A SENSORY EVALUATION OF CITRUS GREENING-AFFECTED JUICE BLENDS

By

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To my parents: for their unconditional love, support and encouragement

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Citrus greening is a devastating disease that kills citrus trees with major economic ramifications in the citrus industry. The main objective of this study was to determine if greening-affected juice could be utilized in the form of juice blends with healthy juice. Blending strategies (by juice and fruit mass) were also investigated in this study. Blending by juice mass could be preferred in small pilot plant scale projects while blending by fruit mass is feasible for large scale industrial juice processing.

This study examined the sensory impact of blends of greening-affected orange juice and healthy orange juice. Sensory evaluation tests were carried out on the University of Florida Campus with an untrained panel (n=60) performing triangle tests, difference-from-control tests and hedonic tests on the following treatments: 5% blend, 10% blend, 20% blend, 50% blend and control (0% blend). Panelists used a 10 point numerical category scale to rate the difference of blends from control and used a 9 point hedonic scale to assess the overall acceptability, sweetness and orange flavor among the blends.

Panelists were able to detect differences in juice blends (Valencia) when blended by juice mass at 10% levels with borderline significance at 5% levels. However,

panelists could not detect differences in juice blends (Valencia and Hamlin) when blended by fruit mass at 5% and 20% (borderline significance in Hamlin) levels. Panelists were able to detect a difference with the 50% blends (Valencia and Hamlin) from the control. In the hedonic tests, only the 50% blend (Valencia) was found significantly different from the control with the lowest sweetness among treatments. The results suggest that greening-affected juice can be blended by fruit mass at 50% levels or lower and be still acceptable to consumers. The 20% blending level or lower is recommended when blending by fruit mass, and the 5% blending level or lower is recommended when blending by juice mass with minimal risk of consumer detection in juice. This is relevant to the Florida citrus industry suffering from the economic impact of citrus greening, specifically as a way to add value to juice yield.

CHAPTER 1
INTRODUCTION

The orange is the world's most popular fruit with orange juice constituting a major portion of the food industry (Tetra Pak 2004, Kimball 1999). In 2005, about 59 million tons of oranges were produced worldwide (FAO 2006). This represents a 45% increase in orange production since 1970 (FAO 2006). As of 2005, 21.8 million tons of orange fruit were processed into orange juice with Brazil (11.9 million tons) and the United States (6 million tons) being the two largest orange juice producing countries in the world (FAO 2006). In the United States, orange juice is also the most popular fruit juice accounting for 60% of all fruit juice sales (Graumlich 1986; Jia 1999). Almost all the oranges produced in the United States come from Florida and California with the former accounting for about 75% of the total oranges (USDA 2007). In Florida, about 92% of all oranges produced are processed into single strength orange juice while 72% of all the oranges are sold as fresh fruit in California (USDA 2007).

The high demand for oranges and their extracted juice is due to their high nutritional value and desirable flavor (Jordan 2003; Polydera 2004). Orange juice is an excellent source of ascorbic acid (vitamin C), sugars (sucrose, fructose and glucose), minerals (potassium, magnesium, calcium), bioactive compounds such as carotenoids (β-carotene, α-carotene, β-cryptoxanthin) and flavanones (hesperidin, narirutin), which have antioxidant properties (Topuz 2005, Plaza 2011, Niu 2008). The daily recommended intake of ascorbic acid is 75 mg for adult women and 90 mg for adult men with insufficient uptake of ascorbic acid leading to scurvy, a disease characterized by bleeding gums, impaired wound healing, anemia, fatigue and depression (Phillips 2010). Regular consumption of orange juice, which contains about 40 mg of ascorbic

acid per 100 ml helps to reduce the risk of the occurrence of scurvy (Norman and Clein 1956). Some research has also shown that bioactive antioxidants found in orange juice have been implicated in the reduction of degenerative human diseases such as cancer (Steinmetz 1993, Slattery 2000). In addition to the nutritional benefits, orange juice has a unique, delicate and desirable taste with over 200 flavor compounds in proper concentration (Jia 1999). While sugars and citric acid are major contributors to the sweetness and sourness of orange juice, a combination of other compounds such as acetaldehyde, citral, ethyl butyrate, d-limonene, linalool and octanal, also contribute to the unique flavor of orange juice (Ahmed 1978_a, Jia 1999).

Although there has been a strong growth in orange juice production worldwide over the past thirty years, the United States has been experiencing a steady decline in demand for orange juice with annual per capita consumption dropping to its lowest point of 4 gallons in the past 11 years (ERS 2011). A part of the decline has been attributed to the rise of low-carbohydrate diet packages such as the Atkins and South Beach diets which categorize orange juice as a high carbohydrate food and may have affected consumer demand (Love 2006). Other causes of the decline include adverse weather conditions and impacts of diseases which reduced overall juice production (ERS 2011). Oranges subjected to freezing conditions are frequently unsuitable for consumption due to the off-flavors generated, the formation of white spots on the fruit surface and the dehydration of fruit (Milliken 1919, Slaughter 2008). Ice crystals grow within the fruit damaging the cells and creating pathways for moisture loss and fruit dehydration (Slaughter 2008). Since 1835, Florida has periodically experienced severe freezes, which have negatively affected the total citrus fruit yield (FCM 2007, Reuters 2010). In

addition to freezes, Florida has also experienced hurricanes (most recently Hurricane Charley, Frances and Jeanne), which have not only damaged crops and trees but also aided the spread of citrus diseases such as citrus canker (Irey 2006). *Xanthomonas citri subsp.* is the plant pathogenic bacterium that causes citrus canker and spreads primarily by wind and rain (Bock 2005). Citrus canker manifests as necrotic lesions on the fruit, leaves and stems of citrus plants with severe infection causing premature fruit drop, blemished fruit and twig dieback (Schubert 2001, Bock 2005).

While citrus canker has long plagued Florida since 1912 with periodic eradications (in 1930 and 1992) and subsequent returns (in 1986 and 1995), it has been usurped in severity and importance by citrus greening (Gottwald 2007_a). Citrus greening also known as huanglongbing, is a bacterial disease that is widely regarded as the most severe and devastating disease of citrus (Gottwald 2007_a, Batool 2007, da Graca 2008, Lin 2008, Tatineni 2008, Shokrollah 2010). The major reasoning behind the shift in focus from citrus canker to citrus greening by researchers is that the later is fatal to citrus crops upon infection as opposed to the former which gradually debilitates the tree (Schubert 2001, Bove 2006). In addition, while citrus canker is primarily a cosmetic injury with lesions that do not penetrate the albedo of the fruit and thus do not affect the quality of the juice (Stall 1983, Dewdeney 2011), citrus greening negatively alters the quality of juice resulting in little or no commercial value of the citrus fruit (Bassanezi 2009, Dagulo 2010). Furthermore, the novelty of citrus greening in Florida (first detected in 2005), and the overall uniqueness of the disease which includes the latency of symptom development, difficulty in isolating in culture and overall potential economic

impact on orange juice production most especially as there is no cure, are all a major cause of concern to the Florida citrus industry (Gottwald 2007_b, Lin 2008).

There is therefore a great incentive to study citrus greening. The general purpose of this thesis research was to explore the utilization of juice from greening affected fruit. Citrus greening, like citrus canker, is not pathogenic to human beings and the lack of consumption or commercialization of greening affected juice is usually due to its poor quality (Dagulo 2010). Specifically, this study will investigate the possibilities of blending healthy juice with greening-affected juice at 5% and 10% weight levels.

CHAPTER 2
LITERATURE REVIEW

Biological and Physical Characteristics

The orange is a spherical shaped fruit that grows best in warm climates from 40 ºN to 40 ºS of the world (Braddock 1999, Spiegel-Roy 1996). Citrus fruit are composed of an outer flavedo layer which showcases the exterior fruit color, as well as containing sesquiterpene oil sacs (Kimball 1999). Beneath this flavedo layer is the albedo layer, which is a white spongy layer that allows the absorption of water and oil (Braddock 1999, Kimball 1999). Both the flavedo and albedo serve to protect the fruit from insects and microorganisms (Kimball 1999). The albedo is a rich source in pectin, carbohydrates, and flavanone glycosides (Braddock 1999).

Beneath the albedo are the fruit sections which are portioned by membrane material with each section containing juice vesicles aligned to the core of the fruit (Kimball 1999). Each juice vesicle contains cells that consist primarily of enlarged vacuoles of juice (Kimball 1999). The juice cell also contains mitochondria, which act through the Kreb's cycle to produce citric acid, constituting over 90% of the organic acid content in juice (Canel 1996, Kimball 1999). Citric acid accumulates in the juice vacuole during maturation of the fruit with high concentrations in the early season fruit and lower concentrations as water and sugars accumulate in the fruit (Bain 1958, Kimball 1999). Water and sugars that accumulate in the juice vacuole come from tree sap and continue to increase during maturation (Kimball 1999).

Citrus Fruit

Citrus belongs to the Rutaceae family with the sweet orange variety (*Citrus sinensis* (L.) Osbeck) regarded as the most important class of commercial citrus grown

(Kimball 1999). Sweet oranges are spherical in shape with an average diameter of 5.7-9.5 cm (Braddock 1999). Sweet oranges are extensively used for fresh fruit around the world although Brazil and the United States utilize them primarily for juicing. About two-thirds of all sweet oranges fall into the common orange cultivar with the rest of the oranges falling into navel oranges, blood oranges and acidless oranges. The harvesting seasons for different varieties overlap allowing citrus processors to operate continuously for eight to nine months each year (Braddock 1999). In Florida, common oranges varieties Hamlin (early season variety), Pineapple (mid season variety) and Valencia (late season variety) account for major orange juice production (Cameron 2000). Hamlin variety has less cloud, a paler color and weaker flavor compared with either Pineapple or Valencia varieties albeit not statistically different (Attaway 1972, Huggart 1975).

Valencia oranges, due to their superior flavor and color are grown primarily for juicing (Zanzig 1999, Kimball 1999). Valencia fruit require about 14-16 months to mature, with harvests usually from February to June depending on crop size, fruit maturity and processing conditions (Tetra Pak 2004). This long growing season allows for Valencia oranges to be affected by winter freezes unlike Hamlin oranges which are harvested from October to January (Cameron 2000, Tetra Pak 2004). Valencia oranges and citrus in general, are also prone to many diseases of the leaves, roots, wood and fruit (Manner 2006). The most severe of these pathogenic diseases is citrus greening.

Citrus Greening

Citrus greening or ‘huanglongbing’ is a devastating disease affecting citrus plants and fruit, eventually leading to the death of the trees (Albrecht 2008). Citrus greening is caused by endogenous, sieve tube restricted bacteria known as *Candidatus* Liberibacter spp. and is vectored from tree to tree by citrus psyllid vectors (Bove 2006).

Only two psyllid vectors have been identified and they are *Diaophorina citri* in Asia and America, and *Trioza erytreae* in Africa (Bove 2006). There has also been some confusion as to whether there exists three 'species' of *Candidatus* Liberibacter named after the three locations spotted, i.e., Ca. L. asiaticus in Asia (da Graca 1991), Ca. L. africanus in Africa (Jagoueix 1994), Ca. L. americanus in South America (Teixeira 2005) or whether there was an adaptation of the one species into new hosts and environments. There is an agreement that all three species spread from their natural asymptomatic hosts to the symptomatic citrus and citrus relatives. However, of the three species, Ca. L. africanus has been identified with a natural host and natural vector, Ca. L. asiaticus has been identified with a natural vector and no natural host while Ca. L. americanus has not been identified with either a host or a vector (da Graca 2008). There is no known control for this disease other than prevention by removal of already infected trees (Bove 2006). In addition, infected citrus trees have a latency period of 6-12 months which impedes effective removal of all symptomatic trees (Bove 2006).

History

Citrus greening was a severe problem in 18th century India where it was called 'dieback' (Gottwald 2007$_a$). Similar problems associated with a decline in citrus were also said to have been noted in 19th century Assam, in India, and by 1912 another similar problem in both symptom and severity was noted in Bombay India (Gottwald 2007). Although this was indexed by Capoor (1963) to be possibly due to Citrus Tristeza virus, Raychaudhuri et al (1969) confirmed that it was indeed citrus greening (Gottwald 2007$_a$, da Graca 2008). In 1919, the disease was identified in southern China and described by farmers in the region as huanglongbing (or yellow shoot) disease (Bove 2006).

From the 1920's citrus greening was reported in several Asian countries. In the Philippines, mottle leaf disease was reported (Gottwald 2007$_a$). In Taiwan, it was known as "Likubun" which means 'decline' and in Indonesia it was reported as citrus vein phloem degeneration (CVPD) (Garnier 1993, Gottwald 2007$_a$). In 1947, Huanglongbing was first reported in South Africa which was similar to the now documented disease in 1943 in Southern China (Tsai 2008). In some parts of South Africa, it was known as the "yellow shoot" disease, while in some other parts it was referred to as "greening" because of the green color of the symptomatic fruit (Gottwald 2007$_a$). It was only in 1995, when the International Organization of Citrus Virologists held a congress in China to officially use the name 'Huanglongbing' to refer to this disease (Gottwald 2007$_a$).

There is a possibility that citrus grown as a staple in India for over 4000 years became infected and was brought into China along the sea trade route (da Graca 2008). From China it may have spread across to other regional countries such as Philippines, Taiwan, and Indonesia either by directly being taken there or through infected propagation and insect transmission (da Graca 2008). An alternate theory proposed by Beattie et al (2006), places Africa as the origin of the disease, possibly through an asymptomatic host such as *Verpris lanceolata* (Gottwald 2007$_a$). It could possibly have been transmitted by an insect to citrus in European settlements in Africa, and then could possibly have been brought to India through infected plants or budwood 300-500 years ago (Gottwald 2007$_a$). According to Beattie et al (2006), this would provide some explanation as to why this disease suddenly appeared in India some 300-500 years ago although, as earlier stated, citrus has been established in India for close to 4000 years (Gottwald 2007$_a$).

Previously it was thought that huanglongbing (from now HLB), was as a result of physiological disorders such as mineral deficiencies, or water logging, or due to soil-borne diseases such as nematode infestation or *Fusarium* infection (Bove 2006). However, all those theories were later jettisoned as it was found by the researcher Lin Kung Hsiang who carried out some surveys in Southern China in 1956 that HLB is a graft transmissible infectious disease (Bove 2006). As it became clear that HLB was spreading due to graft inoculation, there was also some evidence found that HLB could be spreading through vectors (Bove 2006). In South Africa, it was also shown that HLB was spreading through grafting, but more importantly a citrus psyllid, *Trioza erytreae* was identified (Gottwald 2007_a). Soon another citrus psyllid, *Diaphorina citri*, was identified as another vector of HLB in India and the Philippines (Gottwald 2007_a).

