Title

Postharvest Studies to Improve Consumer Quality of Hot Water Treated Mangos (Mangifera indica L.) Imported to the United Sates.

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Postharvest Studies to Improve Consumer Quality of Hot Water Treated Mangos (*Mangifera indica* L.) Imported to the United Sates.

By

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THESIS

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Abstract

Mango (*Mangifera indica* L) is a climacteric tropical fruit that has become popular around the world. US is the largest imported of mango in America, importing mangos from Mexico, Guatemala, Peru, Ecuador, Brazil, Haiti, and Puerto Rico. Mangos most often spend several days (15 to 36) in transit from their country of production to the wholesale markets in the US. Upon arrival at the East Coast (Philadelphia) and West Coast (Los Angeles), mangos are transferred to distribution centers prior to retail stores. Presently 85 to 90 % of imported mangos to the US receive a hot water treatment (HWT) to control Mediterranean and Mexican fruit fly since late 1980s (USDA-APHIS-PPQ, 2022). There are limited postharvest studies of imported mangos to the US available and most of them were carried out using non hot water treated (NHWT) mangos. Over the years, US Mango consumption has increased from 1.7 to 3.6 pounds per capita during the last two decades based on HWT contribution. However, the US mango per capita consumption is still lower than other commodities available in similar yearly cycle. In 2020, bananas, apples, pineapples, and table grapes had a 27.4, 17.6, 7.3, and 8.4 per capita respectively. We believe that for imported mangos to compete with the wide variety of fruits and vegetables available to purchase and satisfy consumers, a clear understanding on consumer quality and postharvest quality deterioration at arrival and distribution in the US is important to propose postharvest handling strategies during transportation, distribution centers, retail stores and consumer education.

As a first step of our work, we surveyed mango quality at arrival to stores across US for one season. Based on these results, we hypothesized that the main barriers to increase US mango consumption further were chilling injury incidence, consumer sensory quality, softening problems, low availability of high flavor ‘ready to eat’, and lack of handlers-consumer education. Therefore, two studies were developed following our previous assessment of arriving quality of HWT mangos imported to the US. These chapters deal with modifications of the current ‘ready to eat’ ripening protocol (Testing the Performance of Exogenous Ethylene Application on Ripening of Hot Water Treated Imported Mangos (*Mangifera indica,* L.) – Chapter 1) and Understanding the Role of the Physiological Maturity and Shipping Temperature on HWT Mango

Arrival Quality (market life potential-chilling injury– Chapter 2). Our results, working with HWT mangos, revealed current potential mango limitations and pointed out at which step(s) during handling quality, problems were occurring. This new information allows assisting postharvest handling changes and establishing strong educational programs to reduce these problems and increase US mango consumption.

Chapter 1 Exogenous ethylene gas treatment is part of the ripening protocol to deliver 'ready to eat' mangos that satisfies consumers preferences and increase mango consumption in the US. In the first chapter, the hypothesis stated that ethylene effectiveness would vary depending on the maturity ripening category of HWT mangos at arrival because mature mangos were already producing ethylene or being exposed to exogenous ethylene from other mangos during handling and transportation to market area. The performance of ethylene was assessed in three batches of 'Tommy Atkins' mango, one batch of 'Ataulfo', one batch of 'Keitt' mangos, all from Mexico, and a batch of 'Kent' mango from Peru. All mangos were treated with hot water expect for 'Keitt' that came from a fruit fly free zone. Data collected during this study supported the hypothesis and suggested that exogenous ethylene treatment is not necessary to achieve the 'ready to eat' stage for HWT mangos. Furthermore, ethylene treatments add handling, cost and delays to the mango postharvest chain that can result in lower fruit quality due to physical damage.

The second chapter was a study in cooperation with CIAD (Culiacan, MX) to find the major defects that impact quality of hot water treated mangos due to low temperature, and temperature exposure during shipment or storage. 'Tommy Atkins', 'Kent', and 'Ataulfo' mangos were collected from a packinghouse in Escuinapa, Culiacan, Mexico. They were classified into three harvest maturity stages using a non-destructive DA meter, and evaluating shape, skin color, shoulder shape, shoulder location in relation to peduncle insertion and skin texture. Quality was assessed at harvest and during cold storage; the initial destructive quality measurements include flesh firmness, flesh color, soluble solids concentration (SSC), and dry matter percentage. Cold storage evaluation was conducted on ripe mangos and assessed external chilling injury (CI) symptoms such as lenticel discoloration, skin pitting, grayish scald, and surface scald. Internal decay was also part of this evaluation and included loss of flesh color, and in severe cases flesh browning. Our results highlighted skin discoloration and fast softening during transportation as the main barriers to deliver high quality mangos at arrival after 30 days.

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Abbreviations

1. 1-Methylcyclopropene (1-MCP)
2. Australian Mango Industry Association (AMIA)
3. Analysis of Variance (ANOVA)
4. Animal and Plant Health Inspection Service (APHIS)
5. Centers for Disease Control and Prevention (CDC)
6. Centro de Investigacion en Alimentacion y Desarrollo (CIAD)
7. Center for the Promotion of Imports from developing countries (CBI)
8. Coefficient of Variation (CV)
9. Chilling Injury (CI)
10. Critical Bruising Thresholds (CBT)
11. Dry Matter (DM)
12. Ethylene Factor (EF)
13. Food and Agriculture Organization (FAO)
14. Foreign Agricultural Service (FAS)
15. Fruit Texture Analyzer (FTA)
16. Good Agricultural Practices (GAP)
17. Hot Water Treated (HWT)
18. Mango Maturity and Ripening Guide (MMRG)
19. Minimum Quality Index (MQI)
20. National Mango Board (NMB)
21. Newton (N)
22. Not Hot Water Treated (NHWT)
23. Plant Growth Regulator (PGR)
24. Plant Protection and Quarantine Program (PPQ)
25. Relative Humidity (RH)

26. Soluble Solids Concentration (SSC)
27. Titratable Acidity (TA)
28. United States (US)
29. United States Department of Agriculture (USDA)

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Introduction

1. Mango Origin and Production

Mango (*Mangifera indica* L.) is a climacteric tropical fruit produced in tropical and subtropical areas worldwide. It has been called "king of the fruits" because of its nutritional value and pleasant taste, aroma and texture (Barbosa Gámez et al, 2017). It is native to the Indo-Burna region and has been cultivated in India for over 4000 years (Paull and Duarte, 2011). The Spanish introduced mango to Hawaii and the west coast of Mexico between 1800 and 1820 (Paull and Duarte, 2011). Since then, mango production has extended to over 100 countries (Evans et al., 2017). Mango is grown on ~ 3.7 million ha, making it the second-most cultivated tropical fruit after banana. There are over 1000 named mango varieties, but only a few are grown commercially (Jahurul et al., 2015). Asia produced 72.5% of the mango, mangosteen and guava cluster crop in 2020, followed by Africa (15.7%), America (11.7%), and Oceania (0.1%). The largest global importers were the United States (545,000 t; 27.0%) and China (380,000 t; 19.0%) (FAOSTAT, 2020).

