $n = 30$	OJ 2014 to 2015 $n = 31$	OJ 2015 to 2016 $n = 27$	MS 2014 to 2015 $n = 20$	MS 2015 to 2016 $n = 27$	OJ 2014 to 2015 $n = 27$	OJ 2015 to 2016 $n = 27$
Prefer AS	8 (36%)	9 (28%)	12 (39%)	12 (44%)	9 (45%)	12 (44%)	12 (44%)	14 (52%)
Prefer SY	14 (64%)	21 (72%)	19 (61%)	15 (56%)	11 (55%)	15 (56%)	15 (56%)	13 (48%)

Only panelists who correctly detected differences between AS and SY oils in a triangle test were asked a preference question.
MS, model solution; OJ, orange juice test solution.

content of SY oil was not consistently different from AS oil, but when it was significantly different, the percent aldehyde content was lower in SY oils. Historically, specific gravity increases with Valencia maturity and is highest in late season samples (Kesterson and others 1971). However, no previous studies could be found where the maximum specific gravity exceeds the USP maximum, as was measured in this study. While there were differences based on harvest time and harvest year for refractive index and UV absorbance, these differences were within USP specifications. Therefore, these differences show that the results of the USP physicochemical tests can vary based on these factors. No trend exists in these results, yet more extensive studies may reveal slight trends only obtainable with much larger sample sizes.

Descriptor:

Sweet:
AS Hamlin in MS (2014-2015 Early)
AS Hamlin in MS (2015-2016 Late)
AS Valencia in MS (2015-2016 Late)
AS Valencia in OJ (2015-2016 Late)

Sour:
SY Hamlin in OJ (2014-2015 Late)
SY Hamlin in OJ (2015-2016 Late)
SY Hamlin in MS (2015-2016 Late)
SY Valencia in MS (2015-2016 Late)

Bitter:
AS Hamlin in OJ (2015-2016 Early)
AS Hamlin in OJ (2015-2016 Late)
AS Valencia in OJ (2014-2015 Early)

Stronger Orange Flavor:
SY Hamlin in MS (2014-2015 Early)
SY Valencia in OJ (2015-2016 Early)

Stronger Aftertaste:
SY Hamlin in OJ (2014-2015 Early)
SY Hamlin in OJ (2014-2015 Late)

Figure 1–Correct panelist descriptor key word summary for taste panels.

Flavor panels

Flavor tests were performed by flavor panels on AS and SY oil in a model solution as well as in an orange juice test solution for both the 2014 to 2015 and 2015 to 2016 harvest years. There were no differences perceived between the flavor of AS and SY oils for any of the samples. Panelists were asked demographic questions including gender, orange juice consumption habits, and race. There were some differences in ability to identify the correct odd sample based on demographics. For gender, females picked the correct odd sample significantly more often than males ($P = 0.020$) for the Hamlin Early 2015 to 2016 model solution. There were consistently more females than males in this data set, and this was the only significant instance for gender. There is likely no overlying trend based upon gender. Orange juice consumption was a significant ($P = 0.034$) factor for the Valencia Early 2015 to 2016 model solution, with individuals who consume orange juice 2 to 3 times per month picking the correct odd sample significantly more often than other consumers of orange juice. Orange juice consumption was also deemed to be unimportant as an overall trend for discriminating SY and AS orange oils, as this was the only significant instance in this data set.

Race was a significant factor for the Valencia Early 2015 to 2016 model solution ($P = 0.021$) and near significant for Valencia Late 2015 to 2016 model solution ($P = 0.089$). For the Valencia Early 2015 to 2016 model solution panel, 47% of Asian panelists picked the correct odd sample in the triangle test while only 16% of White panelists picked the correct odd sample. For the Valencia Late 2015 to 2016 model solution panel, 46% of Black and 42% of Asian panelists picked the correct odd sample while only 16% of White panelists picked the correct odd sample. On average, 20% to 30% of panelists self-identified as Asian and 7% to 15% self-identified as Black. It is possible that Asian and Black panelists are true discriminators of AS and SY orange oils, particularly for Valencia model solution samples.

Panelists who correctly picked the odd sample were presented with a forced choice question where panelists chose which sample they preferred. Of the 8 Valencia panels (4 model solutions, 4 orange juice test solutions), panelists preferred the SY oil in 7 of 8 instances (Table 3). This trend shows a panelist preference for SY oil. No similar trend was seen in the Hamlin panels. Panelists who picked the correct sample were also given the opportunity to submit an optional freeform comment on any differences they perceived between the samples. A few trends emerged from these comments (Figure 1). Overall, 4 AS samples (Hamlin Early 2014 to 2015 model solution, Hamlin Late 2015 to 2016 model solution, Valencia Late 2015 to 2016 model solution, and Valencia Late 2015 to 2016 orange juice) were largely described by panelists as being sweet. Four SY samples (Hamlin Late 2014 to 2015 orange juice sample, Hamlin Early 2015 to 2016 orange juice sample, Hamlin Late 2015 to 2016 model solution, and Valencia Early 2015 to 2016 model solution) were largely described as being sour. Three AS samples (Hamlin Early and Late 2015 to 2016 orange juice, Valencia Early 2015 to 2016 orange juice) were largely described as being bitter. Two SY samples (Hamlin Early 2015 to 2016 model solution and Valencia Early 2015 to 2016 orange juice) were largely described as having strong orange flavor. Two SY samples (Hamlin Early and Late 2014 to 2015 orange juice) were described as having a stronger aftertaste. Overall, these comments suggest that AS samples may enhance perception of sweetness, especially for the model solutions and for 2015 to 2016 Late samples and more bitter, especially in the orange juice test solutions. SY samples may be more sour, especially for Hamlin and 2015 to 2016 samples. SY samples may also have stronger orange flavor as well as stronger aftertaste.