The occurrence of HLB in India, China, and South Africa has posed a serious threat to regional countries that are still free from this disease (Bove 2006). Some of the countries in Africa that have cited cases of HLB include Burundi, Cameroon, Central African Republic, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Nigeria, Reunion, Rwanda, Somalia, South Africa, Swaziland, Tanzania, and Zimbabwe (USDA 2006, Floyd 2006). In Asia, some countries that have cited cases of HLB include Bangladesh, Bhutan, Cambodia, China, East Timor, India, Indonesia, Japan, Laos, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Philippines, Saudi Arabia, Taiwan, Thailand, Vietnam, and Yemen (USDA 2006, Floyd 2006). Recently Iran reported its first occurrence of HLB (Faghihi 2008). This was most likely in line with the high populations of *Diaphorina citri* found in the citrus plantations of Hormozgan and Kerman

provinces of Southern Iran (Bove 2006). In 2004, HLB was detected in Sao Paolo, Brazil (Teixeira 2005). In 2005, HLB was detected in Florida, USA (Bove 2006).

Biology

Citrus Greening (HLB) is named '*Candidatus* Liberibacter spp.' and belongs to the α subdivision of proteobacteria (Jagoueix 1994). HLB received the '*Candidatus*' designations according to rules established by the International Committee on Systematic Bacteriology about uncultured organisms (Murray and Stackenbrandt 1995). However, the generic 'Liberobacter' name chosen by Murray and Stackenbrandt (1995) was changed to 'Liberibacter' following the rules of the International Code of Nomenclature of Bacteria (Garnier 2000, Wang 2006). HLB is a fastidious, phloem limited, gram negative bacteria which inhabits the phloem of citrus plants (Jagoueix 1994, Chiou-nan 1998). HLB causing bacteria has not been cultured on artificial media and is present in very low titers in the host (Jagoueix 1994, Duan 2009). HLB pathogen DNA to host DNA ratios have been shown to be 1:1000 in terms of target copies and 1:13000 by mass (Li 2006).

As mentioned earlier, three forms of the HLB causing bacteria have been found '*Candidatus*. Liberibacter asiaticus', '*Ca*. L. africanus', and '*Ca*. L. americanus'. In South Africa, the Liberibacter africanus and its natural vector, *T. erytreae*, occur in cool areas of Swaziland and Transvaal, but not in hot areas of Swaziland and Transvaal (Bove 2006). This suggests that Liberibacter africanus and *T. erytreae* are heat sensitive. However, in Asia, Liberibacter asiaticus and its natural vector, *D. citri*, are found in areas of hot low altitudes (Bove 2006). This could be seen in the Ningnan county of Sichuan (South China), where 100% of the trees were infected with HLB at altitudes of 1090 to 1200 m above sea level, but only 3% of the trees were found to be infected with

HLB at altitudes of 1385-1620 m above sea level (Bove 2006). This suggests that Liberibacter asiaticus and *D. citri* are heat tolerant. In Sao Paulo, though Liberibacter asiaticus was found in about 10% of HLB affected trees, the remaining 90% of affected trees was due to a new specie, Liberibacter americanus (Bove 2006). The only reported citrus psyllid in Sao Paulo is *D. citri*, which suggests that it possibly spread both Liberibacter asiaticus and Liberibacter americanus (Bove 2006). Also it has been shown that Liberibacter americanus survives in both cool and warm temperatures which suggest it is heat tolerant.

In Florida, *D. citri* was reported in 1998, and HLB from the species Liberibacter asiaticus was subsequently observed in 2005 (Bove 2006). All three species of HLB have shown virtually indistinguishable symptoms in the citrus plant (USDA 2006). In addition, all citrus plants are viable hosts for HLB (USDA 2006). The more susceptible hosts include sweet oranges, tangelos, and mandarins while less susceptible hosts include grapefruits, lemons, rangpur lime, calamondins, and pummelos (USDA 2006). Non-citrus species such as *Murraya paniculata* can also serve as pathogen hosts (USDA 2006).

Spread

HLB can be spread by grafting with diseased budwood (USDA 2006, Wang 2006). This involves utilizing an infected bud and inserting it into a healthy rootstock branch. HLB can also be transmitted through vectors (citrus psyllids) of which two, *T. erytreae* and *D. citri*, have already been identified. As already stated earlier, *T. erytrea* is the primary vector for the African form and *D. citri* is the primary vector for the Asian form. It is being suspected that *D.citri* is also the vector for the Liberibacter americanus (USDA 2006).

D.citri is found only on citrus (crops and ornamentals) and closely related Rutaceae (citrus family) (USDA 2006). *D. citri* can also be found on *Murraya paniculata*, an ornamental rutaceous plant known as jasmine orange (USDA 2006). Adult Asian psyllids are small (3 to 4 mm) with yellowish brown bodies and grayish brown legs. Adults also have mottled brown wings and tend to live up to 6 months (Davis 2005). Adults are hemipteran insects with piercing sucking mouthparts that allow it to feed on the phloem of citrus or other rutaceous plants. Psyllids should not be confused with aphids which are similar in size and affect young citrus leaves, as psyllids are more active than aphids, and fly from shoot to shoot upon any disturbance (Davis 2005). Eggs are bright orange, flattened, oval and are deposited on newly emerging citrus tissue (USDA 2006). Nymphs are yellow or brown and feed on leaves and stems (USDA 2011). Adults feed in a slanted position, with their heads down almost touching the surface, and their rear up at an angle (Davis 2005). The Asian citrus psyllid is most likely to be found on shoots and its population increases during periods of active plant growth (USDA 2006).

T. erytreae is physically very similar to *D. citri* with a slight difference in color as it is black (Pena 2002). Adult African psyllids eggs are oblong in shape, yellow when laid but turn brownish (Pena 2002). The eggs are laid on the edges or main veins of young leaves anchored to the leaf blade by short appendage (Pena 2002). While female Asian psyllids lay up to 800 eggs and live for 3 to 4 months, the female African psyllids lay roughly 600 eggs and live for only 1 month (Pena 2002). There are also slight differences in life cycles with the African psyllid having a slightly longer life cycle (6 weeks) compared to the Asian psyllid (4 weeks) (Pena 2002).

Diagnosis

Historically HLB has been detected primarily by electron microscopy (Folimonova 2011). However, the difficulty in isolating and culturing HLB has hampered efforts to understand its biology and mechanism (Sagaram 2009). There has only been one recorded report of cultivation of HLB which used a medium known as Liber A which contains monobasic and dibasic potassium phosphate, NADP and citrus vein extract (Sechler 2008). Sechler (2008) inoculated the bacteria growing on this medium in young citrus plants and observed similar symptoms with blotted brown leaves. Colonies became visible on Liber A after 3 to 4 days at 28 C and were irregularly shaped and ranged in size from 0.1-0.3 mm (Sechler 2008). However, as these plants were young and unable to bear fruit, there is some uncertainty that the bacterium was in fact HLB (Sechler 2008).

Due to this limitation of cultivation methods, other methods of analysis have been employed in detecting and characterizing HLB. One method involves the use of monoclonal antibodies on different HLB strains to differentiate several serotypes among HLB species (Gao 1993, Hocquellet 1999). The first molecular technique was a DNA hybridization method (Teixeira 2008). The DNA probe As-1.7 recognizes Liberibacter africanus (Planet 1995) while In-2.6 and In-1.9 recognize Liberibacter asiaticus (Villechanoux 1992). However, polymerase chain reaction (PCR), a DNA-based method that involves amplification of 1160 bp fragment of the liberobacter 16S rDNA gene is used most frequently to characterize HLB strains (Jagoueix 1996). PCR has gained popularity compared to other methods due to its simplicity, sensitivity and reliability (Tatineni 2008). Comparisons of the 16S rDNA (Jagoueix 1994) and 16S/23S intergenic region (Jagoueix 1997) have confirmed that HLB is a gram negative

bacterium and more precisely of the alpha subdivision of Proteobacteria (Teixeira 2008). Liberibacter has been tested by conventional PCR as well as nested PCR (Teixeira 2008) and real-time PCR (Li 2008). Li (2008) has shown that real time PCR is at least a 100 fold more sensitive than conventional PCR at detecting 16S rDNA copies from HLB per reaction. Teixeira (2008) has also shown that nested PCR has a similar sensitivity to real-time PCR, both being as much as 1000 times more sensitive than conventional PCR albeit all tested positive for HLB. Although HLB quantified in mottled leaves can amount to 10^7 liberobacter per gram of mottled leaf (Teixeira 2008), it has been shown that HLB is unevenly distributed in the citrus tree and can thus go undetected if the organ being assessed by PCR does not contain any bacteria (Tatineni 2008). Tatineni (2008) found HLB in floral parts (petals, pistils and stamens) despite no obvious symptoms as well as in fruit parts (peduncles, seed coats, and collumella). Suggested organs for detection include the fruit parts with the largest HLB population, followed by the bark, the roots and the leaf midrib (Tatineni 2008). These suggested organs indicate that HLB moves within the phloem direction (Tatineni 2008). Citrus psyllids feed on the phloem from the foliage, simultaneously transmitting the HLB pathogen into the citrus plant. There is some considerable damage done on the leaves which are left pitted, with pits opening to the lower leaf surface (Pena 2002). In severe attacks, the leave blades are cupped, distorted and turn yellow especially when young (Pena 2002).

Symptoms

At an early stage of infection, the citrus shoots take up a characteristic yellow color, and it was consequently identified as ‘huang’ ‘long’ which means “yellow” “shoot” by Chinese farmers (Bove 2006). Sometimes the citrus plant may have only one yellow

shoot which eventually grows into a larger yellow branch (Bove 2006). Although Bove suggests this to be a reason why it was referred to by other researchers as yellow ‘dragon’, because ‘long’ also translates to ‘dragon’, it seems reasonable to suggest that it was merely a translation error into English. This has been confirmed by the Chinese farmers of the Chaosan District of Southern China where HLB was observed as their original and correct intent of the word (Gottwald 2007_a).

At later stages of infection, these yellow branches grow and wrap themselves around the tree eventually canopying the whole tree (Bove 2006). At that point the tree can be said to be fully infected (Bove 2006). In addition to the yellow shoots, the leaves also take a mix of yellow, green hue with no sharp limits between each color (Bove 2006). This is known as “blotchy mottle” and is most characteristic symptom of HLB in all three continents observed (Asia, Africa, Americas) (Bove 2006). The blotchy mottled leaf can be distinguished from mineral deficiencies such as zinc, iron, magnesium, manganese and calcium, which also induce yellow flushes on leaves, by the asymmetry of the blotching in the blotchy mottled leaf (Bove 2006). Generally, yellowing of a leaf by mineral deficiencies is symmetrical on the leaf, while HLB is asymmetrical and irregular (Bove 2006). With enough time the whole leaf may turn yellow (Bove 2006).

In addition to the blotchy mottle, the infected citrus leaves may also become large and leathery with swollen lateral veins (Bove 2006). Eventually, this leads to defoliation and dieback (Bove 2006). Blotchy mottle is usually seen to affect trees with large and healthy leaves (Bove 2006). Although it is the most recognizable characteristic, it sometimes can be difficult to locate especially in cases in later stages with uniform yellowing on the leaves (Bove 2006). In some cases, HLB occurs simultaneously with

other citrus diseases/symptoms and as such this could only add to difficulty in assessing (Bove 2006). Blotchy mottle is best observed on sweet orange trees; however, most citrus species show it including some mandarin varieties such as Tejakula Bali and Clementine in South Africa (Bove 2006).

There are also some fruit symptoms associated with HLB although not specific to HLB, and could be found in stubborn disease (Bove 2006). Symptomatic fruits are usually small, lopsided, asymmetric, and with a bent fruit axis (Bove 2006). The fruit is also poorly colored with the peduncular end of the fruit turning yellowish orange, while the stylar end of the fruit turns pale green (Bove 2006). Also when pressure is exerted with a finger, a silver mark appears on the rind (Bove 2006). Seeds which are brownish black in color are also often aborted in the symptomatic fruit (Bove 2006). Although this can be greatly associated with HLB, it can also be associated with stubborn disease (Bove 2006). These fruit symptoms are more readily noticed in sweet oranges, mandarins and pummelos (Bove 2006).

Juice Flavor Impact

Orange juice is flavor is among the most popular fruit beverage flavors in the world (Shaw 1993, Selli 2004). Orange juice flavor is also the most delicate and complex of citrus flavors (Shaw 1993). Although the sweetness of the sugars and sourness of the organic acids impacts flavor characteristics in orange juice, its fresh and unique flavor is due to the complex combinations between volatile aroma compounds that have an interdependent and quantitative relationship (Kimball 1999, Selli 2004). To further understand the complex nature of orange juice flavor, it is essential to accurately quantify as many volatile compounds as possible (Moshonas 1994). Other factors that affect the flavor of the orange juice are the different proportions of the volatile

compounds (Shaw 1979), the taste thresholds of volatiles (Patton 1957), and synergistic effects between volatiles (Shaw 1980) (Nisperos-Carriedo 1990). Shaw (1977) identified over 150 volatile compounds in orange juice or in flavor fractions derived from orange juice (Ahmed 1978_b). Johnson (1996) identified over 200 volatile compounds in the juice of sweet oranges (Selli 2004). The volatile compounds present in fresh orange juice originate from either the juice contained in the juice sacs during extraction, or from the two oil sources (peel and juice) (Hui 2010). Peel oil is found primarily in oval shaped sacs in the flavedo of the peel and its constituents include d-limonene (about 90 percent), a sesquiterpene, with other monoterpenes and sesquiterpenes in trace amounts (Kimball 1999). D-limonene, b-myrecene and α-pinene are the major constituents of peel oil (Ahmed 1978_c). There is also oil found in globular bodies in juice sacs get dispersed in juice during extraction (Davis 1932, Hui 2010). Valencene is the second most abundant terpene after d-limonene found in orange juice, particularly juice oil (Maarse 1991, Elston 2005, Dagulo 2010)

The flavor compounds in orange juice are 0.02% of its weight and important contributors to orange flavor are hydrocarbons (75-89%), aldehydes (0.6-1.7%), esters (1%), Ketones (1%) and alcohols (1-5%). (Nisperos-Carriedo 1990, Jia 1998, Selli 2004). In addition, only a small fraction of volatile compounds present in food have an primary impact on the aroma and flavor (Qiao 2008). Ahmed (1978) identified acetaldehyde, citral, ethyl butyrate, limonene, linalool, octanal and α-pinene as major contributors to orange juice. There is some disagreement about the contribution of D-limonene to orange juice flavor. Kimball (1999) has suggested that D-limonene acts as a carrier of flavors than as an actual contributor itself. However, Fan (2009) reports that

d-limonene actually has a citrus like aroma. B-myrcene is the second most abundant terpene in free form after d-limonene and contributes a lemon-musty, flavor to orange juice (Fan 2009). Linalool and Octanal are responsible for fruity flavors and herbal notes respectively (Rega 2003). Ethyl butyrate has a fruity, sweet aroma while α-pinene has a fruity and piney aroma (Hognadottir 2003).