The United States imports mangos from Mexico (65.0%), Peru (10.0%), Ecuador (9.0%), Brazil (7.1%), Haiti (2.3%), Guatemala (4.6%), and Puerto Rico (USDA Foreign Agricultural Service, 2018). These countries provide continuous availability of six major cultivars to US markets (Figure 1) (National Mango Board, www.mango.org). 'Tommy Atkins' is the most important cultivar grown in the Western Hemisphere. It is a round-shaped cultivar bred in Florida, characterized by firm flesh, medium juiciness, medium fiber and thick, orange-yellow skin with a dark red blush. Its resistance to handling and shipping stress make it a popular choice for export (Knight et al., 2009). 'Kent', 'Keitt', and 'Haden' are other round cultivars bred in Florida (Brecht et al., 2014). 'Ataulfo' from Mexico and 'Francis' from Haiti are flat cultivars (Brecht et al., 2014) characterized by thin skin and soft, sweet and very juicy flesh (Knight et al., 2009). Over the past two decades, mango consumption in the US has increased steadily, reaching 3.6 pounds per capita in 2020 (Shahbandeh, 2021). As US consumers become more informed, providing high-quality, flavorful ripe fruit that is rich in nutrients is crucial to maintain and increase the demand for mango.

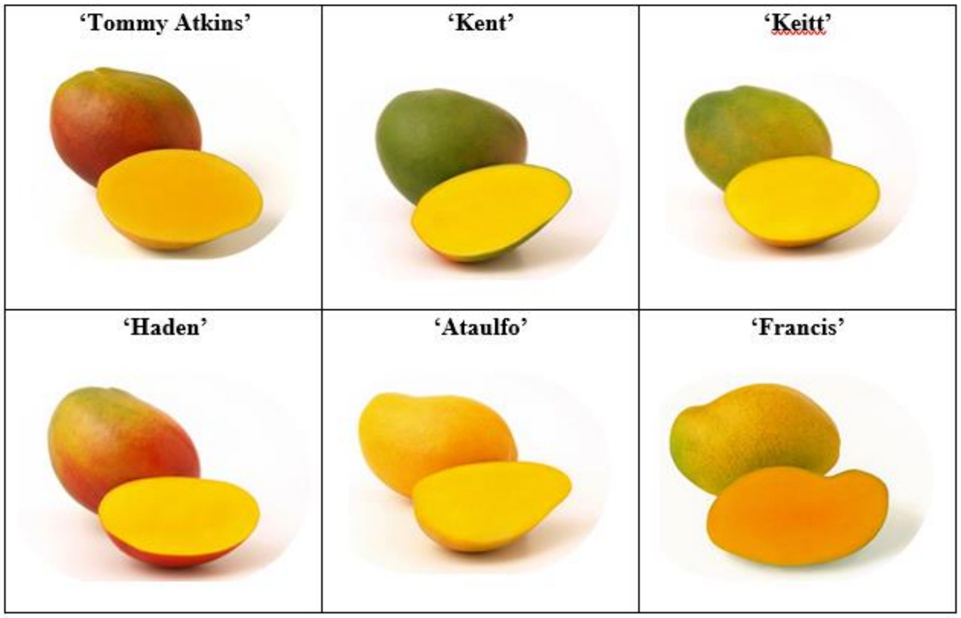

Figure 1. Major mango cultivars imported to the US market. (National Mango Board, mango.org)

2. Postharvest Handling

Postharvest handling includes all operations from harvest until consumption or fruit death. Its primary goal is to deliver fruits that meet consumer preferences. External characteristics like fruit size and skin color are critical because these attributes are observable at the time of purchase. However, to fully satisfy consumers and earn additional purchases, internal factors such as flesh firmness, sugar content, acidity, and aroma are critical (Crisosto and Costa, 2008).

2.1 Consumer quality

Consumer quality encompasses external (fruit size, shape, and skin color) and internal (flesh firmness, sugar content, acidity and aroma) sensory attributes (Crisosto and Costa, 2008) in addition to nutritional value and food safety to achieve high consumer quality, mangos are harvested at the firm mature green (unripe) stage to withstand transportation and postharvest handling (Figure 2). Several postharvest handling steps prepare mangos for foreign markets. Hot water treatment (HWT), temperature management, and ripening protocols enhance eating quality, ensure food safety, and mitigate early quality decay when conducted properly. Otherwise, they can cause external and internal disorders such as lenticel spotting, uneven ripening, skin pitting and internal browning (Figure 3).

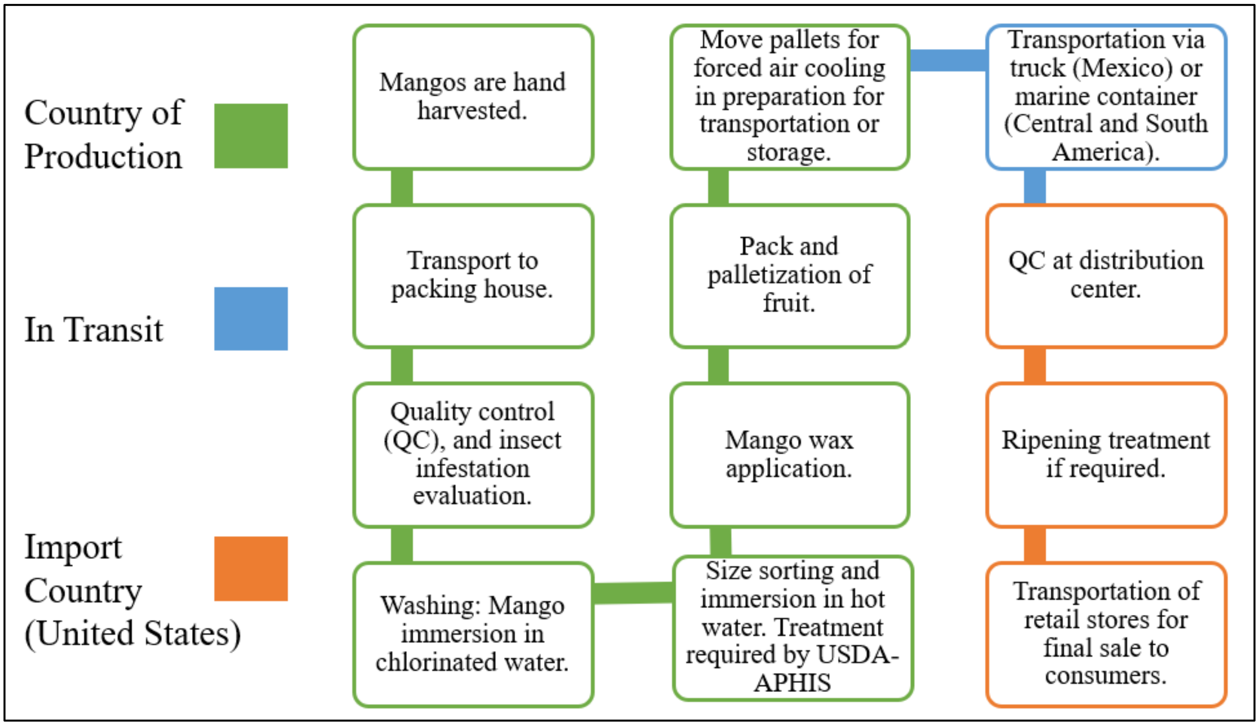

Figure 2. Major steps in mango postharvest handling of hot water treated mangos imported to the US market. (Brecht et al., 2014; Kader et al., 2002).