The results of our study, which indicate no significant differences for orange oil taste, differ from a previous study on orange juice (Plotto and others 2010). Plotto and others reported significant differences between the control and HLB positive affected juices. These differences are attributable to not only volatile but also nonvolatile compounds. Panelists in Plotto and others (2010) described HLB positive juice as being sweeter, but also more sour, fermented, and having an aftertaste. While no significant differences were found in our study, panelists who picked the correct odd sample did indicate that SY samples had a stronger aftertaste and were more sour. Unlike Plotto and others' (2010) orange juice samples, panelist comments indicated that AS orange oils may be more sweet than SY orange oils. While it is useful to look for similarities between orange juice and orange oil, ultimately these are 2 different substances with some shared similarities. No previous studies have investigated the effect of HLB on the taste imparted by citrus CP oil when added to orange juice or model solutions.

Aroma panels

Aroma panels were performed on AS and SY oil for the 2015 to 2016 harvest year. There were significant differences between AS and SY samples for Hamlin Early ($P = 0.01$) and Valencia Late ($P = 0.001$). There were also significant effects for the race demographic variable for Hamlin Early ($P = 0.040$) and near significant effects for Hamlin Late ($P = 0.054$) samples. For Hamlin Early oils, 75% of Asian panelists picked the correct different sample while only 42% of White panelists picked the correct different sample. For Hamlin Late oils, 33% of Asian and 30% of Black panelists picked the correct odd sample while only 18% of White panelists picked the correct odd sample. These results agree with the taste panel results, and suggest that Asian and Black panelists have better ability to discriminate between SY and AS samples. There were no significant effects for gender or orange juice consumption.

Panelists who picked the correct odd sample were presented with a forced choice question where they chose which sample they preferred. There was a trend for the aroma of AS samples being preferred over SY samples. For Hamlin Early and Late as well as Valencia Late, the aroma of AS samples was preferred. Panelists who picked the correct odd sample were also given the opportunity to submit an optional freeform comment on the perceived differences between the samples. Only comments from the panels with significant differences (Hamlin Early and Valencia Late) were analyzed. Panelists described SY samples for both Hamlin Early and Valencia Late as being more sour smelling. Valencia Late AS samples were additionally described as being sweeter smelling while Valencia Late SY samples were described as having a stronger aroma.

A previous study (Plotto and others 2010) found significant differences between the aroma of HLB unaffected (control) and HLB affected orange juices. The aroma of HLB affected Hamlin juices was described by panelists as being fresher, fruitier, and slightly fermented. Some HLB affected Valencia orange juices had aromas described as sour, off, or fermented (Plotto and others 2010). Our results also showed SY samples as smelling more sour, although none of the other descriptors from Plotto and others (2010) orange juice study were corroborated for our orange oils samples. Much like taste, while it is useful to look for similarities between orange juice and orange oil, ultimately these are 2 different compounds with some shared similarities. No previous studies have investigated the aroma of orange oil from fruit affected by HLB.

Conclusion

This study showed that there are differences in the USP mandated physicochemical properties of orange oils based on whether the oils come from fruit SY or AS for HLB, the harvest time, and the harvest year. All Hamlin oils had mean aldehyde contents below the USP minimum and Late AS Valencia oils had specific gravities above the maximum. Significant differences based on harvest year were seen for aldehyde content, refractive index, optical rotation, and UV absorbance. While none of these changes led to an oil being out of USP specifications, they indicate a need to monitor the quality of oil every year to ensure a consistent product.

There were no significant differences between AS and SY oils for flavor when oil was added to an orange juice or model solution. Race was a significant factor for ability to discriminate between AS and SY samples for 1 panel and near significant for a 2nd, and may warrant future investigation. Some trends emerged from the taste panels, including tendencies for discriminators to describe AS oil as sweet and bitter and SY oil as sour, stronger, and having an aftertaste in model solutions or orange juice test solutions. Additionally, there was a trend for discriminating panelists to prefer the taste of SY Valencia oil to AS Valencia oil.

Significant differences in aroma were observed between AS and SY samples for 2015 to 2016 Hamlin Early and Valencia Late oils. Race was also a significant factor in one of the aroma panels and near significant in a 2nd panel. There was a trend for panelists to prefer the aroma of AS oil. There was also a trend for discriminating panelists to describe AS Late Season Valencia oil as being sweeter smelling, SY Late Season Valencia oil as having a stronger intensity of aroma scent, and SY samples for both Hamlin Early and Valencia Late as being more sour smelling.

Overall, these results show that HLB can have an effect on the aroma and USP properties of Florida orange oils, although flavor may be unaffected by this plant disease. For such a devastating disease, we may have expected to see more significant quality defects based upon disease stage than those seen in this study.

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Author Contributions

Brittany M. Xu collected the data, interpreted the results, and drafted the manuscript. Charles A. Sims provided advice and expertise in the area of sensory analysis and edited the manuscript. Edgardo Etxeberria provided advice and expertise in the area of horticulture and edited the manuscript. Renée M. Goodrich-Schneider assisted in designing the study, edited the manuscript, and provided general expertise and advice.

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