The progress in the quantification of key volatile compounds has been largely dependent on available analytical methods (Nisperos-Carriedo 1990). Gas chromatography (GC) analysis is a valuable method to detect flavor compounds in orange juice (Qiao 2008). Prior to analysis, volatile compounds are isolated, extracted and concentrated, adsorbed and separated in a capillary column before purification (Lindsay 1996). There are four main types of GC analysis methods that vary in the transfer of samples into the column and they include direct injection, static headspace, dynamic headspace and solid microextraction (Snyder 1988, Jordan 2005). The direct injection method consists of using a gas syringe to extract aromas from the juice into the GC injector (Reineccius 2006). This method was used by Schreier (1977) to quantify 39 volatile compounds that are primary contributors to orange juice from a single sample (Moshonas 1994).In addition, Moshonas (1987) quantified 24 volatile constituents from one sample each of Valencia and Temple oranges also using direct injection gas chromatography (Moshonas 1994). While the direct injection method has been used successfully, it uses a low amount of gas sample which ensures only volatiles of a sufficiently large concentration are analyzed (Reineccius 2006, Grodowska 2010). Hence, the static and dynamic headspace methods were introduced to capture more volatiles of lower concentration. The static headspace method involves the

establishment of a thermodynamic equilibration of volatiles with an inert gas above the sample enclosed in a vial (Wu 1998). An aliquot of the equilibrated headspace is then injected into the gas chromatography system (Wu 1998). Similarly, dynamic headspace uses a carrier gas, to purge the volatiles in the headspace unto an adsorbent or cryogenic trap (Nielsen 2010). The cryogenic trap is less sensitive than the adsorbent trap and captures most headspace vapors (Nielsen 2010). However, the disadvantage is that water vapor is usually the most abundant head space vapor and is captured in large amounts in a cryogenic trap (Nielsen 2010). Nisperos-Carriedo (1990) quantified 20 volatiles from 15 fresh orange juices and 14 processed orange juice products using static headspace GC analysis. Moshonas and Shaw (1992) also compared static and dynamic headspace GC to quantify 16 volatiles in four fresh orange juice samples (Moshonas 1994). Also static headspace analysis was used by Shaw (1993) to quantify 19 volatiles from another set of four fresh orange juice samples (Moshonas 1994). Although static headspace GC analysis is successful at quantifying compounds and relatively easy, it is not effective in measuring the more volatile orange juice compounds such as octanal and α-pinene (Cadwallader 1994). Therefore, dynamic headspace or purge and trap GC analysis was the considered. Moshonas (1994) quantified 46 volatile constituents from 13 samples of both hand extracted and mechanically extracted orange juice using dynamic headspace GC analysis. Moshonas (1997) also assessed the quantitative and qualitative differences between freshly squeezed juice and pasteurized juice using dynamic headspace GC analysis and subsequently quantifying 46 volatiles that were not significantly different in either of the juices.

A relatively new method of GC analysis called solid phase microextraction (SPME) technique (Zhang 1993, Jia 1998). Solid phase microextraction is a solvent free sample preparation technique where a fused silica fiber coated with polymeric organic liquid is immersed in the headspace above the sample to adsorb the volatiles by concentration on the coating, and consequently to be transferred to a GC instrument for desorption and analysis (Zhang 1993). The principle behind SPME is the equilibrium partitioning of flavor compounds between the headspace and the coated fiber, and this mainly depends on the heating time, temperature, sample volume and volatile headspace concentration (Jia 1998). The lack of sample preparation and solvent extraction makes SPME GC analysis simple, fast, low cost and portable compared to dynamic headspace, liquid-liquid simultaneous extraction and distillation, and traditional static headspace (Zhang 1993, Jia 1998, Bazemore 1999). SPME has been applied to orange juice by several researchers (Yang 1994, Jia 1998, Bazemore 1999). It has been shown that an increase in temperature of orange juice amounts to a decrease in absorption of volatile compounds on the coated fiber (Jia 1998).

There has been some confusion as to flavor of greening-affected juice. Early reports by Mclean and Oberholzer (1965) of greening affected juice described its flavor as ‘poor’ and ‘bitter’ (Dagulo 2010). Plotto (2008) reported that a consumer panel could not detect the difference between asymptomatic and healthy juice, albeit an expert panel noted the former as being sweeter than the later. This sweetness was largely due to a lower acidity and a higher ºBrix/acid ratio than healthy juice (Plotto 2008). ºBrix levels were lower in asymptomatic juice than in healthy juice albeit non significant (Plotto 2008). In addition, asymptomatic juice from late harvest (Valencia) had higher

levels of acetaldehyde, methanol, α-pinene, and 2-methylpropanol than healthy juice (Plotto 2008). Dagulo (2010) however reported that ºBrix levels in symptomatic juice were significantly lower than healthy juice and acidity levels in symptomatic juice were significantly higher than healthy juice. Dagulo (2010) also confirmed that the ºbrix/acid ratio in asymptomatic juice from a late harvest (Valencia) were higher than healthy juice. However, ºBrix/acid ratios of asymptomatic juice were in general similar to healthy juice (Dagulo 2010). Dagulo (2010) reported symptomatic juice had lower level of esters particularly ethyl butyrate than control juice and a higher level of terpenes such as α-pinene and myrcene, alcohols like linalool and aldehydes such as hexanal and nonanal. Symptomatic juice also has lower valencene levels than control and asymptomatic juice (Dagulo 2010). Valencene has no aroma activity (Elston 2005) and is used as a marker for fruit quality/maturity because its concentration increases as fruit matures (Sharon-Asa 2003, Dagulo 2010). This suggests that greening-affected fruit might not have matured in a normal way (Dagulo 2010).

As greening-affected juice has often been described as ‘bitter’, Dagulo (2010) investigated the presence of bitter flavanone glycosides to see if it was responsible for the bitterness. Flavanone glycosides are a specific form of flavonoids found in citrus juices and are widely used as a differentiation of species, varieties and in cases of juice adulteration (Coffin 1971, Mouly 1994, Mouly 1998). Flavonoids are yellow pigments found widely in nature and are the most important pigments in nature along with carotenoids and tetrapyrrole derivatives (Ooghe 1994). Among the common flavanone glycosides are hesperidin, narirutin, naringin and neohesperedin (Mouly 1998). Hesperidin and narirutin are found in sweet oranges and are tasteless (Rapisarda

2003). Naringin and neohesperidin are not found in sweet oranges (Rouseff 1987) and cause bitterness in grape fruits (*Citrus* × *paradisi* Macfad.) and sour oranges (*Citrus aurantium*), where they are primarily found (Kometani 1996, Dagulo 2010). Dagulo (2010) confirmed that no concentration of naringin or neohesperidin was found in either the control, asymptomatic or symptomatic juices. However, as expected, narirutin, hesperidin and didymin were found in all the juices albeit not contributing to the overall flavor (Dagulo 2010). Dagulo (2010) also investigated the presence of polymethoxylated flavones which have been reported by Swift (1965) to impart bitterness in juice. Polymethoxylated flavones are mainly found in the flavedo portion of the peel and corresponding peel oil (Chen 1997, Green 2007, Dagulo 2010). The most common polymethoxylated flavones are nobiletin, sinensetin and tangeretin (Braddock 1999). Dagulo (2010) found that polymethoxylated flavones were far below their bitterness thresholds in the control, asymptomatic and symptomatic juices. Dagulo (2010) then investigated the impact of limonin on the control, asymptomatic and symptomatic juices. Limonin is a member of a group of triterpenoid compounds known as limonoids (Hasegawa 1982). Limonin although intensely bitter is found largely as a non-bitter limonin precursor in oranges in juice sacs known as limonoate A-ring lactone (LARL) which converts to limonin under acidic conditions and heat after extraction, and is accelerated under the effects of limonin D-ring lactone hydroxylase (Hasegawa 1982, Abbasi 2005). This is known as delayed bitterness and is occasionally observed in sweet orange (*Citrus sinensis*) (Abbasi 2005, Dagulo 2010). The concentration of LARL decreases as fruit matures, and is often used as a marker for fruit maturity (Fong 1992). In addition, as fruit matures, some of LARL is converted to limonin 17-β-D-

glucopyranoside (LG), another tasteless compound, leaving less LARL to convert to limonin (Fong 1992, Dagulo 2010). Dagulo (2010) found that limonin concentrations were higher in symptomatic juice than in control juice. Dagulo (2010) suggested the possibility of the conversion of LARL to LG being inhibited or delayed in symptomatic fruit, giving the appearance of fruit immaturity. Gaudagni (1973) reported the threshold level of limonin to be 6.5 µg/ml. Dagulo (2010) found that the threshold levels in symptomatic juice to be 2.41 to 5.41 µg/ml which is lower than what an 'average' person would detect as bitter, albeit bitter sensitive people might find symptomatic juice bitter. In addition, nomilin is a limonoid found in the seeds of oranges, lemons, and in the vesicles of grapefruit (Rouseff 1982). Nomilin is reported to be twice as bitter as limonin (Rouseff 1982). Similar to limonin, nomilin is at its highest concentration in the early parts of the season and drastically reduces as the fruit matures (Rouseff 1982). Baldwin (2010) reported that nomilin concentration is high in symptomatic juice albeit not above the taste threshold.

Control

HLB has no known cure. When it was found that HLB was a bacteria and not a virus, injections of infected trees with tetracycline were tried in some countries such as South Africa, Taiwan and Indonesia, but was soon disbanded not only for ecological reasons but also because tetracycline is bacteriostatic, only limiting the growth of the bacteria, rather than bactericidal which would kill the bacteria outright (Bove 2006). In addition to the aforementioned reasons for the disbandment, tetracycline had to be applied yearly rather than just once. Similarly, treatment with rolitetracycline only reduces symptom expression and can be said to be bacteriostatic as well (Eppo 2011).

As such the most effective way of control is by preventing infection of the trees through vector control programs (Bove 2006). Since areas not infected with HLB are posed a most severe threat, it is important for those areas to establish strict quarantine measures to keep it out (Bove 2006). For areas newly infected with HLB, Bove (2006) suggests that there are only two ways to reduce the potential damage that could be caused after a rapid survey of the extent of infection.

The first way Bove suggests is by eliminating all infected citrus trees. This can be difficult to accomplish as HLB has a latency period, and sometimes early infected trees do not express symptoms until roughly 6-12 months after infection (Bove 2006). As such removal of all symptomatic trees does not guarantee a successful removal of the disease in the area (Bove 2006). The second way Bove (2006) suggests is by keeping psyllid populations as low as possible. This is usually carried out by using systemic insecticides usually applied to the tree trunks. In Florida, *D. citri* is usually susceptible to chlorpyrifos, fenpropathrin, imidacloprid, and kaolin (Sullivan 2010). These insecticides reduce the psyllid population to a low level but may require repeated application (Sullivan 2010). In Taiwan, the psyllid population is usually controlled by the combination of insecticides and nymph parasitoids which have been reported to keep the psyllid population low (Chiou-nan 1998).

Economic Impact

Huanglongbing is a devastating disease to citrus trees and the citrus industry in general. In addition to its rapid spread and symptom manifestation, there is no cure for this disease at the moment with diseased trees or asymptomatic trees in diseased orchards felled, and psyllid populations kept low with the aid of parasitoids and insecticides. As such, it is clear that when HLB is present it has a very severe impact on

costs to farm growers and processors. Even when not present, HLB still provides considerable costs to stakeholders in the form of prevention and routine inspections.

Since its discovery there have been close to 100 million infected trees that have been destroyed in areas such as South and South East Asia, South Africa, India, the Philippines, Indonesia and the Arabian Peninsula (Gottwald 2007_a). This has generally reduced the yield for domestic consumption in these countries, and for exports. HLB has also reduced the citrus yield worldwide. As of 2005/2006, Brazil, China and the United States were the world's leading producers of citrus with a combined amount of 44.2 million metric tons (FAS 2006). In Sao Paulo, Brazil, 18.2 million metric tons of citrus fruit were produced in 2006 with estimated yearly earnings of $5.6 billion and also providing up to 400,000 jobs (Lopes 2010). However, with the discovery of Liberibacter americanus in 2004, HLB has spread to 268 municipalities with approximately 24% of the 96,000 citrus blocks infected (Lopes 2010).

Similarly, the United States produced 11.58 million metric tons of citrus in 2006. The top two major citrus producing regions of the United States which are Florida and California account for 96% of total production in the US. Florida has estimated earnings of $9.3 billion, supporting 100,000 jobs (National Research Council 2010) and producing 7.83 million metric tons in 2006 (FAS 2006). However, since the discovery of Liberibacter asiaticus in 2005, HLB has spread throughout most of Florida with about 60,000 acres of citrus trees decimated which is equivalent to 10% of the total yield (ERS 2007). It is projected that in the next 7-12 years virtually all current citrus plantings will be affected by HLB disease (Stover 2008). Also, despite the fact that California is at the moment HLB free, its yield capacity of 3.29 million metric tons which it produced in 2006

and it's $1.88 billion yearly earnings supporting 26,000 jobs is at a severe risk (Chavez 2010).

China which produced 14.4 million metric tons of citrus in 2006, has been dealing with HLB for close to 100 years. China has therefore been forced to expand its production areas to places where citrus psyllid vectors find unsuitable (high altitude regions) trying to meet the burgeoning domestic demand (PAMCO 2006). Although this has resulted in an overall expansion in production and yield of citrus over the past 50 years, potential earnings could be much higher with a more utilization of land (Xinlu 2001).

Blending

Harvested oranges vary naturally in juice characteristics (soluble solids, acidity, peel oil, etc.) and as such blending is performed by juice processors as a way to provide some consistency to processed juice which has well regulated standard parameters (Kimball 1991). Juice of different varieties can be blended to optimize flavor quality in juice thus giving juice processors more flexibility during processing (Kimball 1991). Another benefit of blending is the ease of manipulation of the juice characteristics as it is more difficult to physically manipulate sugar and acid content in juice (Bates 2001). Perhaps the most important advantage of blending lies in its ability to utilize juices defective in sensory or nutritional attributes thus adding value to the juice (Bates 2001). Several researchers have investigated the benefits of blending of juices as a means to overcome their sensory or nutritional defects. Bhardwaj and Mukherjee (2011) employed blending as a strategy to utilize kinnow mandarin juice which becomes bitter and undesirable under storage as limonate-a-lactone, a non bitter compound, is converted to limonin which is bitter in taste. Hence, Kinnow juice was blended with

pomegranate juice and Aonla juice, both with added spice extracts, to improve the sensory and nutritional characteristics of the juice (Bhardwaj and Mukherjee 2011). Suitable blending ratios were found by the authors who optimized flavor and improved nutritional content in the kinnow blend (Bhardwaj and Mukherjee 2011). Similarly, Raj et al. (2011) investigated the feasibility of blending sand pear juice which is undesirable to consumers due to its high acidity, astringency and grittiness. Sand pear juice was blended with apple juice which is considerably milder in acidity while higher in sweetness, with an aim to producing a more favorable flavor and nutritionally adequate juice (Raj 2011). The authors also found suitable blending ratios that achieved this aim (Raj 2011).

Juice blending is performed usually to meet certain specified juice characteristics of interest such as ^{0}Brix, percent acid, ºBrix/acid ratio, percent oil, percent pulp and sometimes limonin concentration in parts per million (Kimball 1991). However, as shown in the literature cited above, the converse is also frequently used, i.e., blending of different juices in varying proportions, often with the aim of finding a suitable ratio. These blending proportions, which can either be by volume or mass, are usually carried out in situations where specific juice characteristics may not be entirely representative of the complex citrus juice flavor or nutrition quality. In such cases, a group of blend ratios are determined prior to testing by sensory evaluation as well as analytical methods, with successful blends having strong preference ratings by consumers.

Sensory Evaluation Overview

Discrimination tests are often useful when trying to demonstrate that two similar products have perceivable differences (Meilgaard 2007, Stone and Sidel 2004).