2.2 Hot Water Treatment (HWT)

HWT is a non-chemical phytosanitary method approve by United States Department of Agriculture (USDA) to control Mediterranean fruit fly (*Ceratitis capitata* or Medfly) and Mexican fruit fly (*Anastrepha ludens*) (Brecht et al, 2014; Hernández et al., 2017). This treatment also minimizes several microorganisms on mango surfaces and anthracnose decay caused by *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* (Kader et al., 2002; Johnson and Hofman, 2009). Medfly is found throughout the Mediterranean region, Southern Europe, the Middle East, Western Australia, South and Central America and Hawaii; thus, 85 to 90% of mangos arriving in US markets are HWT (Brecht et al., 2014; Hernández et al., 2017). According to the treatment manual from the Animal and Plant Health Inspection Service - Plant Protection and Quarantine program (APHIS-PPQ), this treatment can only take place in a certified facility and monitored by APHIS personnel. Mangos must be pre-sorted by size, weight and shape (Table 1). Pulp temperature must be 21.1°C (70°F) or above prior to treatment and the hot water bath temperature must be at 46.1°C (115°F) within the first five minutes of immersion. Immersion time varies between 65 and 110 minutes depending on the size and shape of the fruit (Table 1). If mangos are hydrocooled within 30 minutes after the hot water treatment, the regulations request the immersion be prolonged for an additional 10 minutes (USDA-APHIS-PPQ, 2022). Water quality should be monitored to avoid spreading other diseases. Mango has no history of causing outbreaks in the US, except for a *Salmonella enterica* outbreak between November and December, 1999, that was traced back to a single mango farm in Brazil. The investigation cited water for immersion as a possible point of contamination (Sivapalasingam et al., 2003) highlighting the importance of water quality controls in packing houses.

Table 1. Guidelines for hot water treatment by mango cultivar according to the USDA-APHIS-PPQ treatment manual. (USDA-APHIS-PPQ, 2022). Available at: https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf.

Mango Shape	Fruit Weight (grams)	Time Required (Minutes)
Rounded: ‘Tommy Atkins’, ‘Kent’, ‘Haden’, ‘Keitt’. 	≤ 500	75
	501-700	90
	701-900	110
Flat: ‘Frances’, ‘Ataulfo’. 	≤ 375	65
	376-500	75

2.3 Temperature Management

Cold temperature during storage and transportation is a common postharvest technique that slows deterioration and ripening to maintain fruit quality and extend shelf life. Tropical fruits like mango are highly susceptible to chilling injury (CI). Major chilling injury symptoms in mango include poor taste and aroma, grayish, scald-like skin discoloration, lenticel spotting, skin pitting, uneven ripening and flesh browning (Brecht and Yahia, 2009; Sivakumar, 2011; Figure 3). CI symptoms do not always develop during cold storage, but become visual when fruits are brought to warmer display temperatures (Rymbai et al., 2012). CI symptoms are a major concern for the mango industry because they affect fruit appearance and flavor, reducing consumer acceptance and market value. The current recommended temperature range to prevent chilling injury during mango transportation and storage is 10 to 13°C (50 to 55°F). However, it is important to consider maturity, and cultivar susceptibility. For instance, ‘Haden’ and ‘Keitt’ are more CI susceptible than other cultivars (Kader et al., 2002; Brecht and Yahia, 2009).

An important concept when studying CI in fruit is the critical chilling temperature threshold, or the temperature (over a specific time) below which irreversible injury may occur. This must be established for each fruit or vegetable and is often time- and cultivar-specific. The threshold temperature is the lowest temperature at which a susceptible commodity can be held with no symptoms of CI ever developing. In peach, the relationship between temperature and time of exposure is more important than temperature itself; therefore, the safe temperature and exposure period must be determined (Crisosto et al., 1999). The observed discrepancy among limited existing reports of a threshold temperature for mangos may be due to differences in cultivar susceptibility and fruit maturity. No previous study was undertaken at a production site to systematically compare the responses of different major mango cultivar at different maturity stages to a range of potentially CI-inducing time and temperature combinations. Knowing the critical combinations of time and temperature that induce CI in the most important mango cultivar imported to the US would allow shippers to decrease the incidence of CI and thus deliver higher-quality, better-tasting mangos to the consumer. Further research is needed to understand how transportation time, shipping temperature, cultivar and maturity affect the onset and severity of chilling injury symptoms.

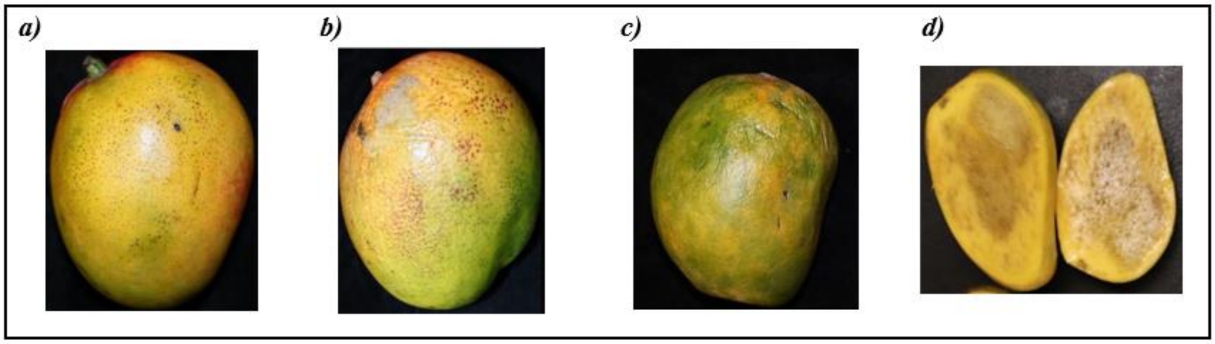

Figure 3. Major chilling injury symptoms; a) lenticel spotting, b) skin pitting, c) uneven ripening, d) flesh browning (Photo courtesy: Velasquez and Crisosto, 2019).

3. Maturity

Maturity at harvest is especially important to assure mango consumer quality. There are two terms for maturity that are important to distinguish: *physiological maturity*, defined as "the step, in development when a plant or plant part (fruit) will continue ontogeny even if detached" (Watada et al., 1984) and *horticultural maturity*, defined as "the stage of development when a plant or plant part possesses the prerequisites for utilization by markets and consumers for a particular purpose" (Watada et al., 1984). A definition specific to mangos is provided by the United States Standards for Grades of Mangos and regulates marketing. It defines *maturity* as "the stage of development that will ensure the completion of the ripening process" (USDA/AMS, 2007), referring to physiological maturity. This definition does not assure consumer satisfaction.

Ideally, mangos should be consumed when ripe to appreciate flavor. *Ripe* is a late stage of fruit development when fruits, on or off the tree, express characteristic aesthetic and flavor qualities. These include changes in composition, color, texture, and other sensory attributes (Watada et al., 1984). Mangos are harvested physiologically mature and firm to tolerate postharvest treatments, handling, and long-distance transportation from their country of production to foreign markets such as the US To provide guidance in selecting mature mangos, the National Mango Board (NMB) has established five maturity stages based on flesh color development at the production site (one of the best indicators of maturity) and ripeness (Figure 4) (NMB, 2010; Yahia, 2011).

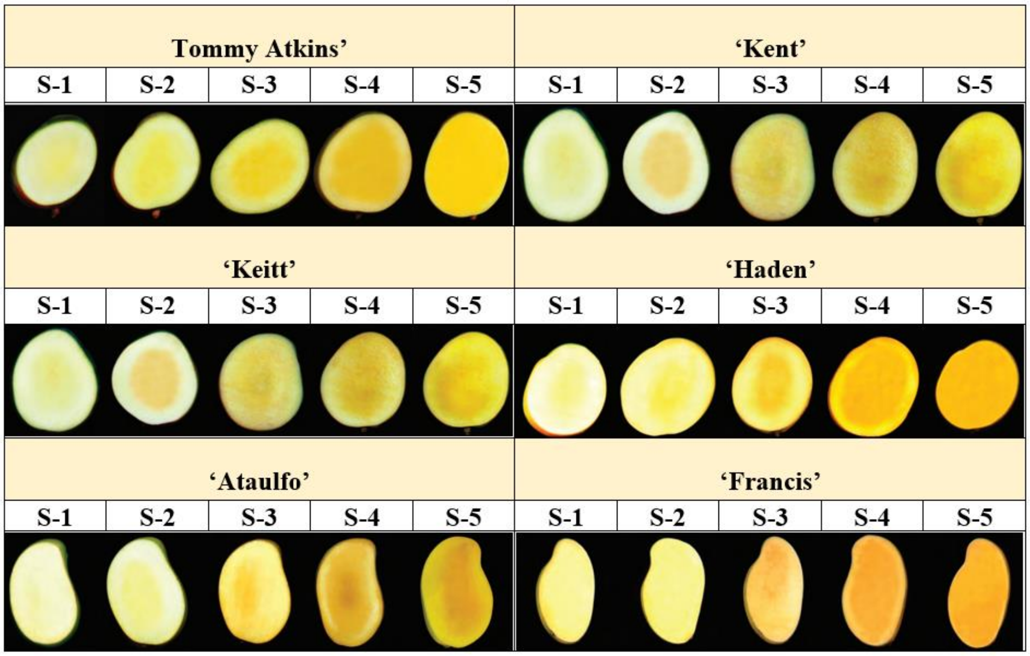

Figure 4. Flesh color development of major US imported cultivars (S-1: immature; S-2: mature (unripe); S-3: firm ripe; S-4: soft ripe; and S-5: over ripe) (NMB, 2010; Brecht et al., 2014). Available at: https://www.mango.org/wp-content/uploads/2017/10/Mango_Maturity_And_Ripeness_Guide.pdf.

In addition to this flesh color guide, texture (firmness) and soluble solids concentration (SSC) provide additional reference for evaluating maturity and ripeness (NMB, 2010; Crane et al., 2009; Yahia, 2011). Firmness is an objective measure of the force required to penetrate fruit flesh (fruit softening) (Nassur et al., 2015), determined objectively using a penetrometer. SSC is the soluble solids concentration of a juice solution, commonly used to assess maturity and/or quality of fruits, vegetables, wines, and other beverages. SSC encompasses sugars, acids, soluble pectins, and other soluble constituents, but is considered equivalent to sugar content (Son et al., 2009). Fully rounded shoulders are also a helpful visual indicator of physiological maturity for pickers (Yahia, 2011; Brecht et al., 2014). Implementing these parameters to select mature firm unripe mangos at harvest elevates ripe mango quality potential and consumer quality.

3.1 Changes During Maturation and Ripening

Mango maturation and ripening occur from day 49 to day 77 after fruit set (Tharanathan et al., 2006; Maldonado-Celis et al., 2019). Reaching physiological maturity prior to harvest is important because by this stage of development, the fruit has accumulated vitamins, starch, sugars, and phenolic compounds that will allow proper ripening and flavor (Maldonado-Celis et al., 2019). Ripening is an irreversible process of biochemical and physiological changes such as flesh softening, starch degradation, accumulation of soluble solids (sugars, acids, soluble pectins, and other soluble constituents), production of aromatic volatiles and increased carbon dioxide and ethylene production (Pandit et al., 2010; Liu et al., 2015). During ripening, starch decreases due to hydrolysis, allowing soluble sugars to accumulate in the pulp and enhancing fruit flavor (Figure 5). Aromatic volatiles are synthesized from terpenoids, fatty acid-derived C_6-volatiles, phenylpropanoid aromatic compounds and alkanes, alkenes, alcohols, esters, aldehydes, and ketones (Pandit et al., 2009). As chlorophyll is degraded, skin color changes from green to yellow, orange, and/or red. At the same time, carotenoids and anthocyanins are synthesized and accumulate in cell vacuoles to enhance flesh and skin color (Yashoda et al., 2006; Yahia, 2011; Maldonado-Celis et al., 2019). In addition to these chemical changes, hydrolysis of structural polysaccharides causes fruit softening (Yashoda et al., 2006; Gill et al., 2017). Starch degradation, soluble solids accumulation, color development and fruit softening determine the expression of desirable characteristics at ripening.

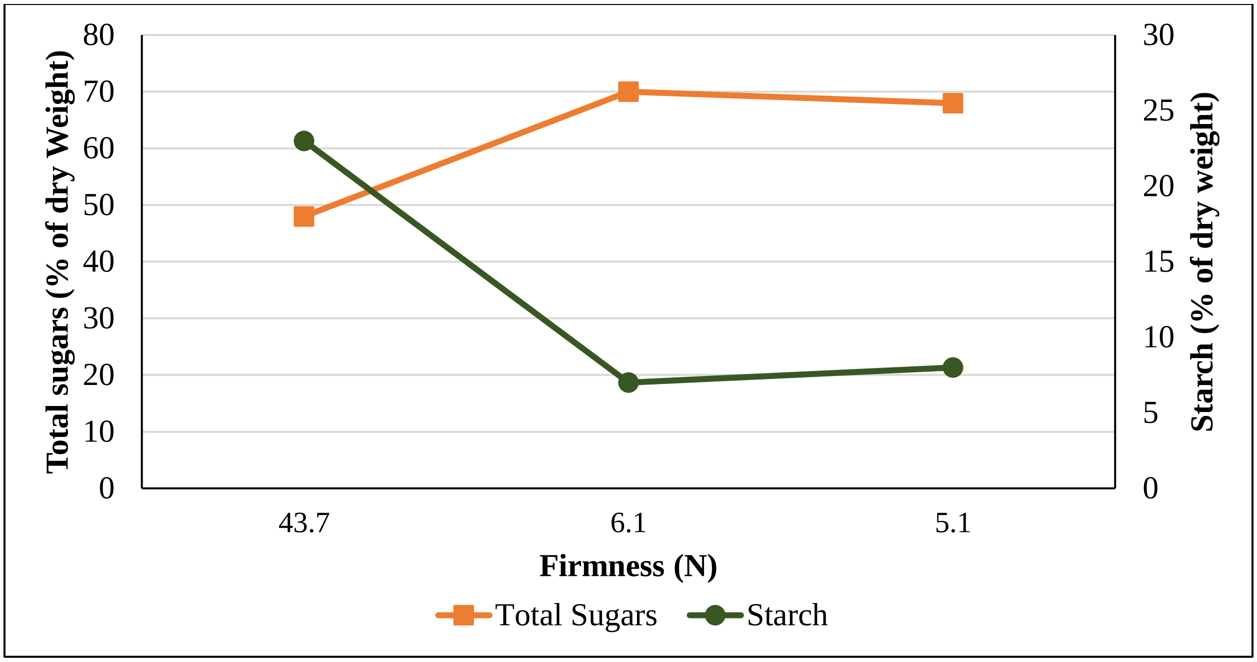

Figure 5. Total sugars and starch concentration of imported 'Haden' mangos measured at different firmness stages at 20°C (68°F) (Gonzalez-Moscoso, 2014).

4. Consumer Quality

There are three key components of consumer quality: sensory quality, nutritional quality, and food safety.

4.1 Sensory Quality

Sensory quality is the set of attributes that are perceived by the consumer and includes fruit size, skin color flesh color, flesh firmness, sugar content, acidity, and aroma (Crisosto and Costa, 2008; Delgado et al., 2013). It has been assessed by measuring SSC, dry matter (DM), firmness, flesh color, and visual appraisal of external and internal defects. Sensory evaluations by trained panels and consumer surveys should be correlated with these objective measures to further understand and predict consumer preferences (Kader, 2002). Following this recommendation, sensory analysis and consumer preference surveys were carried out by our group (Makani, 2013; Gonzalez-Moscoso, 2014; Nassur et al., 2015). This data provided the basis of the 'ready to eat' mango program, that combines controlled ripening with a minimum quality index (MQI) for consumer acceptance.