Conversely, discrimination tests can also help determine if two products are sufficiently similar to be used interchangeably (Meilgaard 2007). If the differences between products compared are too large then it becomes obvious to detect and discrimination testing is not useful (Lawless 1999). As such, perceived differences would have to be subtle to increase the usefulness of discrimination testing (Lawless 1999).

There are numerous discrimination tests available to a sensory scientist but the most used tests include paired-comparison test, duo-trio test and the triangle test (Stone and Sidel 2004). There are two forms of the paired comparison test namely the directional paired comparison (2-alternative-forced-choice; 2-AFC) and the difference paired test (simple difference test). The 2-AFC test is utilized by a sensory scientist aware of the specific sensory attribute difference between the two samples (Lawless 1999). The subject's task is to identify which is different by that specific sensory attribute (Lawless 1999). In the simple difference test, however, the subject's task is just to identify which is different often with the sensory scientist unaware of if the two samples differ (Lawless 1999). Although Lawless (1999) finds 2-AFC test as comparatively more efficient than the simple difference test, Stone and Sidel (2004) are wary of the limitations of the 2-AFC test especially when ingredient differences may result in more than one to one (multiple) impact on the sensory attributes in the sample. In addition, the subjects (panelists) may not fully understand the specific sensory attributes to identify in a 2-AFC test (Stone and Sidel 2004). Statistically there is a one in two chance of guessing correctly that both samples are different, and therefore the probability of the null hypothesis that both are not different is $P_{null} = 0.5$ (Lawless 1999). This is

comparatively less efficient than the triangle test requiring more correct responses (and more participants) to achieve statistical significance.

The triangle test was developed by Helm and Trolle (1946) of Carlsberg Breweries, Copenhagen, Denmark, where it was used for control work and for the selection of taste panel members (Mounts and Warner 1980, Stone and Sidel 2004). In a triangle test, a panelist is presented with three samples, two of which are identical and one is different, and asked to identify the different sample (Meilgaard 2007). The chance probability of identifying the correct sample (different sample) is 0.33 and is more statistically efficient than either the duo-trio test or the paired comparison test. However, limitations exist for the triangle test and it is not effective for products with high sensory fatigue, products that involve carryover or adaptation (Meilgaard 2007). In addition, some subjects may find testing three samples too confusing or difficult as the subject has to recall the sensory characteristics of two products before evaluating the third and then making a decision (Stone and Sidel 2004,Meilgaard 2007).

The Duo-Trio test was developed by Peryam and Swartz (1950) as an alternative to the triangle test (Stone and Sidel 2004). The duo-trio test involves a subject presented with three products, one of which is identified as reference (or control) product. The subject's task is to identify the difference between the two samples (non-reference) with the aid of the third reference sample. Similar to the paired comparison test, the duo-trio test statistically has a one in two chance of guessing correctly that both samples are different and is less efficient than the triangle test (Meilgaard 2007)

Juice Analysis

A portion of the juice treatments (blends) was set aside to determine the soluble solids content (^{0}Brix), percent acidity and color.

Soluble solids

Orange juice contains a variety of chemicals with sugars or carbohydrates being the most predominant chemicals (Kimball 1999). These carbohydrates represent roughly 80% of the soluble solids in orange juice (Kimball 1999, Kelebek 2009). The main carbohydrates in orange juice are sucrose, glucose and fructose in the ratios of 2:1:1 (Kimball 1999, Kelebek 2009). The sucrose molecule consists of one molecule of glucose and one molecule of fructose (more specifically an α-D-glucopyranosyl unit and a β-D-fructofuranosyl unit) linked head to head (reducing end to reducing end) (Kimball 1999, BeMiller and Whistler 1996). As sucrose consists of one part glucose to one part fructose, the density of aqueous solutions of sucrose mixed with equal parts of fructose and glucose are similar to densities of 100-percent sucrose (Kimball 1999).

Juice density is a very important quality control parameter in the juice industry (Kimball 1999, Cepeda 1999, Zuritz 2004). Juice densities are used in weight and volume parameter adjustments, standardizing laboratory results, managing inventories and marketing (Kimball 1999). Insoluble solids such as cloud and pulp contribute little to the orange juice density (Kimball 1999). As orange juice is a sugar containing solution, its density can be determined by scales that apply to pure sugar solutions (Kimball 1999). However, soluble solids in orange juice include carbohydrates and non-carbohydrates (Kimball 1999). Organic acids and their salts contribute to about 10% of soluble solids in orange fruit (Cayuela 2008). In order to account for noncarbohydrates in soluble solids, a correction is usually applied to density measurements as sugar scales and tables are used (Kimball 1999).

The current scale used by the processed juice industry is based on relating the concentration of sucrose solution to solution density at a temperature of 20 ºC (Kimball

1999). The term '^{0}Brix' is used in expressing the percent weight of sucrose in a pure sucrose solution (Redd 1986). ^{0}Brix is usually determined by a hydrometer or weighted spindle calibrated to read directly in percent sucrose (Redd 1986). The principle behind the hydrometer lies with the buoyancy of the spindle which is directly proportional to the density of the solution (Kimball 1999). Therefore, less dense juice would have a lower lying spindle while more dense juice will have a higher lying spindle. However, dissolved gases could affect the accuracy of spindle readings because it affects the buoyancy of the spindle (Kimball 1999). In addition, foam on the brim of the juice makes hydrometer readings difficult (Kimball 1999). As temperature affects the density of a solution, ^{0}Brix hydrometers have built in thermometers along with a temperature correction scale used to correct ^{0}Brix readings (Kimball 1999). The viscosity of concentrated orange juice however makes the weighted ^{0}Brix spindle impractical; as such the refractometer is preferred as a more convenient method of determining the ^{0}Brix of orange juice (Redd 1986).

The principle behind the refractometer is that light travels fastest in a vacuum. However when light passes through a medium (a liquid for instance) it moves slower and is bent (refracted) at an angle. The size of this angle depends on the density of the medium. Hence in a refractometer, light passes through its fogged prism and is refracted upon encountering the juice sample. The critical ray is the ray that travels parallel to the surface of the prism representing the minimum angle at which the scattered light can strike the prism (Kimball 1999). The refractometer is ^{0}Brix calibrated with the critical ray representing the ^{0}Brix value of the medium (Kimball 1999). All scattered light necessarily falls below this critical ^{0}Brix value as they all have a larger

refraction angle. The shadow or dark area above the critical ^{0}Brix value helps provide a contrasting boundary layer to the critical ^{0}Brix for better visibility (Kimball 1999).

The refractometer, although more convenient than the spindle, also has some sources of errors. One of the greatest sources of error is the fact that citrus juices, especially concentrates, produce an indistinct shadowy layer between the light and dark zones of the refractometer, allowing for a fairly wide range of possible ^{0}Brix readings (Kimball 1999). Another source of error is reading the ^{0}Brix without proper calibration (Kimball 1999). Distilled water can be used to calibrate the refractometer and is sufficient in most cases (Kimball 1999). In addition, temperature fluctuations affect ^{0}Brix readings and as such temperature corrections are made for ^{0}Brix readings from refractometers that do not account for temperature. Equation 3.1 below, adopted from Kimball (1999), is used to correct for temperature:

$$\begin{aligned} Cor_T = {} & B^2\left[\left(+1.425\times10^{-4}\right)-\left(8.605\times10^{-6}T\right)+\left(7.138\times10^{-8}T^2\right)\right]+ \\ & B\left[\left(-2.009\times10^{-2}\right)+\left(1.738\times10^{-3}T\right)-\left(1.857\times10^{-5}T^2\right)\right]+ \\ & \left[\left(-7.788\times10^{-1}\right)+\left(1.700\times10^{-2}T\right)+\left(1.100\times10^{-3}T^2\right)\right] \end{aligned} \qquad (0.1)$$

However many modern refractometers make this correction automatically (Kimball 1999).

In addition to temperature corrections, acid corrections are also necessary to account for the soluble acid and their salts in the juice. As mentioned earlier, citric acid accounts for over 90% of the organic acid content in juice. It must be remembered, however, that other organic acids such as malic acid account for the remaining 10% of the organic acid content in juice. Also, titration does not account for acid salts which may account for 20% of the total salts and acids and can affect the ^{0}Brix reading (Shaw

1983, Kimball 1999). However, the error in ^{0}Brix reading from undetected salts is usually insignificant on an industrial basis and is usually ignored by industry (Kimball 1999). Equation 3.2 below, adopted from Kimball (1999) accounts for citric acid:

$$Cor_A = 0.014 + 0.192A - 0.00035A^2 \quad (0.2)$$

Modern refractometers do not account for this correction and manual corrections are made to ^{0}Brix readings.

Percent acidity

Titratable acid is a measure of the acid content of orange juice by titration with an aqueous alkali solution (Redd 1986).In this experiment, titratable acid was determined by acid titration with sodium hydroxide (NaOH) according to the method outlined by Kimball (1999) in Equation 3.3 shown below:

$$\%acid = \frac{(Normality)(ml \text{ titrated})(equivalent \text{ weight of citric acid = 64})(100)}{(Volume \text{ of sample taken for estimation})(1000)} \quad (0.3)$$

Although there was some controversy as to what pH should be taken as the endpoint for acid titration of orange juice, Kimball (1991) reports that the pH of 8.2 is used by the USDA in determining grade standards for citrus juices. The Association of Official Analytical Chemists (AOAC) recommends a normality of 0.1N (N = normality = number of moles of H^+ or OH^-) for acid titration (Kimball 1991).

^{0}Brix /acid ratio and BrimA index

The ^{0}Brix /Acid Ratio is an empirical ratio found by dividing the acid-corrected, temperature-corrected ^{0}Brix by the percent titratable acidity (Kimball 1999). Equation 3.4 is shown below:

$$B/A = \frac{^\circ Brix}{Acid} \quad (0.4)$$

Fellars (1991) reports that the ^{0}Brix/acid ratio is the most commonly used indicator of fruit maturity and palatability (Yoon 2006). In California, the fresh fruit market requires a ^{0}Brix/acid ratio of 8:1 or 8, while the fresh fruit market in Florida requires a ^{0}Brix/acid ratio of at least 10:1 or 10 (Kimball 1999). However, the ^{0}Brix/Acid ratio of commercial juice in Florida is required to be at least 13 and can be usually accomplished through blending (Kimball 1999). Consumers prefer juices with ^{0}Brix/Acid ratio ranging from 15 to 18 depending on the product and individual tastes (Kimball 1999).

The mammalian tongue tastes a variety of compounds but can only discriminate between sweet, bitter, sour, salty and umami (taste of sodium monoglutamate) (Adler 2000). About 50 to 100 taste receptor cells are clustered in taste buds distributed on the surface of the tongue and on the palate (Alder 2000, Kinammon 1992). Each receptor is about 60 μm in diameter and has numerous microvilli about 2 μm long that extend into the taste pores on the tongue (Kimball 1999). Taste is detected when chemicals (taste stimuli) diffuse through the taste pores and reach the apical membranes of taste receptor cells (Kinammon 1992). The chemicals interact with the microvilli resulting in a membrane conductance change in the taste receptor cell, depolarization, action potential initiation, and release of the transmitter onto gustatory afferents (Kinammon 1992). Taste receptors are very sensitive and are replenished every 12 to 17 days (Kimball 1999). In orange juice, the sourness of the organic acids and the sweetness of the sugars compete for the same receptor sites which makes the ºBrix/Acid ratio important measure of juice flavor quality (Kimball1999). However, the difficulty with ºBrix/acid ratio is that a single ratio can have varying amounts of soluble solids content and acidity leading to inconsistencies in flavor (Obenland 2009). Jordan (2001)

proposed a new parameter known as the BrimA index as a means to meet this difficulty (Obenland 2009). The BrimA index proposed multiplying titratable acidity by a constant which varies according to fruit type and then subtracting that overall value from the ^{0}Brix (Obenland 2009). Equation 3.5 is shown below:

$$BrimA = {}^{\circ}Brix - \left(k \times \text{total acid}\right) \tag{0.5}$$

Jordan (2001) reported that the constant 'k' = 5 is suitable for citrus fruit, however Obenland (2009) reports that k = 3, 4 or 5 give very similar flavor correlations. The constant 'k' reflects the tongue's higher sensitivity to acidity than to sugar (Jaya 2003). The index allows smaller amounts of acid than sugars to make the same amount of numerical change to the index (Jaya 2003). Jordan (2001) proposed this index based on the fact that sugars and acids have opposite effects on flavors and that the tongue is more sensitive to acidity (Jaya 2003, Obenland 2009)

The BrimA index is relatively new and has not enjoyed broad consensus as a better alternative to the ºBrix/acid ratio. Harker (2002) reported that BrimA index did not improve flavor prediction in comparison to ºBrix/acid ratio. Jayasena (2008) also reported that BrimA index poorly predicted flavor in comparison with the ºBrix/acid Ratio. However, Obenland (2009) found the BrimA index a better alternative for flavor prediction of California navel oranges and recommended the BrimA index over the ºBrix/acid ratio as a flavor quality standard especially for low acid poor tasting fruit. It is therefore important to utilize both parameters in assessing the flavor of juice treatments in this experiment. For the purpose of this thesis research, the 'k' coefficient of 3 was selected because it accounted for an assumed minimal sensitivity of acidity in the juice blends.

Juice color

The natural bright color of citrus juices has long been regarded as one of the major qualitative advantages over other food products (Kimball 1999). Juice color is a key factor in influencing consumer acceptance (Calvo 2001, Tiwari 2008). Tepper (1993) notes that the color of orange juice is used as a quality control parameter for commercial classification of the product (Kimball 1999, Cortes 2008). Juice color is mainly due to carotenoid pigmentation with produces bright and cheerful associations complementing the sweet and tart flavors as well as pleasant aromas of orange juice (Kimball 1999, Melendez-Martinez 2005).

Carotenoids are responsible for many fruit colors and also promote healthy benefits in humans by exerting potential action against certain cancers, preventing gastric ulcers, stimulating the immune system, preventing cardiovascular disease and protecting against age-related muscular degeneration and cataracts (Gama 2005). Gross (1987) reports that citrus is a complex source of carotenoids with the largest number of carotenoids in any fruit (Rouseff 1996, Melendez-Martinez 2005). The main carotenoids responsible for the orange color of orange juice are lutein (23% of total carotenoids, yellow-green color), α-carotene (7%, orange-yellow color), β-carotene (8%, orange-yellow color), zeaxanthin (20%, yellow color), violaxanthin (11%, yellow color), and β-cryptoxanthin (21%, orange color) (Kimball 1999). The above carotenoids can be grouped into pro-vitamin A carotenoids (α-carotene, β-carotene and β-cryptoxanthin) and antioxidant carotenoids (β-carotene, zeaxanthin, lutein) (de Ancos 2002).

Juice color can be measured by two main methods: USDA color scoring and Tristimulus tests. The USDA has an established standard for grading orange juice color using six plastic tube colors (Kimball 1999, USDA 1983). Each plastic color tube

represents a standard from OJ1 (lightest) to OJ6 (darkest) (Kimball 1999). These tubes can be used for direct color comparisons or to calibrate approved colorimeters (Kimball 1999). USDA assigns 40 points out of a total of 100 points to color, for classification purposes (Melendez-Mendez 2005, Stewart 1977). Therefore grade A juice must have a color number between 36-40 points whereas grade B juice must have a color number between 32-35 points (Melendez-Mendez 2005, Stewart 1977). Any color number lower than 31 points is regarded as substandard (Stewart 1977).