4.2 Nutritional Quality

Another aspect of consumer quality is nutritional value and health benefits for consumers. Mango is a source of carbohydrates such as starch and pectins. It offers small amounts of protein, amino acids and fatty acids. Mango is a good source of sugars, including glucose, fructose and sucrose, although sucrose is the major sugar in 'ready to eat' (ripe) mangos. These sugars are a major contributor to the nutrition and flavor of this commodity. Mango provides vitamins C, A, B-6, E and K. The high concentration of vitamin A aids vision and some metabolic functions (Rymbai et al., 2013). Mango also provides minerals such as calcium, magnesium, and phosphorus (Maldonado-Celis et al., 2019). Mango nutrients can vary depending on area of cultivation, management of the orchard, and overall health of trees. The major nutrients and quantities available in mango based on USDA FoodData Central analysis are describe below (Table 2).

Table 2. Raw mango nutritional values in a 100 g portion of 'Tommy Atkins', 'Keitt', 'Kent' and 'Haden' cultivars. (USDA FoodData Central, 2019). Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/169910/nutrients.

Name	**Quantity**	**Units**
Sugars (glucose, fructose, and sucrose)	13.7	g
Fiber, total dietary	1.6	g
Proteins	0.8	g
Total lipids	0.4	g
Calcium	11.0	mg
Magnesium	10.0	mg
Phosphorus	14.0	mg

Potassium	168.0	mg
Vitamin C	36.4	mg
Vitamin B-6	0.1	mg
Folate	43.0	µg
Vitamin A	54.0	µg

Mango is considered a functional food because it provides dietary fiber, minerals, fatty acids, vitamins and bio-active compounds such as polyphenols, ascorbic acid, and carotenoids (Rymbai et al., 2013; Barbosa Gamez et al., 2017; Maldonado-Celis et al., 2019) Lupeol in mango pulp has anti-inflammatory, anticancer, antimicrobial, and anti-mutagenic properties that help to reduce the incidence of tumors (Rymbai et al., 2012; Liu et al., 2021). The antioxidant activity of carotenoids helps prevent degenerative diseases such as cancer muscular diseases and neurological, inflammatory and immune disorders (Rymbai et al., 2013). Mango polyphenols, particularly gallotannins, promote beneficial gut microbiota that metabolize unabsorbed food components and prevent chronic inflammatory bowel disease (Kim et al., 2021).

4.3 Food Safety

While mangos are not a common cause of disease outbreaks in the US, S*almonella enterica* contamination is a challenge for the mango industry. *S. enterica* uses water as a mode of transport (Liu, 2018) and mango packers use water for fruit washing and for the HWT required since 1988 by USDA/APHIS (Sivapalasingam et al., 2003). This treatment increased water utilization in the packing line and thus the probability of *S. enterica* contamination. *S. enterica* outbreaks linked to mangos in the US were reported in January 2000 and October 2012 by the Centers for Disease Control and Prevention (CDC) (Sivapalasingam et al., 2003; CDC, 2012). Following Good Agricultural Practices (GAP) from the USDA including water quality controls, and preventive practices such as water sanitization can minimize cross contamination and prevent future outbreaks (USDA/AMS, 2022).

5. 'Ready to eat' Program

The 'ready to eat' program uses controlled ripening under ethylene and temperature management to provide ripe mangos, using a minimum quality index (MQI) to assure consumer satisfaction. The relation between fruit firmness and consumer acceptance was determined for different cultivars to express maximum flavor (Nassur et al., 2015). Consumer sensory studies based on flesh firmness revealed acceptance of 87.3% for ripe (4.5 to 13.3N) and 89.2% for partially ripe (15.6 to 26.7N) mangos, but only 39.1% acceptance for mature green mangos (51.2 to 71.2N). Thus, the ideal ripening stage based on firmness for 'ready to eat' mangos is between 4.5 and 13.3 N (Nassur et al., 2015; Crisosto et al., 2017).

A successful 'ready to eat' program also uses a minimum quality index (MQIs) based on dry matter (DM; Table 3) to ensure consumer acceptance and future purchases (Makani, 2013; Gonzalez-Moscoso, 2014; Nassur et al., 2015). Consumer 'in store' sensory tests confirmed that sensory quality is important for mango purchase and consumption. SSC can be measured at the ripe, 'ready to eat' stage (RSSC) to predict consumer quality. However, SSC does not predict consumer quality upon mango arrival at the import country because it increases throughout postharvest handling due to starch degradation, making SSC an unreliable measure (Figure 5, Gonzalez-Moscoso, 2014).

Dry matter (DM) is the weight of all mango pulp components excluding water (Anderson et al., 2017), including starch, soluble sugars, organic acids, pectins, cell walls, and minerals. It was proposed as minimum quality index in kiwifruit and mango because it remains constant throughout postharvest handling and ripening and it is highly correlated with both RSSC and consumer acceptance (Crisosto, 2001; Gonzalez-Moscoso, 2014; Anderson et al., 2017). 'In store' consumer tests demonstrated that DM is an accurate and reliable quality index that predicts mango consumer quality potential upon fruit arrival (Table 3; Gonzalez-Moscoso, 2014). Marketing 'ready to eat' mangos that meet MQIs based on cultivar-specific

DM percentages provides a useful strategy to monitor imported mango quality in the US market and set quality goals for the industry to achieve by implementing ripening protocols. Upon arrival in the US, mangos currently go through quality control and a ripening treatment consisting of 100 ppm exogenous ethylene exposure for 24 to 48 hours at 20°C (68°F) and 90 to 95% relative humidity (RH; Kader et al., 2002; Crisosto et al., 2017; Brecht et al., 2014).

This study's objectives were to identify measurable parameters of imported mangos that predict consumer acceptance, determine barriers to delivering 'ready to eat' fruit, and recommend change in postharvest handling to overcome those barriers.

Table 3. Proposed minimum quality indices for the 'ready to eat' program, based on DM for major cultivars in the US market. (Gonzalez-Moscoso, 2014; Nassur et al., 2015).

Cultivar	DM (%) MQI
Ataulfo	15.0
Francis	15.0
Tommy Atkins	14.0
Haden	14.0
Kent	15.0
Keitt	15.0

6. Mango Quality Survey

To identify potential obstacles to meeting MQIs and the 'ready to eat' stage standard, our laboratory conducted a quality survey of imported mangos from East and West Coast stores. Mango samples from Peru, Mexico, Guatemala, Ecuador and Brazil were collected from four different stores in Davis–Woodland, California and from three stores in Philadelphia, Pennsylvania. 'Tommy Atkins', 'Kent', ‘Keitt’, ‘Haden’, and ‘Ataulfo’ samples were collected every other week during an 11-month cycle (February to December, 2019). For each mango sample, the flesh temperature, fruit firmness, flesh color, soluble solids concentration, dry matter and internal and external defects were determined (Velasquez et al., 2020).

Most mangos surpassed the proposed MQIs at arrival to US retail stores (Figure 6); the average dry matter percentages for all cultivars were greater than the proposed MQI, indicating that a large proportion of mangos in the sample lots exceeded the proposed MQI. Only a small fraction of the mango samples had low DM values, potentially caused by early harvest. This is a common practice to reach early markets and/or to extend shelf life during long shipment by harvesting hard mangos (Anderson et al., 2017). The percentages of fruit that did not reach their cultivar's MQI were: ‘Tommy Atkins’: 22.3%; ‘Keitt’: 3.7%; ‘Kent’: 3.2%; ‘Haden’: 8.8%; and ‘Ataulfo’: 5.6%. This problem is easily corrected by training and supervising pickers, aided by enforcement of MQIs by packers and marketers to avoid marketing immature and/or low DM fruit.