The standard method of color scoring in the food industry recommended by the International Committee of Illumination in 1931 is based on the 'standard observer' or simulated standard eye that consists of three primary colors referred to as X (red), Y (green) and Z (blue) (Kimball 1999, Mrak & Stewart 1954, Mendoza 2006). Hunter came up with some parameters that measured redness(a) on a green (-) to red (+) scale, yellow-ness (b) on a blue (-) to yellow (+) scale and lightness (L) stimulus parameters (Hunter 1958, Kimball 1999, Sanchez-Moreno 2003). Kramer and Twigg (1970) have shown that Tristimulus parameters can be related to Hunter Parameters as shown below (Kimball 1999):

$$L = 100\sqrt{Y} \tag{0.6}$$

$$a = \frac{175\left(1.20X - Y\right)}{\sqrt{Y}} \tag{0.7}$$

$$b = \frac{70\left(Y - 0.847Z\right)}{\sqrt{Y}} \tag{0.8}$$

In this experiment, the color of all the juice samples were determined using Hunterlab ColorQuest XE spectrophotometer (Virginia, USA) equipped with a light source D65 and observation angle of 10º. The instrument was calibrated using a white

(X= 81.01, Y=85.77, Z=89.24) standard. In addition to L*a*b parameters, Chroma that quantifies color intensity in comparison to pure white background and hue angle that represents the attribute of color that is related to perceived colors (yellow, blue, red, green or a combination of each) are determined (ETS 2011). Chroma, C, and hue angle, hº are defined as follows (Brewer 2001, Sanchez-Moreno 2003):

$$C = \sqrt{\left(a^2 + b^2\right)} \tag{0.9}$$

$$h^{\circ} = \tan^{-1}\frac{b}{a} \tag{0.10}$$

Purpose

Although HLB is extremely harmful to citrus trees, it is harmless to humans (USDA 2012). This knowledge has not been applied by citrus growers who theoretically would discard off deformed or green fruit during sorting. In fact, very sparse literature exists on the effects of greening on citrus juice parameters. Newly carried out research on the effects of greening on juice quality suggest that consumers find symptomatic juice "bitter" and "acidic" compared with asymptomatic juice from infected trees and control (non-infected) juice (Plotto 2010). This finding corresponds with a chemical analysis of symptomatic juice which shows higher acidity levels and lower sugar levels as compared with asymptomatic juice and control juice which were both comparatively similar (Dagulo 2010). It was also observed that symptomatic juice had a much reduced amount of the compound valencene as compared with asymptomatic juice and control juice (Dagulo 2010). The valencene compound has been identified as a marker of maturity in fruit and is associated with juice flavor quality (Elston 2005). It is clear that a

significant opportunity exists to investigate the effects of greening on citrus juice parameters.

The proposed research objectives will be to determine from an array of prepared samples whether greening affected juice (both symptomatic and asymptomatic juice) can be blended at a level of 5-10% in the control juice. The aim would be to determine if any blending levels exist at which consumers would be unable to differentiate between the greening affect juice and the control juice. Finally, a proposed blending strategy would be developed utilizing old and new industry parameters to achieve an acceptable orange juice product.

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CHAPTER 3
RESEARCH METHODS

Citrus greening affected juice was blended with non-affected juice at 5 and 10% levels with the aim to determine whether consumers will be unable to differentiate between greening-affected juice blends and control (healthy) juice. Juice was extracted from Valencia and Hamlin variety oranges affected by greening collected from three harvests and will be immediately stored in a freezer. Consumer sensory testing were conducted via a taste panel and include difference-from-control and triangle tests. The primary hypothesis (Hypothesis 1) is that consumers will be unable to differentiate citrus greening affected juice blended at 5% levels with non-affected juice but will detect a difference at 10% levels. The foundation behind this hypothesis comes from anecdotal citrus industry knowledge that different juices can be blended into each other at a 5% level without noticeable sensory differences. Hypothesis 2 is that consumers will be unable to differentiate greening juice blended with Valencia juice rather than Hamlin Juice at 5% levels. The foundation behind this hypothesis is that Hamlin oranges have less sugar, are watery and also more astringent than Valencia oranges.

Blending Strategy

The blend ratios of healthy control juice (Valencia juice) to greening affected juice by juice mass will be investigated at 95:5 and 90:10 ratios. In addition, blending by fruit mass will also be investigated. Blending by fruit mass involves collecting a portion of greening-affected fruit (specifically 5% and 10%) from the total fruit amassed from the harvest, juicing and blending with healthy control juice in the already stated proportions. As mentioned earlier, blends (95:5 and 90:10) from both juice and fruit manipulation will

be compared with control juices to see if consumers are able to detect a difference between the blends and the control juices.

Sensory Evaluation

Sensory evaluation of juice samples was carried out by using a triangle test to determine if there is a difference between the juice blends and control juice. The choice of using a triangle test is not only for its statistical superiority to similar difference tests (pairwise comparison and duo-trio) but also due to the nature of greening juice which lies more with an imbalance of chemical constituents rather than one or two specific attribute differences from healthy juice. A sample ballot of the triangle test is shown in Fig 3 in the appendix section. In order to determine the sample size for this experiment, a few parameters such as the type I and II risk level as well as the true proportion of distinguishers have to be determined. In reality, the type I or 'α' risk is the most important parameter, i.e., the risk of determining that there is a perceptible difference when there is none (Meilgaard 2007). In this thesis research, a 5% level of type I risk has been selected. The type II risk level i.e. the risk of concluding no perceptible difference when it exists, is less important when testing for difference. In this experiment a 10% level of type II risk has been selected. The true proportion of distinguishers is arbitrarily chosen and reflects assumed sensory differences between treatments. In this experiment, 0.30 or 30% proportion has been selected based on an assumption that sensory differences will be moderate. According to Meilgaard (2007), the minimum number of assessors based on these parameters is 53 tasters. This panel will consist of at least 53 tasters that are untrained and regular (at least once a month) orange juice drinkers.

Once differences were established, then a subsequent consumer acceptability test using a hedonic 9-point scale was employed on the blends. This consumer acceptability test assessed for sweetness, orange flavor, and overall acceptability. After hedonic testing determining consumers' preferences of the juice blends, a difference from control test was carried out to estimate the size of the differences among the blends. A sample ballot of the hedonic test is shown in Fig 4 in the appendix. A difference from control test utilizes a control sample as a reference for comparison with the juice blends (Meilgaard 2007). The objective of the difference from control test is to compare how different the various juice blends are from the control. The difference from control test was used for juice blends from fruit (5%, 10%, 20% and 50% blends) utilizing a 10 point numerical categorical scale (1 = No difference and 10 = Extreme difference). A sample ballot of the difference from control test is shown in Fig 5 in the appendix.

Sensory analysis was conducted in the University of Florida Food Science and Human Nutrition (FSHN) Sensory Laby which consists of 10 separate and private booths with 10 computers. Sixty panelists were asked demographic questions about their age, gender and frequency of consumption. Panelists were then directed to take a bite of a cracker and a sip of water before tasting every sample. For the triangle test, three 30 ml cups were presented to the panelists at room temperature (20 C) with orange juice consisting roughly 20 ml of the cups, and asked to identify which amongst the three cups is different, as two are of the same source. For the difference-from-control test, 60 panelists were presented with a 30 ml cup filled with a reference sample (healthy juice) and then provided with three 30 ml cups filled with different juice blends (5%, 10%, 20%, 50% and control (0%)) and blind coded with a three digit number.

Panelists were asked to rate how different each of their juice samples (treatments) was from the control on the 10 point scale.

Data from the difference-from-control test were statistically analyzed using analysis of variance and significant means separated using Tukey's method at a 0.05% alpha level.

ºBrix Analysis

^{0}Brix readings were taken with the aid of a hand held refractometer (Fisher Scientific, Catalog number 13-946-21, Range 0-32% ^{0}Brix). A few drops of juice from each sample were placed on the refractometer prism and ^{0}Brix readings measured.

Acid Analysis

Approximately 10 ml of orange juice was utilized as the standard orange juice quantity and diluted with 10 ml of distilled water. This dilution was then titrated with 0.1N NaOH to an endpoint pH of 8.2. The pH readings were measured using a Fisher Scientific AB 15 plus pH meter. Equation 3 was then utilized after titration to determine the percent acidity of each sample.

Color Analysis

Juice color analysis was carried out on all samples including blends.

Preliminary Studies

Evaluation of the level (%) of greening-affected juice to be blended with healthy juice: To determine the proper level of greening-affected juice to be blended with healthy juice, 5% and 10% levels were chosen based on anecdotal industrial practices. Experiment 1 was carried out as a triangle test with 37 panelists sampling pasteurized juice from the Valencia harvest (01-13-2010) at both 5% and 10% levels. Results shown below suggest that 5 and 10% levels were appropriate for blending greening-

affected juice with healthy juice. The results suggest that 5% blends are barely detectable whereas it appears to be much easier to detect 10% blends.

Table 3-1. Triangle tests on pasteurized valencia juice (01-13-2010)

Significance	5%	10%
Correct Responses	18	17
Actual Responses	17	23

An analysis of the pasteurized Valencia juice was carried out to determine ^{0}Brix , titratable acidity, ^{0}Brix/acid ratio and color (L*, a*, b*) with results shown below:

Table 3-2. Analysis of pasteurized valencia juice (01-13-2010)

	Healthy	5% Blend	10% Blend	Greening
pH	3.920±0.016	3.840±0.002	3.872±0.002	3.743±0.004
^{0}Brix	10±0.0	11±0.0	6.6±0.0	8±0.0
Corr. ^{0}Brix	10.07±0.0	11.15±0.0	6.71±0.0	8.15±0.0
Acidity	0.307±0.046	0.751±0.025	0.527±0.013	0.738±0.030
^{0}Brix /Acid[i]	32.8	14.8	12.7	11.0
BrimA	9.2	8.8	5.1	5.9
L	53.76±0.940	49.62±0.904	37.09±4.701	48.60±1.932
a	-0.77±0.367	-1.01±0.227	-2.06±0.208	-2.23±0.235
b	24.95±1.262	22.15±1.058	4.46±1.704	17.6±1.876

[i] Values are presented as averages of three replicates

According to Table 3-2, healthy juice and greening-affected juice had, as expected, the highest and lowest pH values reflecting the lowest and highest acidity levels respectively in the juice. However, curiously the 5% blend had a lower pH and higher acidity compared with the 10% blend which had a higher pH and lower acidity. This suggests an error in blending. In addition, the ^{0}Brix levels of the 5% blend and 10% blend were the highest and lowest respectively. This is inconsistent and suggests soluble solids were not properly distributed. The ^{0}Brix levels of two juices that are blended should never mathematically be above the higher ^{0}Brix level juice or

alternatively below the lower ^{0}Brix level juice. The ^{0}Brix/acid ratios were high due to the extremely low acidity levels of the juices and it is difficult to assess perceived sweetness among the treatments with such varied ^{0}Brix and acid levels. As such, the BrimA index developed by Jordan (2001), as an alternative to ^{0}Brix /acid ratios was also utilized. BrimA scores show that the 5% blend has the highest perceived sweetness followed by healthy juice, the 10% blend and greening juice respectively. As stated above, this is highly suspicious as the parameters of blends lie within the range of the parameters of their constituent component juices. There was great homogeneity among all of the juice treatments except for the 10% blend which was considerably lower in brightness (L) and yellower than other juice treatments. The slight inconsistencies were not critical to establishing the 5% and 10% blending levels. However, results outlined in Table 3-1 suggested that 10% blends levels were clearly detectable by panelists, while 5% blend levels were barely detectable. Further evidence was necessary with a larger number of panelists to ascertain whether 5% levels of greening-affected juice could be successfully blended with healthy juice.

Therefore a second preliminary triangle test, Experiment 2, was carried out with 59 panelists using unpasteurized Valencia juice (01-13-2010) at only 5% levels. Results shown in Table 3-3 suggest that blending greening-affected juice at 5% level with healthy juice is not only appropriate but could possibly be minimum threshold level of detection.

Table 3-3. Difference tests on unpasteurized valencia juice (01-13-2010)

Significance	5%
Correct Responses	27
Actual Responses	27

Table 3-4. Difference tests on unpasteurized valencia juice

	Healthy	5% Blend	Greening
pH	3.847±0.010	3.827±0.010	3.721±0.006
^{0}Brix	9.8±0.0	9.6±0.0	7.6±0.0
Corr. ^{0}Brix	9.92±0.0	9.74±0.0	7.81±0.0
Acidity	0.588±0.019	0.663±0.019	1.051±0.013
^{0}Brix /Acid[i]	16.8	14.7	7.2
BrimA	8.1	7.8	4.6
L	47.54±1.040	47.03±0.959	49.14±1.251
a	-1.09±0.367	-1.47±0.747	-2.28±0.235
b	19.93±1.598	18.06±3.904	18.31±1.439

[i] Values are presented as averages of three replicates

According to Table 3-4, healthy juice and greening-affected juice had the highest and lowest pH values reflecting the lowest and highest acidity levels respectively in the juice. The 5% blend had a pH value and acidity level at an intermediate level compared with healthy juice and greening-affected juice. In addition, healthy and greening-affected juice had the highest and lowest ^{0}Brix levels with the 5% blend having a ^{0}Brix level in between both juices. This suggests proper blending as the 5% blend has acidity and soluble solid content that is within the range of healthy juice and greening-affected juice. The ^{0}Brix /acid ratios show that healthy juice is sweeter than both the 5% blend and greening-affected juice. The 5% blend is also sweeter than the greening-affected juice. This was also evident in BrimA scores which were similar to the ^{0}Brix /Acid ratios, with perceived sweetness the highest in healthy juice, then the 5% blend and finally greening-affected juice respectively. There was great homogeneity in color parameters (L*, a*, b*) among all of the juice treatments.

A summary of the preliminary studies shows that the immature taste of greening-affected juice is clearly discernible at 10% levels while barely discernible (threshold level) at 5% levels blended with healthy juice.