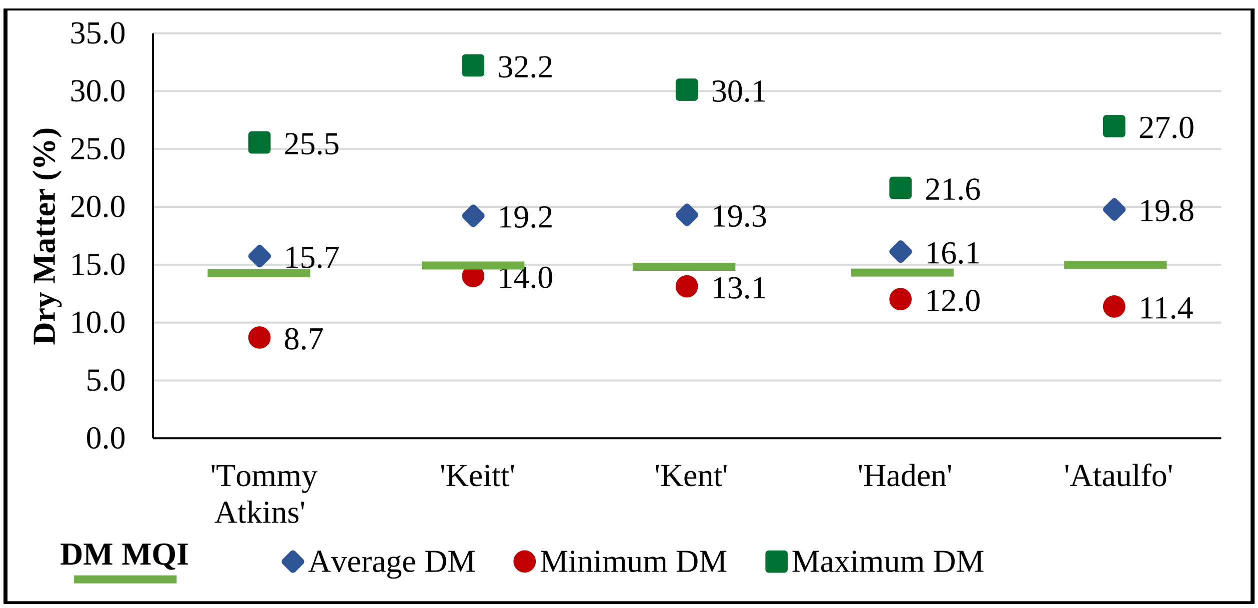

Figure 6. Average, minimum and maximum dry matter percentages of mangos arriving to US retail stores in 2019 (Velasquez et al., 2020), including dry matter minimum quality indices proposed by Gonzalez-Moscoso (2014) and Nassur, et al. (2015).

MQIs should not be considered a major barrier for the industry as they are easily exceeded. Most mangos in US markets can express high flavor and other attributes expected by consumers if ripening is completed prior to fruit consumption.

Firmness is a good indicator of ripening and the 'ready to eat' stage. The firmness of mangos arriving at retail stores was evaluated throughout the entire 11- month cycle (Figure 7).

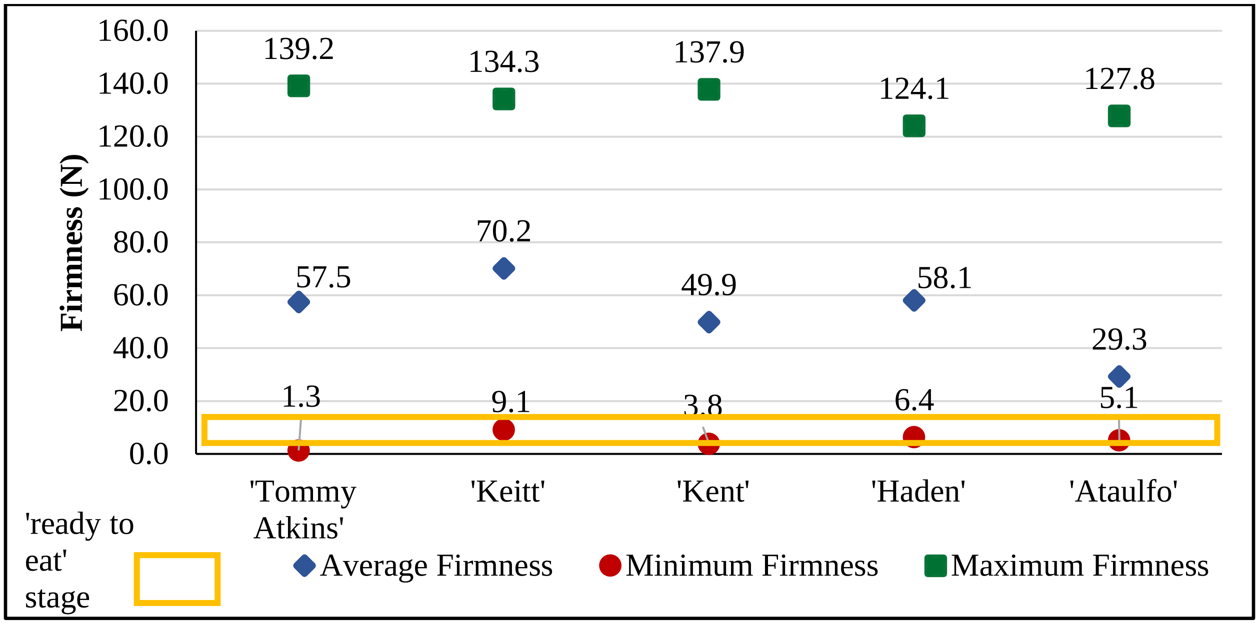

Figure 7. Firmness of major mango cultivars upon arrival at US retail stores (Velasquez et al., 2020).

Our survey found a high variability in firmness at arrival to retail stores (Velasquez et al., 2020), despite previous commercial ripening. Only an average 17.5% of mangos arrived at the 'ready to eat' stage after commercial ripening, with a consumer acceptance rate of ~ 87.3% (Nassur et al., 2015). Also, 34.6% of mangos had firmness lower or equal to the critical bruising threshold ($\leq$ 22.3 N) previously established by our group (Baez et al., 2018). Mangos below this threshold do not require further softening because they are already at or past the desirable 'ready to eat' stage. Critical bruising thresholds based on firmness (CBT) were established previously to predict the impact of these soft mangos on postharvest losses (Gonzalez-Moscoso, 2014; Nassur et al., 2015). Carefully handling should be encouraged to prevent compression and/or impact bruises. Illustrations of physical and other damage observed during this quality survey are available in “Appendix A” of this thesis. The survey also found 38.8% firm mangos (within mature green range, 51.2 to 71.2N or above) arriving at retail stores, reaching as high 139.2 N (Figure 7). Unfortunately, the average firmness of each cultivar arriving at retail stores was within their mature green unripe firmness range (51.2 to71.2N). Mangos within this firmness range have only 39.1% acceptance among consumers (Nassur et al., 2015; Figure 7). These mangos will benefit from a ripening treatment to reach the ‘ready to eat’ stage. To increase mango consumption, it is critical to identify potential obstacles to delivery of ‘ready to eat’ fruit. The high variability in firmness at store arrival and incidence of very firm and soft mangos suggested uneven ripening as an obstacle toward consumer acceptance. This directed us to conduct further research to evaluate the effectiveness of exogenous ethylene treatment in triggering ripening and reducing the firmness variability of HWT and non-HWT mangos. Following current commercial guidelines for ripening treatment (Crisosto et al., 2017), we evaluated how fast, even ripening can be attained (Chapter 1).