CHAPTER 4
RESULTS AND DISCUSSION

Blending by Juice

The purpose of these experiments is to determine whether greening-affected juice could be successfully blended by mass in healthy juice at 5% and 10% levels. Experiment 3 was a triangle test with 60 panelists sampling unpasteurized Valencia juice from the April 14th 2008 harvest. Results of the taste panel are shown below:

Table 4-1. Difference tests on pasteurized valencia juice (04-14-08)

Significance	5%	10%
Correct Responses	27	26
Actual Responses	27	38

As expected, panelists were able to significantly recognize the presence of greening-affected juice at 10% levels while barely able to recognize the presence of greening-affected juice at 5% levels. This corresponds with results from the preliminary studies. An analysis of the each of the treatments of pasteurized Valencia juice (04-14-08) to determine ^{0}Brix , titratable acidity, ^{0}Brix/acid ratio, BrimA index and color (L*, a*, b*) is shown below:

Table 4-2. Analysis of unpasteurized valencia juice (04-14-08)

	Healthy	5% Blend	10% Blend	Greening
pH	3.715±0.002	3.769±0.011	3.719±0.004	3.391±0.002
^{0}Brix	12±0.0	10.4±0.0	10.6±0.0	8±0.0
Corr. ^{0}Brix	12.18±0.0	10.55±0.0	10.77±0.0	8.26±0.0
Acidity	0.887±0.013	0.745±0.009	0.823±0.024	1.314±0.046
^{0}Brix /Acid[i]	13.7	14.2	13.0	6.3
BrimA	9.5	8.3	8.3	4.3
L	51.81±0.940	54.29±0.959	52.15±0.446	54.11±1.832
a	9.51±0.654	3.31±0.308	5.09±0.465	6.71±1.365
b	37.54±0.663	33.08±0.026	34.95±0.861	34.46±2.899

[i] Values are presented as averages of three replicates

According to Table 4-2, Greening-affected juice had the lowest pH value and highest acidity level among all juice treatments. There was some inconsistency among the healthy juice, 5% blend and 10% blend as pertaining to acidity. The 5% blend had the highest pH value and lowest acidity level. This was unexpected as healthy juice should have the lowest acid level. The 10% blend, as expected, had a higher acidity than the 5% blend but unexpectedly had a lower acidity than healthy juice. With respect to soluble solids content, healthy and greening-affected juice had the highest and lowest ^{0}Brix levels. The 5% and 10% blends had ºBrix levels in between both healthy and greening-affected juices with the later having a higher ^{0}Brix than the former. The ^{0}Brix/acid ratios and BrimA index values suggest that healthy juice had the highest sweetness among all treatments while greening-affected juice had least sweetness among all treatments. There was some discordance among the 5% blend and 10% blend in terms of sweetness perception by both ^{0}Brix/acid ratios and BrimA index values. The ºBrix/acid ratios suggest that 5% blend is sweeter than the 10% blend while the BrimA index indicates both blends having equal sweetness. Albeit there was some homogeneity in color parameters (L*, a* and b*) among all of the juice treatments, both a* and b* were statistically significantly different among all treatments with L*, a*, and b* having p values of 0.06, 0.00008, and 0.04, respectively. No explanation is offered as to why the 5% blend had a lower acidity than the healthy juice. Nevertheless, it is useful to observe that the 10% blend had fairly similar acidity and ^{0}Brix values despite overwhelmingly separated by panelists via sensory evaluation as different from normal juice. This seems to suggest that ºBrix/acid ratio and BrimA index may not entirely account for the off-flavor differences in juice blends. In addition, the 10% blend had a

slightly similar color (L*, a*, b*) to the healthy juice compared with the 5% blend. It is therefore unclear as to the effects of color as an enabling factor for panelists to significantly differentiate the 10% blend from healthy juice. As this is a sensory difference test, it is hazardous to speculate on specific factors that led consumers to discern the 10% blend from the healthy juice. However, a suspected primary factor that could perhaps have played a significant role in showcasing the differences is the imbalance of flavor compounds found in greening-affected juice. A plausible explanation for the results is that the mass of greening juice added to the healthy juice at 10% levels was perhaps excessive in terms of negatively impacting the flavor of healthy orange juice. However, there is a strong plausibility that the 5% levels could be a threshold level showing the maximum amount of greening juice that could be added to healthy juice without negatively impacting the flavor.

A repeat triangle test, Experiment 4, with 60 panelists was then carried out testing unpasteurized Valencia juice dated from April 4th, 2008 (04-04-08). Results are shown below:

Table 4-3. Difference tests on unpasteurized valencia juice (04-04-08)

Significance	5%	10%
Correct Responses	27	26
Actual Responses	17	32

Results from Table 4-3 confirmed expectations that panelists are able to significantly recognize the presence of greening-affected juice at 10% levels while barely able to recognize the presence of greening-affected juice at 5% levels. These results, however, reveal that panelists overwhelmingly could not differentiate the 5% blend from healthy juice. An analysis of each of the treatments of unpasteurized

Valencia juice (04-04-08) to determine ^{0}Brix, titratable acidity, ^{0}Brix/acid ratio, BrimA index and color (L*, a*, b*) is shown below:

Table 4-4. Analysis of unpasteurized valencia juice (04-04-08)

	Healthy	5% Blend	10% Blend	Greening
pH	3.625±0.005	3.549±0.002	3.537±0.003	3.353±0.001
^{0}Brix	12±0.0	11.2±0.0	10.8±0.0	5.6±0.0
Corr. ^{0}Brix	12.18±0.0	11.39±0.0	10.99±0.0	5.83±0.0
Acidity	0.868±0.035	0.941±0.006	0.955±0.019	1.154±0.020
^{0}Brix /Acid[i]	14.0	12.1	11.5	5.1
BrimA	9.6	8.6	8.1	2.4
L	52.06±0.727	51.70±0.610	51.72±0.779	52.31±0.167
A	4.75±1.300	4.82±0.444	4.72±0.534	4.08±0.568
B	33.67±2.078	33.36±0.944	32.79±0.833	33.23±1.230

[i] Values are presented as averages of three replicates

The results outlined in Table 4-4, confirms expectations of acidity and ^{0}Brix levels due to blending. These expectations are that healthy juice would have the lowest acidity levels followed by the 5% blend, 10% blend and finally greening-affected juice respectively. These expectations are due to the tendency of greening-affected juice to have higher acidity levels than healthy juice. As such, it makes sense that any addition of greening-affected juice to the healthy juice in blend formation would increase acidity. This is the case in Table 4-4, where pH and acidity levels where decreasing and increasing respectively, in healthy juice, 5% blend, 10% blend and greening-affected juice. In addition, the ^{0}Brix levels in healthy juice are shown to be the highest followed by the 5% blend, 10% blend and eventually greening-affected juice having lowest ^{0}Brix levels. The ^{0}Brix/acid ratio of healthy juice is the highest among the four treatments and suggests the highest rated in perceived sweetness. Following healthy juice in a decreasing ºBrix/acid ratio and sweetness perception include the 5% blend, 10% blend and greening-affected juice respectively. The BrimA index also follows the same trend

as the ^{0}Brix/acid ratio. The color coordinates (L*, a*, b*) in Table 4-4 are very similar among all treatments. In addition, all color parameters were not significantly different from each other with L, a, and b having p values of 0.24, 0.65, and 0.88, respectively. Perez-Lopez (2005) reported commercial Valencia orange juice having coordinates (L= 52.99±0.02, a=5.50±0.01, b=33.81±0.02) which is very similar to color coordinates in Table 4-4.

Blending by Fruit

The purpose of the following experiments was to determine whether greening-affected juice could be successfully blended by fruit mass in healthy juice at 5% and 10% levels. Experiment 5 was carried out as a hedonic test with 55 panelists sampling pasteurized Valencia juice from the April 7th, 2011 harvest. Results of the taste panel as well as analysis of the four treatments are shown below.

Table 4-5. Hedonic test of pasteurized valencia juice (04-07-11)

	5% Blend	10% Blend
Overall Acceptability	7.02±1.340[a]	6.73±1.592[a]
Sweetness	6.96±1.387[a]	6.45±1.597[a]
Orange Flavor	6.96±1.290[a]	6.73±1.394[a]

[a] Means with the same letter show no significance at alpha = 0.05

Results from Table 4-5 show that panelists rated the 5% and 10% blend the same in terms of overall acceptability, sweetness and orange flavor. However, it is important to note that the 5% blend had the highest ratings in overall acceptability, sweetness and orange flavor. In addition, there was a significant difference in sweetness between the 5% and 10% blends at an alpha level of 10% or 0.10. Albeit it can be said with greater

confidence (95%), that there is no significant difference in sweetness between both blends.

An analysis of the each of the blends (both 5% and 10%) as well as control juice and greening-affected juice treatments from the pasteurized Valencia juice (04-07-11) to determine ^{0}Brix, titratable acidity, ^{0}Brix/Acid ratio, BrimA index and color (L*, a*, b*) is shown below:

Table 4-6. Analysis of pasteurized valencia juice (04-07-11)

	Healthy	5% Blend	10% Blend	Greening
pH	3.856±0.005	3.886±0.015	3.886±0.015	3.853±0.005
^{0}Brix	12.8±0.0	12.2±0.0	11.2±0.0	11±0.0
Corr. ^{0}Brix	12.96±0.0	12.35±0.0	11.34±0.0	11.17±0.0
Acidity	0.770±0.016	0.712±0.061	0.695±0.016	0.829±0.028
^{0}Brix /Acid[i]	16.8	17.3	16.3	13.4
BrimA	10.7	10.2	9.3	8.7
L	52.60±1.261	52.05±0.733	51.66±0.575	53.77±1.576
a	2.07±0.587	3.37±0.630	2.19±0.296	2.09±0.130
b	36.99±2.782	37.15±1.408	35.30±0.718	38.60±2.730

[i] Values are presented as averages of three replicates

The results from Table 4.6 shows acidity and ^{0}Brix levels for pasteurized Valencia juice from April 7th, 2011. Healthy juice is shown to have the highest ^{0}Brix levels followed by the 5% blend, the 10% blend and greening-affected juice respectively. However, both the 5% and 10% blends had lower acidities than healthy juice and greening juice. Several comments have been mentioned earlier about the inherent impossibility of this occurring and the strong likelihood of blending errors when dealing with juice blends. However this is a fruit blend. As such, it is entirely plausible that inconsistencies in blend compositions may occur. This is because the choice of both greening-affected fruit and healthy fruit varies. In other words, in fruit blending, there is not a set of oranges that are consistently used to form blends. As such there could be

variability ensuing among blends from fruit with variable acidity or ^{0}Brix levels. In this case, the 5% blend because of a combination of its comparatively lower acid level and higher ^{0}Brix , has the highest ^{0}Brix/Acid ratio among all the juice treatments. Greening-affected juice has the lowest ^{0}Brix/Acid ratio due to having the highest acid content and lowest ^{0}Brix level amongst all the juice treatments. The BrimA index, which by definition is closely aligned with the ^{0}Brix, shows a steady decline in index value from healthy juice to the greening juice which is consistent with ^{0}Brix levels. Color coordinates (L*, a*, b*) also remained similar among all treatments albeit only a* was significantly different among all the treatments with L*, a*, and b* having p values of 0.18, 0.02, and 0.35, respectively. The close similarities in ^{0}Brix/acid ratios (of the 5% blend and 10% blend) and color is reflected in prior hedonic testing (Table 4.5) which shows no significance at alpha = 0.05 in overall acceptability, sweetness or overall flavor.

After hedonic testing, a difference by control test (Experiment 6) was carried out with 59 panelists on the same pasteurized Valencia juice (04-07-2011). The following treatments which were contrasted with each other in this experiment include: control (healthy juice), 5% blend and the 10% blend. Results are shown in Table 4.7 below:

Table 4-7. Difference-from-control test of pasteurized valencia juice (04-07-11)

	Control	5% Blend	10% Blend
Mean Score	4.58±2.119[a]	4.03±2.251[a]	4.34±2.223[a]

[a] Means with the same letter show no significance at alpha = 0.05

The results from the difference-from-control test shown in Table 4-7 showcases how different the treatments were rated from the control. On the 10 point categorical scale with, there was no significant difference among the treatments with a range of means from 4.03-4.58 (5% Blend, 10% Blend, and Control respectively). All the

treatment means were closer to the ‘no difference’ end of the scale, albeit curiously the control treatment, which was included blindly, was rated not different. In addition, panelists could not find any difference between the blind control and the blends (both 5% and 10%) ranking them even lower than the control (closer to the ‘no difference’ end of the scale) albeit the range of means was towards the middle of the scale. This phenomenon of avoiding the ends of scales is known as error of central tendency (Meilgaard 2007). The error of central tendency is a psychological error whereby panelists score products in the mid-range of a scale, avoiding the extreme ends (Stone and Sidel 2004). This inevitably ends up giving the effect that products are more similar than they really are (Stone and Sidel 2004). In this case, prior hedonic testing shows us that the 5% blend and 10% blend were very similar to the panelists, which may have also influenced the panelists’ decisions in rating them so closely together on the 10 point scale.

As there was no significant difference between the 5% and 10% blends and the reference control, it was important to determine if there would be a difference if the treatments were higher blends (20%, 50%). Experiment 7 was therefore also a difference from control test with 60 panelists assessing the following treatments alongside the reference control: 10% blend, 20% blend, 50% blend and the control juice. The 5% blend was not included in this experiment as prior preliminary testing as well as recent hedonic testing showed that the 5% was barely discernible from the control juice. It is important to state that a 5% blend by fruit is not equivalent to a 5% blend by juice. A 5% blend by fruit simply means 5% (by mass) of greening fruit were added to a batch of 95% (by mass) healthy fruit and juiced to form a blend. However,

greening-affected fruit tend to be smaller in size and deformed shape compared to healthy fruit (Bove 2006). This would mean a smaller than stated proportion of greening-affected juice is mixed with healthy juice. In other words, a 5% blend by fruit would be probably slightly less than 5% blend by juice. This would therefore mean that a 5% blend by fruit would have even more similar to control juice than the 5% blend by juice which was barely discernible from control juice. Therefore in Experiment 7 the 5% blend was neglected. Also, similar to previous difference-from-control test, a blind control was also placed among the treatments unknown to panelists. Results from this experiment are shown below:

Table 4-8. Difference-from-control test of pasteurized valencia juice (04-07-11)

	Control	10% Blend	20% Blend	50% Blend
Mean Score	3.87 ± 2.332^{c}	5.08 ± 2.842^{ab}	4.45 ± 2.332^{bc}	5.57 ± 2.770^{a}

^{abc} Means with the same letter show no significance at alpha = 0.05

Table 4-8 shows how differently the treatments, particularly the high blends (20% and 50%) vary from reference control. The results show that the 50% blend is significantly different from the control and the 20% blend. In addition, the 10% blend was found to be significantly different from the control juice. As expected the control juice was ranked closest to the 'no difference' end of the scale i.e. after comparing with a reference sample. Curiously, panelists ranked the 20% blend closer than all other blends (including the 10% blend) to the control juice. This may appear inconsistent but a plausible explanation exists when considering the fact that in fruit blending, there is minimal control of the composition of the juices to be blended. There is a potential for variation in compositions of the juices available from blending, as fruit may be utilized from different trees in the grove. In contrast with blending by juice, where a specific

known composition of healthy juice is blended with a specific, known composition of greening-affected juice, blending by fruit involves using different fruit for the composition of each blend. The selection of fruit from the same grove does not guarantee the same composition from each fruit. In addition, the fact that for each blend a separate batch of fruits are used, which is a key feature in fruit blending, ensures the possibility of composition variation in blends. In this case, it seems entirely plausible that fruits (either healthy or greening-affected) involved in producing the 20% blend may have had a higher ^{0}Brix or lower acid composition than the 10% blend. This could explain why panelists ranked the 10% blend higher than the 20% blend, as well as finding the 10% blend significantly different from the reference sample. An analysis of the higher blends (20% and 50% blends) is shown below:

Table 4-9. Analysis of pasteurized valencia juice (04-07-11)

	Healthy	10% Blend	20% Blend	50% Blend
pH	3.85±0.005	3.93±0.015	3.87±0.005	3.82±0.011
^{0}Brix	12.8±0.0	11.8±0.0	12.2±0.0	11.2±0.0
Corr. ^{0}Brix	12.96±0.0	11.97±0.0	12.38±0.0	11.38±0.0
Acidity	0.770±0.016	0.753±0.079	0.814±0.018	0.902±0.019
^{0}Brix /Acid[i]	16.8	15.7	15.2	12.6
BrimA	10.7	9.7	9.9	8.7
L	52.60±1.261	51.17±0.248	52.95±0.562	52.68±0.681
a	2.07±0.587	1.36±0.040	2.30±0.281	2.15±0.110
b	36.99±2.782	33.28±0.384	36.60±0.700	36.71±0.362

[i] Values are presented as averages of three replicates

In Table 4-9, there was some variation in the pH values. The pH values did not seem to reflect the acidity content in each blend. Probable reasons for this variation could be the presence of high levels of sinking pulp in most of the blends. Healthy juice had the highest ^{0}Brix level among the treatments while the 50% blend had the lowest ^{0}Brix level among the treatments as expected. In addition, the 20% blend had a higher

^{0}Brix and slightly higher acidity than the 10% blend. As such the 10% blend had a slightly higher ^{0}Brix/Acid ratio while the BrimA index which is more correlated to ^{0}Brix was slightly higher for the 20% blend than the 10% blend. The results of Table 4-9, correlate with the sweetness levels in each blend, with the healthy juice very similar to the 20% blend, followed by the 10% blend and 50% blend. This is also reflected in the BrimA index values for each blend. However, the closeness in sweetness between the 10% blend and the 20% blend is also mirrored in the ^{0}Brix/acid ratio where there is a slight difference between both blends. As stated earlier, it is not unusual for higher blends to have a sweeter or less acidic content compared with lower blends. The reason for this is variation of juice characteristics as different batches of fruit are blended together. All treatments had similar color coordinates albeit only a* and b* was significantly different among all the treatments with L*, a*, and b* having p values of 0.08, 0.03, and 0.04, respectively. The 10% blend had a slightly lighter (less yellowish and more light greenish) color.

After testing Valencia juice, it was important to see if any similar patterns emerge in Hamlin juice blends. Therefore a repeat of the experiments (difference-from-control test, hedonic test and juice analysis) was conducted for Hamlin juice from the December 2nd, 2011, and pasteurized on January 19th, 2012. Experiment 8 was a difference-from-control test on pasteurized Hamlin juice (01-19-12) carried out on 60 panelists. The following treatments in this experiment include: control (healthy juice), 5% blend and the 10% blend. Results are shown below:

Table 4-10. Difference-from-control test of pasteurized hamlin juice (01-19-12)

	Control	5% Blend	10% Blend
Mean Score	3.67±2.267[a]	4.32±2.418[a]	4.34±2.440[a]

[a] Means with the same subscripts show no significance at alpha = 0.05

The results shown in Table 4-10 show that there was no significant difference among the control, 5% blend and 10% blend. These results confirm earlier results shown in Table 4-7, which used Valencia juice. The results in Table 4-10 show that panelists were unable to detect a difference between among any combination of blends and control. In other words, panelists could not detect a difference between 5% blends and either the control juice or the 10% blend. Similarly, consumers could not detect between the 10% blend and either the control or the 5% blend. This result is also significant because it reflects the differences in blending techniques, i.e. between blending by juice and blending by fruit. Blending by fruit involves juicing the fruit first, and since the juice is a fraction of the fruit component, then a percentage (e.g. 5%) of fruit mass ultimately results in less than 5% of juice mass. In addition, greening-affected fruit tend to be smaller in size and ultimately juice mass than healthy fruit. Therefore, a 10% blend by fruit is actually less than stated and thus explains why panelists are able to clearly detect 10% blends by juice and are just barely able to detect 5% blends by juice.

A juice analysis of the treatments (5% blend, 10% blend, and control juice) involved in the difference-from-control test of Hamlin juice (01-19-12) as well as greening-affected juice was conducted and the results are shown below.

In Table 4-11, the pH values do not seem to correlate well with the acidity content in each blend. One probable reason for this variation in pH could be due to the presence of high levels of sinking pulp in most of the blends if not well agitated. The 5% blend has the highest ºBrix level among the treatments while the 10% blend has the lowest ºBrix level among the treatments. As stated earlier, this variation in ºBrix levels

Table 4-11. Analysis of pasteurized hamlin juice (01-19-12)

	Healthy	5% Blend	10% Blend	Greening
pH	3.83±0.021	3.90±0.020	3.90±0.015	3.82±0.005
Brix	9.4±0.0	10.4±0.0	8.4±0.0	9±0.0
Corr. Brix	9.51±0.0	10.51±0.0	8.54±0.0	9.15±0.0
Acidity	0.535±0.019	0.544±0.064	0.665±0.019	0.733±0.019
Brix/Acid[i]	17.8	19.3	12.8	12.5
BrimA	7.9	8.8	6.5	6.9
L	55.77±0.271	59.52±0.708	53.54±0.272	56.09±0.522
a	-1.24±0.050	-0.60±0.119	-1.74±0.070	-1.75±0.034
b	29.52±0.236	33.54±0.678	27.57±0.535	28.17±0.375

[i] Values are presented as averages of three replicates

whereby the blends have a higher ºBrix than the control or lower ºBrix than the greening juice is not unusual in the blending by fruit strategy. When balanced by the acidity levels, the 5% blend has the highest ºBrix/Acid ratio followed closely by the healthy juice. The 10% blend has a much lower °Brix/Acid ratio compared with the healthy juice and the 5% blend, and is closely followed by the greening juice which has the lowest ºBrix/acid ratio. Usually, a gap so large in the difference between the ºBrix/Acid ratios of the 10% blend and the sweeter 5% blend and healthy juice is expected to be noticeable by panelists. However, the results reflected in Table 4-10 suggest that the panelists were unable to detect a difference possibly because the ºBrix/acid ratio for the 10% blend was still fairly acceptable by the panelists. Kimball (1999) reports that commercial juice should have a ºBrix/acid ratio of 13.0 which is very similar to the 10% blend Brix/acid ratio of 12.6. The BrimA values indicate how sweet each treatment tastes, and has a stronger correlation with ºBrix values as shown above where the 10% blend has the lowest ºBrix and lowest sweetness while the 5% blend has the highest ºBrix and highest sweetness. There was some variability among the treatments had all color coordinates significantly different among all treatments with L*, a*, and b* having p

values of <0.001 for each coordinate. This variability among the treatments in terms of color could be attributed to the 5% blend having noticeably positively higher L*, a*, b* coordinates which reflected the pulpiness and dark orange color of the blend compared with other treatments.

Experiment 9 was a difference-from-control test with higher blend treatments (20% blend, 50% blend) including the 10% blend and control juice. This experiment was carried out on 60 panelists and the results are shown below:

Table 4-12. Difference-from-control test of pasteurized hamlin juice (01-19-12)

	Control	10% Blend	20% Blend	50% Blend
Mean Score	3.85 ± 2.169^{b}	3.88 ± 2.179^{b}	4.50 ± 2.508^{ab}	5.17 ± 2.395^{a}

[a] Means with the same letter show no significance at alpha = 0.05

The results reflected in Table 4-12, suggest that panelists can clearly distinguish between the 50% blend and either the 10% blend or control juice. This is important because for both Valencia and Hamlin juice, when blended by fruit mass, panelists have been consistently able to detect 50% blends and discern a difference from control juice. The results also suggest that the 20% blend might be a probable threshold value in detection of greening juice in blends with control juice as the base. The reason for this suggestion is that the 20% blend is marginally discernible as different from both the 10% blend and the control on the one hand, or the 50% blend on the other hand. In this way, the 20% blend by fruit mirrors the 5% blend by juice, which was also barely discernible as different from the control juice. Surprisingly, the 10% blend was ranked very similar to the control despite its lower ^{0}Brix and ^{0}Brix/acid ratio, as shown in Table 4-12. It was therefore important to analyze the higher blends (20%, and 50%) and contrast with the already analyzed healthy juice and greening-affected juice. This is shown below:

Table 4-13. Analysis of pasteurized hamlin juice (01-19-12)

	Healthy	20% Blend	50% Blend	Greening
pH	3.83±0.021	3.89±0.010	3.89±0.011	3.82±0.005
^{0}Brix	9.4±0.0	8.6±0.0	9.4±0.0	9±0.0
Corr. ^{0}Brix	9.51±0.0	8.72±0.0	9.53±0.0	9.15±0.0
Acidity	0.535±0.019	0.558±0.020	0.654±0.009	0.733±0.019
^{0}Brix /Acid[i]	17.7	15.6	14.5	12.5
BrimA	7.7	7.0	7.6	7.0
L	55.77±0.271	55.15±0.681	57.22±0.256	56.09±0.522
a	-1.24±0.050	-1.73±0.120	-1.57±0.160	-1.75±0.034
b	29.52±0.236	27.05±0.808	29.93±0.807	28.17±0.375

[i] Values are presented as averages of three replicates

Observing Table 4-13, it's not immediately clear that the 50% juice blend is clearly different from healthy juice as noted by panelists in Table 4-12. The 50% blend has the same ^{0}Brix level as the healthy juice. However, the higher acidity content in the 50% blend means it has a slightly reduced ^{0}Brix/acid ratio and sweetness compared with the healthy juice. Similarly, there is no immediately clear reason why the 20% blend is barely discernible from the healthy juice despite having the second lowest ^{0}Brix level of all the treatments (the lowest being the 10% blend). However, because the acidity content of the 20% blend was lower than the 50% blend, the former had a slightly higher ^{0}Brix/acid ratio than the later blend. However, BrimA values which show that the 50% blend was indeed sweeter than the 20% blend, reflecting the higher ^{0}Brix level of the 50% blend. Although color coordinates (L*a*b) for all treatments were similar they were all significantly different from each other with L, a, and b having p values of 0.004, 0.001, and 0.001, respectively. All the treatments had varying intensities of a greenish-yellow hue with the 20% blend closest to the greening-affected juice in color.

The results seem to suggest that the ^{0}Brix/acid ratio or BrimA may not be the best tool to detect differences among blends. The off flavor of greening juice, as stated

earlier, results from an imbalance in flavor compounds and is independent of soluble solid content or acidity levels. The comparatively higher acidity of the blends due to the presence of greening juice did not result in unfavorable ^{0}Brix/acid ratios that were not comparable to those of healthy juice. The results emphasize that blending masks off flavors not necessarily associated with ^{0}Brix and acidity, and the 20% blend could represent the minimum amount of flavor compound imbalance noticeable to panelists. Thus the 50% blend, containing a higher level flavor compound imbalance as a result of a higher level of greening juice was noticeable to panelists. It is important to emphasize that the flavor compound imbalance refers to possibly lower levels of esters particularly ethyl butyrate, and possibly higher levels of terpenes, alcohols and aldehydes, compared with healthy juice (Dagulo 2010). It is important to emphasize that the sensory evaluation results of Table 4-12 are empirical, and that no chromatographic testing was done on any of the treatments in this study to determine the quantity and quality of flavor compounds present. Nevertheless, this study is important as it builds on research which clearly has outlined the flavor imbalance as a critical factor in the development of off flavors associated with greening juice.

The next step in the research study was to assess whether panelists found any of the blends favorable. To assess this favorability, a hedonic test was carried out on 60 panelists with the following treatments: 10% blend, 20% blend and 50% blend. The 10% blend was selected for two reasons; to assess its favorability and secondly, to serve as a control based on the results from Table 5-0, which suggested that panelists could not determine a difference between the 10% blend and the healthy juice. The results of this hedonic test are shown below:

The results of Table 4-14 show that there is no significant difference among all treatments in overall acceptability, sweetness, and orange flavor. This suggests that although panelists can detect a difference between the 50% blend and the control, as reported in Table 4-12, it was not a statistically significant unfavorable difference from the control (or 10% blend). Similarly, although panelists could barely detect the difference between the 20% blend and the control, also reported in Table 4-10, the barely detectable difference was not a statistically significant unfavorable difference compared with the control. In fact, there was no significant difference between the control (the 10% blend) and any of the blends (20% and 50% blends).

Table 4-14. Hedonic test of pasteurized hamlin juice (01-19-12)

	10% Blend	20% Blend	50% Blend
Overall Acceptability	6.03±1.785[a]	5.87±1.751[a]	5.75±1.847[a]
Sweetness	5.95±1.770[a]	5.68±1.780[a]	5.52±1.790[a]
Orange Flavor	5.72±1.869[a]	6.03±1.438[a]	5.87±1.692[a]

[a] Means with the same letter show no significance at alpha = 0.05

The significance of these results (Table 4-14) is that for Hamlin oranges, in this particular season, up to 50% of the total fruit mass of greening-affected fruit can be blended alongside healthy fruit to form a juice blend that is still acceptable to consumers. A lower blending percentage of 20% or lower, greening fruit mass can also be blended alongside healthy juice and would be the better option considering that panelists would still be able to detect a difference at 50% or higher blends.

The high percentage (up to 50%) of greening-affected fruit capable of being blended may seem surprising but is not unusual. Wagner (1978) showed that 15-35% tangerine juice blended in grapefruit juice was significantly different from, and as well as

preferred to, unblended grapefruit juice. Inyang (1979) showed that the astringency of cashew apple juice could be masked by blending in orange juice at a ratio of 60:40, which was also the most preferred blend ratio by the panelists. It is also important to re-emphasize that 50% greening-affected fruit may translate to a lower percentage of juice blended with healthy juice, due to the tendency of greening fruit to be smaller in size and thus lower in volume of juice available for extraction from greening-affected fruit.

Experiment 10 was a hedonic test using Valencia juice with 61 panelists asked to assess the overall acceptability, sweetness and orange flavor of control juice (healthy juice), the 20% blend, and the 50% blend. The results are shown below:

Table 4-15. Hedonic test of pasteurized valencia juice (04-07-11)

	Control	20% Blend	50% Blend
Overall Acceptability	7.03 ± 1.505^{a}	6.97 ± 1.169^{a}	6.69 ± 1.397^{a}
Sweetness	7.05 ± 1.499^{a}	6.95 ± 1.617^{ab}	6.39 ± 1.626^{b}
Orange Flavor	6.82 ± 1.607^{a}	7.02 ± 1.284^{a}	6.69 ± 1.373^{a}

[a] Means with the same letter show no significance at alpha = 0.05

The results outlined in Table 4-15 suggest that there is no significant difference between control juice and the 20% blend or 50% blend in overall acceptability and orange flavor. There was however, a significant difference between the 50% blend and the Control with respect to sweetness with the former rating the lowest in sweetness according to the panelists. Despite the comparatively low sweetness of the 50% blend, it is important to note that it was still found acceptable and comparable to control juice by panelists.

This confirms our results from Table 4-14, that the 50% blend although noticeably different from control juice, is still comparatively acceptable to control juice by panelists.

Similarly, the 20% blend was not significantly different from either the control juice or the 50% blend in terms of sweetness, which confirms our suspicion that the 20% blend could be a minimum threshold of detecting a difference between itself and control juice.