We also examined chilling injury incidence because of its important role in postharvest deterioration and consumer acceptance. Consumers decide initially to purchase mango based on external visual factors such as skin color and freedom from defects. Lenticel spotting, an external CI visual damage, affects mango quality perception and market value. This damage is created by deterioration of lenticels, pores on mango peel that facilitate gas exchange, which are affected by biotic (diseases) and abiotic stresses such as low and high temperatures (Rymbai et al., 2012). Our quality survey revealed lenticel spotting as the primary CI symptom expressed by major cultivars in the US market (Table 4). ‘Keitt’ mango had the most severe lenticel spotting incidence (80.1%), which confirmed its high CI susceptibility (Velasquez et al., 2020) and highlighted chilling injury damage as a significant obstacle to increased mango consumption.

Table 4. Chilling injury symptom incidence in major cultivars available in the US market. Defect incidence and severity was evaluated in ripe mangos. Severity was classified as light (L; ≤ 25% affected area) or rejections (R; > 25% affected area) (Velasquez et al., 2020).

Chilling injury symptoms	**Cultivar**									
	‘Tommy Atkins’ n = 1090		**‘Keitt’** n = 141		**‘Kent’** n = 635		**‘Haden’** n = 182		**‘Ataulfo’** n = 672	
	L (%)	R (%)	L (%)	R (%)	L (%)	R (%)	L (%)	R (%)	L (%)	R (%)
Lenticel spotting	25.5	13.1	9.9	80.1	15.4	14.2	16.5	24.7	21.3	18.0
Skin pitting	0.7	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Uneven ripening	0.6	0.4	0.0	0.0	0.0	0.5	0.6	2.8	0.7	0.3
Flesh browning	1.1	1.2	1.4	0.0	1.7	0.3	0.0	0.0	1.6	2.5

Knowledge of CI temperature thresholds is critical to mitigate CI; thus, understanding the influence of shipping time, fruit maturity and shipping temperature on CI symptom severity allows these parameters to be adjusted for better postharvest handling based on cultivar and maturity stage. These survey results justify our detailed study on the relationship between maturity stage and shipping temperature on the onset of chilling injury in HWT mangos.

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Chapter 1

Testing the Performance of Exogenous Ethylene Application on Ripening of Hot Water Treated Imported Mangos (*Mangifera indica,* L.)

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Abstract: Commercial applications of plant growth regulator (PGR) improve tree growth and production performance in several tropical and subtropical commodities. However, there is not enough published information on how PGRs affect consumer quality. The most valuable PGR impact on climacteric fruit consumer quality is the role of exogenous ethylene and 1-MCP on fruit softening. Ripening protocols have been developed and used to deliver 'ready to eat' climacteric fruits such as avocados, kiwifruit, pears, and stone fruit. The biological roles of ethylene and temperature on ripening changes including softening are the bases for the design of successful commercial 'ready to eat' protocols. These protocols, when well executed, allow fruit to ripen evenly, express their maximum flavor at the retail stores and increase consumption, thus, allowing consumers to benefit from a healthy diet. Recent detailed observations on imported mangos arriving to the Netherlands and United States, mostly hot water-treated, suggested that exogenous ethylene may not be required.

Our hypothesis was that the role of exogenous ethylene on imported mango ripening varies depending on mango ripening stages. Thus, imported mangos are either producing endogenous ethylene (physiologically mature), and/or have been exposed to exogenous ethylene from other mangos, during handling-transportation from production to consumption areas, and undergoing different stages of ripening. We discovered that exogenous ethylene was not necessary to ripen imported hot water treated mangos, as fast and proper ripening is only triggered by warming allowing 'ready to transfer or buy' and/or 'ready eat' stages to be reached within one to three days. The use of ethylene application during the ripening protocol required extra facilities, equipment, and handling costs. In addition, ethylene application triggers softening of immature and low-flavor mangos, lowering consumer quality in retail stores. We propose enforcing maturity quality standards to assure consumer quality, proper packaging to avoid bruising, and sorting to segregate mangos into different ripening stages prior to ripening and fruit delivery to retail stores.

Key words: ‘ready to ripen’, ‘ready to eat’, firmness variability, temperature management, sorting, consumer quality.

1. Introduction

Avocados, bananas, breadfruit, cherimoya, durian, guava, jackfruit, kiwifruit, mangos, mangosteen, papayas, passion fruit, plantain, rambutan, sapodilla, sapote, soursop, and pineapple are tropical-subtropical fruits that are of interest to consumers across markets (Watada et al., 1984; Kader, 1997; Wills et al., 2001). Over the last decade, mangos (*Mangifera indica* L.) have expanded marketing share in the United States (US) and Europe (FAOSTAT, 2016; CBI Market Intelligence, 2021) and are now the second-most important tropical fruit crop worldwide after banana (Singh et al., 2013). Mangos are produced in Australia, Southeast Asia, Hawaii, Egypt, Israel, South Africa and Central and South America. The US is the largest single-country retailer of fresh mangos, and the Netherlands is the largest importer in Europe (FAOSTAT, 2016; CBI Market Intelligence, 2021). Mangos imported to the US come from Brazil, Ecuador, Guatemala, Haiti, Mexico, Puerto Rico, and Peru. The most popular cultivars available in the US market are ‘Ataulfo’, ‘Francis’, ‘Haden’, ‘Kent’, ‘Keitt’ and ‘Tommy Atkins’ that have the potential to provide high nutritional value to consumer in addition to excellent sensory attributes. Mango consumption per capita in the US has increased steadily in the past two decades reaching 3.63 pounds per capita in 2020 (Shahbandeh, 2021). Therefore, delivering high quality ripe mangos will attract new consumers and will allow to keep increasing consumption in foreign markets such as the US and Europe.

In mangos, as in avocados, fruit on the canopy cannot ripen properly until removed from the tree. The role of ethylene tree factor (inhibition of the climacteric) while fruit is on the canopy and ethylene factor (internal ethylene and ethylene sensitivity) are poorly understood (Blanpied,1993). Studies have shown that ethylene presence is crucial to initiate and maintain the ripening process on climacteric fruits such as tomatoes (Hoeberichts et al., 2002; Pech et al., 2012). Removal from the tree onsets endogenous ethylene production, ethylene receptors activation, and autocatalytic ethylene production that triggers and coordinates the enzyme systems responsible for the physical and chemical changes associated with ripening (Burg and Burg, 1965; Reddy and Srivastava, 1999). The skin and flesh colors change from green to yellow-orange, flesh softens, and flavor develops during ripening allowing for the expression of sensory quality that fulfills consumers satisfaction (Kader, 1999; Brecht and Yahia, 2009). Based on this climacteric biological concept, fruits are harvested at physiological maturity, defined as 'The stage of development when a plant or plant part will continue ontogeny even if detached' (Watada et al., 1984; Sivakumar et al., 2011). Mangos, that are harvested at physiological maturity, should ripen properly, and reach the fully ripe or 'ready to eat' stage in eight to ten days at 20 to 22ºC (68 to 72°F) (Singh et al., 2013) while immature mangos treated with ethylene will soften, they will develop poor flavor (Brecht and Yahia, 2009). Softening of textural firmness is one of the main changes during ripening that influences market life and quality (Yashoda et al., 2007); the enzyme-mediated alterations to the structure of cell wall, partial or complete solubilization of cell wall polysaccharides, and hydrolysis of starch and other polysaccharides allow for softening to occur. Cell wall-degrading enzymes increase activity at the onset of ethylene biosynthesis, and some tend to decrease activity after the climacteric. (Prasanna et al., 2003; Gill et al., 2017). Fruit firmness has shown to be highly correlated to maturity and quality of fruits (Roe and Bruemmer, 1981; Cherng and Ouyang, 2003), thus, it can be used to evaluate maturity and ripening stages. In addition to firmness, there are other parameters such as flesh color development (Mango Maturity and Ripeness Guide - MMRG; NMB, 2019) and rounded shoulders (Brecht and Yahia, 2009 and Yahia, 2011) to determine physiological maturity of each mango cultivar. Mangos are harvested mature (unripe) and firm to withstand the postharvest handling required to bring them to retail market. Thus, ripening-softening occurs during shipment, distribution centers, retail stores, and consumers' homes. Regrettably, consumers encounter inconsistent mango maturity and ripening stages even within a single mango lot (Sivakumar et al., 2011).