It is still important to perform a juice analysis of each treatment in the study testing for juice parameters. The results are shown below:

Table 4-16. Analysis of pasteurized valencia juice (04-07-11)

	Healthy	20% Blend	50% Blend
pH	3.76±0.000	3.75±0.011	3.69±0.011
^{0}Brix	12.4±0.0	12.2±0.0	11±0.0
Corr. ^{0}Brix	12.56±0.0	12.37±0.0	11.18±0.0
Acidity	0.789±0.059	0.814±0.013	0.876±0.019
^{0}Brix /Acid[i]	15.9	15.2	12.8
BrimA	10.2	9.9	8.5
L	52.47±0.862	52.97±0.890	52.47±0.495
a	2.12±0.279	2.74±0.517	2.02±0.170
b	35.50±0.992	37.23±1.834	34.88±0.551

[i] Values are presented as averages of three replicates

The results in Table 4-16, show that the healthy juice is very similar to the 20% blend in ^{0}Brix and acidity, and consequently having very similar ^{0}Brix/acid ratios. The results also show that the 50% blend has the lowest ^{0}Brix as well as the highest acidity among all treatments, thereby giving it the lowest ^{0}Brix/acid ratio among all treatments. Although the 50% blend for both Hamlin and Valencia juice had a ^{0}Brix/acid ratio that was lower by a factor of three, than either the healthy juice or control juice, it seems for Valencia juice panelists were able to detect a difference in sweetness, while for Hamlin juice panelists were unable to detect a difference in sweetness. This suggests that differences in sweetness may be more pronounced in Valencia juice that has a more pronounced deeper flavor than that an early season fruit juice such as Hamlin juice. As expected, the BrimA scores correlated strongly with the ^{0}Brix of each treatment, with

healthy juice having the highest BrimA score followed by the 20% blend and finally the 50% blend having the lowest BrimA score and sweetness. The color coordinates were fairly similar with L*, a* and b* values not significantly different among treatments having p values of 0.6, 0.09, and 0.13 respectively.

Finally, a Pearson correlation was performed to assess the relationship between objective flavor indicators (ºBrix/acid ratio and BrimA index) with hedonic parameters (overall acceptability, sweetness and orange flavor) of the greening-affected juice blends (Control, 5% blend, 10% blend, 20% blend and 50% blend). The BrimA index will be assessed at three different 'k' values (3, 4 and 5). The 'k' coefficient of a BrimA index accounts for the tongue's higher sensitivity to acids than sugars, normally varying from 2-10 (Jaya 2007). There is some confusion as to what the appropriate 'k' value is for orange juice. Jordan (2001) finds the BrimA with k = 5 as appropriate for citrus fruits, while Obenland (2009) finds the BrimA with k = 3, 4 or 5 to be similar when correlated with hedonic parameters. It is therefore important to investigate the BrimA at k = 3, 4 and 5, to determine which sensitivity index ('k') bests correlates with the hedonic parameters of the greening-affected juice blends. The purpose of these correlations is to determine how effective these indicators (ºBrix/acid ratio and BrimA index) are at assessing the hedonic parameters of greening-affected juice blends. Table 4-17 shows the correlation of ºBrix/acid ratio and BrimA with hedonic parameters of greening-affected juice blends from Valencia fruit.

Table 4-17. Correlation of °brix/acid ratio and brimA with hedonic parameters for valencia juice (4-07-11)

	Overall Acceptability	Sweetness	Orange Flavor
^{0}Brix/Acid[i]	0.31	-0.35	0.44
BrimA			
k = 3	0.80	0.18	0.11
k = 4	0.73	0.07	0.14
k = 5	0.38	-0.36	0.22

The results in Table 4-17 show that the ºBrix/acid ratio was a poor predictor of overall acceptability, sweetness (negatively correlated) and orange flavor of juice blends from Valencia fruit. The BrimA index had a strong positive correlation with overall acceptability at k = 3, albeit having a very poor positive correlation with sweetness and orange flavor. The BrimA index at k = 4 was slightly lower, with very poor positive correlations with sweetness and orange flavor. The BrimA index at k = 5 had the weakest correlation with overall acceptability with poor correlations with sweetness (negatively correlated) and orange flavor. There was a strong similarity between the BrimA index correlations at k = 5 and the ºBrix/Acid ratio correlations.

Correlations (Pearson's r) were also found for Hamlin juice blends, similarly assessing the relationship between the ºBrix/acid ratio and BrimA against the hedonic parameters obtained from Hamlin fruit. These correlations are shown in Table 4-18.

Table 4-18. Correlation of °brix/acid ratio and brimA with hedonic parameters for hamlin juice (4-07-11)

	Overall Acceptability	Sweetness	Orange Flavor
^{0}Brix/Acid[i]	0.64	0.66	0.78
BrimA			
k = 3	0.61	0.62	0.73
k = 4	0.62	0.64	0.75
k = 5	0.64	0.66	0.77

[i] Values are presented as averages of three replicates

The results in Table 4-18 show that the ºBrix/acid ratio correlated fairly strongly and positively with overall acceptability, sweetness and orange flavor of juice blends from Hamlin fruit. Similarly, the BrimA index had a fairly strong correlation with all hedonic parameters at k = 3. The correlations tended to get slightly stronger with increasing k values and at k = 5 the correlations closely mirrored the ºBrix/acid ratio correlations. This corresponds with Table 4-17, where correlations of BrimA at k = 5 with hedonic parameters was also the most similar to the Brix/acid ratio correlations compared with different 'k' values (3 and 4) of the BrimA index. This seems to suggest that the BrimA at k = 5 is the most equivalent to ºBrix/acid ratio.

The correlation results of Table 4-17 and Table 4-18 are inconclusive in determining the efficacy of the indicators at assessing the hedonic parameters of the juice blends. While the ºBrix/acid ratio had poor correlations with all hedonic parameters in Valencia juice blends, it had moderately better correlations with all hedonic parameters in Hamlin juice blends. The BrimA index (at k = 3, 4, and 5) also had poor correlations with sweetness and orange flavor, but much stronger correlations with overall acceptability in Valencia juice, while it had similarly moderate correlations with all hedonic parameters in Hamlin juice. Part of the variability apparent in the weak and moderate correlations is partly due to the fact that neither of the indicators accounts for the role of volatile compounds, which is very influential in greening-affected fruit as stated earlier. Nevertheless, an explanation of the variability of sensory data is naturally limited until more experiments with juice from more harvest seasons can be done. This would be beneficial in providing a more balanced assessment of the efficiency of each indicator in relation to hedonic parameters of the juice blends.

Summary and Conclusion

The purpose of this study was to determine whether greening affected juice could be successfully blended in the control juice. For the preliminary studies, 5% and 10% levels were chosen based on undocumented industrial practices. Preliminary results suggested that 5% and 10% blending levels are appropriate for blending by juice. Once blending levels were determined, a triangle test was conducted using pasteurized Valencia juice blends at 5% and 10% levels along with a control (healthy juice). The results confirmed preliminary findings that the 5% blend was just barely statistically significant in difference from the control, while the 10% was clearly significantly different from control juice. In Experiment 3, some inconsistencies in blending occurred such that the 10% blend had a higher ^{0}Brix than the 5% blend, leading to a repeat of the experiment, with similar conclusions except this time the 5% blend was clearly not significantly different from the control juice. The conclusion from both experiments is that 5% blend could represent a flavor threshold for greening juice. In Experiment 4, the 5% blend was clearly not significant from the control juice while in Experiment 3 it was border line significantly different from the control juice. Nevertheless, the conclusion favors the borderline conclusion when considering preliminary results which in all cases were borderline significant from the control at the 5% blending level. Similarly, it was clear that blending at 10% levels was clearly significantly different from the control juice in both experiments and in all preliminary findings. Investigating the analytical profiles of both the 5% and 10% blends, it was noticeable that both blends were similar in ^{0}Brix levels, titratable acidity, ^{0}Brix/acid ratios, BrimA index values and even in some color

coordinates (L*). This suggests that ^{0}Brix/acid ratios and BrimA index may not be as effective in detecting blend differences from control juice.

The next objective was to assess consumer acceptance of the juice blends. Since the consumers could detect a difference in the blends, it was important to determine how different the blends were compared to the control juice. Once that was determined, then it would be necessary to assess how preferable the blends are to consumers. In addition, it was also important to assess new blending strategies, in this case blending by fruit mass, and contrast that with the previous strategy of blending by juice mass. The results of the difference from control test and hedonic test on both 5% and 10% blends from Valencia fruit shows no significance between both blends and control juice. This means that for blends by fruit, both the 5% and 10% blends were not as significantly different from the control. In addition, the 5% and 10% blends were as preferable as the control juice. At this point, a difference between blending strategies seems to reflect the difference in results obtained from similar blending levels. This difference in blending strategies can be attributed to two reasons; first, a lower amount of greening-affected juice is utilized in blends by fruit due to the smaller fruit size of symptomatic fruit as well as lower volume of juice, and secondly, variation that is present and intrinsic in blending by fruit, as not the same fruit and consequently juice is blended into healthy juice.

It was at this point that higher blends (blends higher than 10% greening juice) were considered. The following treatments were then utilized in the study: 20% blend and 50% blend, which were both blends by fruit. The difference-from-control and hedonic tests were repeated using those blends as well as the 10% blend and control

juice. The results showed that the 50% blend was significantly different from the control. The 20% blend was not significantly different from the control juice while curiously the 10% blend was significantly different from the control but not significantly different from the 20% or 50% blend. The hedonic test results (without the 10% blend) showed that panelists found the 50% blend less sweet albeit still acceptable overall when compared with the control juice. The 20% blend was found preferable and not significantly different from control juice in overall acceptability, sweetness and orange flavor. Therefore, the conclusion derived from results is that consumers can definitely detect the 50% blend as being different from the control juice. That difference was shown to be that of a lower sweetness comparatively to the 20% blend and control juice. Nevertheless, as a blending strategy for Valencia fruit it seems riskier to blend greening fruit at 50% or higher mass level. It is more conservative and thus recommended to blend at a 20% or lower level which has been shown to be hedonically preferable to control juice.

Lastly, it was important to confirm previous results with new tests using Hamlin juice. It had been previously hypothesized that Hamlin juice having a bland and poorer flavor would have been affected in a bigger way by citrus greening, with differences and off-flavors from greening-affected juice more accentuated in the blends. A repeat of difference-from-control and hedonic tests showed similar results with Valencia fruit, with the 5% and 10% blends not significantly different from control juice and hedonically similar to control juice. Higher blends (20% blend and 50% blend) of Hamlin greening-affected juice were next assessed, again with difference-from-control and hedonic tests, with the results showing that the 50% blend was significantly different from the control

juice having the largest difference among all treatments compared to the control juice. The 20% blend was not significantly different from the 10% blend, and the 50% blend. This suggests the 20% blend as a probable flavor threshold for panelists. The flavor threshold in this study was not defined but can be explained as the point (or range) of marginal detection of a difference by a panelist. The hedonic results showed no significant difference for any of the blends (20% and 50% blends) in overall acceptability, sweetness and orange flavor when compared with control juice. From these results, it can be concluded that there was no clear difference in accentuation of off-flavors prominent in greening-affected juice in Hamlin blends when compared to Valencia juice blends. Albeit the 50% blend was hedonically similar to the control juice, it is recommended that blending at that level may be too risky when considering seasonal variation in addition to intrinsic variation inherent in the blending design. It is recommended that the 20% blend or lower be used by juice processors as a conservative way to utilize greening-affected juice with less risk of consumer detection in juices.

APPENDIX
BALLOT TEMPLATE

To begin test, please click on the continue button below:

Please indicate your gender.

- ❍ Male
- ❍ Female

Male:
Please indicate your age range.

- ❍ Under 18
- ❍ 18-29
- ❍ 30-44
- ❍ 45-65
- ❍ Over 65

Question # 3.

Female:
Please indicate your age range.

- ❍ Under 18
- ❍ 18-29
- ❍ 30-44
- ❍ 45-65
- ❍ Over 65

Question # 4.

How often do you drink orange juice?

- ❍ Once per day
- ❍ Once per week
- ❍ Once per month
- ❍ Once per year
- ❍ Never

Fig 2. Sample demographic questions used in sensory tests

Triangle Test

Today you will be tasting orange juice.

Take a bite of cracker and a sip of water to rinse your mouth.

Remember to do this before you taste each sample.

WHEN ANSWERING ANY QUESTION, MAKE SURE THE NUMBER ON THE CUP MATCHES THE NUMBER ON THE MONITOR.

Please click on the 'Continue' button below.

In front of you are three samples. Two samples are identical, and the other is different. Taste the samples in the order indicated below and identify the *DIFFERENT* sample.

<<Sample1>> **<<Sample2>>** **<<Sample3>>**

Fig 3. Sample ballot for orange juice triangle test using a nine-point hedonic scale

HEDONIC TEST

Take a bite of cracker and a sip of water to rinse your mouth.

Remember to do this before you taste each sample.

WHEN ANSWERING ANY QUESTION, MAKE SURE THE NUMBER ON THE CUP MATCHES THE NUMBER ON THE MONITOR.

Please click on the 'Continue' button below

Please indicate how much you like or dislike each **Orange Juice** sample **OVERALL**.

Sample <<Sample1>>

Overall Liking

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

Sweetness

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

Orange Flavor

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

Fig 4. Sample ballot for orange juice hedonic test using a nine-point hedonic scale

Difference-From-Control Test

Today you will be tasting orange juice.

Take a bite of cracker and a sip of water to rinse your mouth.

Remember to do this before you taste each sample.

WHEN ANSWERING ANY QUESTION, MAKE SURE THE NUMBER ON THE CUP MATCHES THE NUMBER ON THE MONITOR.

Please click on the 'Continue' button below.

Please take a sip of Sample 000.

You will be comparing the other samples to this one.

Click the Continue button below to proceed.

Please indicate how different each sample is when compared to the control (**Sample 000**).

Difference from Control

Sample <<Sample1>>

No different									Extremely different
1	2	3	4	5	6	7	8	9	10

Sample <<Sample2>>

No different									Extremely different
1	2	3	4	5	6	7	8	9	10

Sample <<Sample3>>

No different									Extremely different
1	2	3	4	5	6	7	8	9	10

Fig 5. Sample ballot for orange juice difference-from-control test using a ten point scale

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BIOGRAPHICAL SKETCH

Chinedu Ikpechukwu was born in the vibrant city of Lagos, Nigeria. Chinedu's interest in food science sprang from visiting a superstore for the very first time and culminated in a genuine curiosity about food production and processing. This spurred him on to pursue a bachelor of science in chemical engineering from New Jersey Institute of Technology. Upon graduating in May 2009 and with thoughts of pursuing a higher degree, Chinedu was eager to satisfy his now insatiable hunger for food science. Luckily, he became aware of University of Florida offering a masters degree in food science and human nutrition, thus applying and getting admission in January 2010. Chinedu has greatly enjoyed his experience as a Food Science graduate student, gaining some knowledge in Food Product Development, Food chemistry and Citrus Processing. In addition, Chinedu has been buoyed by the opportunity to participate in research that has helped hone his analytical and sensory skills. Graduating in May 2012, Chinedu hopes to obtain a career in food product development with an eye to starting a food business in the long term.