In the efforts of reducing immaturity, and ripening inconsistency, flesh color charts (NMB, 2019) and ripening protocols have been developed (Kader et al., 2002; Brecht et al., 2014; Crisosto et al., 2017). Ripening protocols have become standard postharvest handling for avocados, bananas, nectarines, peaches, pear, plum, kiwifruit, and mango to ensure ‘ready to eat’ (ripe) fruit of quality satisfactory to consumers (Crisosto et al., 2004; Crisosto and Valero, 2006; Eaks, 1978). To optimize ripening protocols and educate consumers, three ripening stages have been identified for peaches, nectarines, pears, plums, mangos, and kiwifruit (Crisosto et al., 2001a; Crisosto and Crisosto 2001b; Crisosto et al., 2004). The ‘ready to ripen’, ‘ready to transfer or buy’, and ‘ready to eat’ stages were defined using in-store consumers tests (Crisosto et al., 2001a; Crisosto and Crisosto 2001b) and fruit physical damage laboratory tests (Valero et al., 2007). The ‘ready to transfer or buy’ stage was determined based on the relationship between fruit firmness and susceptibility to mechanical-physical damage and is used to determined when to stop ripening protocols and transport mangos to retail display. Communication between ripening protocol operator and retail outlet is important, as mangos at an advanced ripening stage are highly susceptible to physical damage and decay. Consumer sensory surveys defined the ‘ready to eat’ stage for mango as 4.5-13.3 Newtons flesh firmness, measured on the cheek with an eight mm tip (Nassur et al., 2015). Ripening protocol performance depends on proper ripening conditions (temperature, RH, air velocity and ethylene), monitoring, determination of the ripening stage, and consumer quality of the incoming fruit.

Plant growth regulator (PGR) applications can improve commercial tree growth and production in several commodities. However, there is little information available on PGR benefits to tropical fruit consumer quality. The most valuable PGR studies have addressed using exogenous ethylene to soften climacteric fruits, but there is little information on the effects of using 1-MCP and exogenous ethylene on consumer quality of tropical and subtropical commodities such as mango and avocados (Yahia, 2011 and Wang et al., 2009). Exogenous ethylene has been used in pears, kiwifruit, avocados, and mangos to complement current commercial retail ripening protocols. Under specific conditions, avocado (Eaks, 1978), kiwifruit (Ritenour et al., 1999; Hertog et al., 2016), and pears (Villalobos-Acuña et al., 2011) require exogenous ethylene application to complete ripening. The benefits of exogenous ethylene for fast and uniform softening were examined in freshly non hot water treated harvested 'Kensington' mangos (Wills et al., 2001). Mangos were highly sensitive to ethylene air concentrations ranging from 0.005 to 10 µl L^{-1} at 20°C (68°F). Ethylene treatment decreased the time required to ripen from 12.8 to 7.5 days. However, this study on the response of mangos to exogenous ethylene used freshly harvested mangoes without hot water treatment (HWT) at the production sites. Currently, most imported mangos to the US required HWT by United States Department of Agriculture (USDA) to control Mediterranean fruit fly (*Ceratitis capitata* or Medfly) and Mexican fruit fly (*Anastrepha ludens*) (Brecht et al., 2014; Hernandez et al., 2017). Medfly is spread throughout the Mediterranean region, Southern Europe, the Middle East, Western Australia, South and Central America and Hawaii. Consequently, 85 to 90% of mangos arriving to the US markets are HWT. A study evaluated the ethylene application at 100 µl L^{-1} for 12 hours at 25°C (77°F) on hydrothermal treated 'Ataulfo' and found a decrease of 13 to 9 days of ripening period (Montalvo et al., 2007). Even though, this set of conditions is more similar to commercial mango postharvest handling, it is important to account for the several days fruit is in transit from country of production to wholesale markets in the US that vary from 15 to 36 days depending on country of production (Velasquez et al., 2020) because HWT mangos are already producing ethylene in response to the stress caused by the hot water treatment (Brecht and Yahia, 2009; Baez et al., 2018a and 2018b) thus ethylene cross-contamination is occurring after HWT, and throughout days in transit.

Recent models based on physiological studies utilize firmness, internal ethylene, and ethylene sensitivity as variables to proposed and describe softening of mangos imported into the Netherlands (Schouten et al., 2018); researchers recognized the potential implication of hot water treatment and chilling injury in the ethylene factor. After identifying some of the main factors that play a role in the production of endogenous ethylene and ripening of mangos, we decided that the evaluation of exogenous ethylene application should be conducted upon arrival to the US, because commercial mango ripening occurs now, thus, all the factors (maturity, hot water treatment, and cold transportation) of the commercial setting are accounted for.

The current commercial mango ripening treatment consists of exogenous ethylene exposure at 100 ppm concentration for 24 to 48 hours at 20°C (68°F), and 90 to 95% relative humidity (RH) (Kader et al., 2002; Yahia, 2011; Brecht et al., 2014). Nevertheless, detailed observations on mangos arriving after transportation to the Netherlands (Schouten et al., 2018) and the US (Nassur et al., 2015; Velasquez et al., 2020) suggest that exogenous ethylene may not be necessary. In imported HWT mangos, there is no published data to recommend or justify using exogenous ethylene for commercial ripening.

We anticipated that HWT imported mangos are producing endogenous ethylene (physiological mature) and/or have been exposed to exogenous ethylene during handling and transportation to undergo ripening. Thus, the application of exogenous ethylene as part of the ripening protocol may not be necessary to improve the speed and uniformity of mango ripening. Besides the cost involved in applying ethylene, the extra handling associated with ethylene application may increase losses and/or introduce immature mangos with low minimum quality indices (MQI) to the retail distribution system. Our hypothesis is that the role of exogenous ethylene on mango triggering ripening will vary, depending on the maturity ripeness categories of imported HWT mangos; therefore, the benefits of using ethylene as part of the ripening protocol to speed and synchronize softening must be investigated.

2. Materials and Methods

2.1 Plant Material and Fruit Preparation

Imported mangos from four major cultivars in the US market were selected; ‘Tommy Atkins’, ‘Ataulfo’, ‘Keitt’, and ‘Kent’. Three lots correspond to ‘Tommy Atkins’ mangos, one lot of ‘Ataulfo’ mango, and one lot of ‘Keitt’ mango all coming from Mexico, and one lot of ‘Kent’ mangos from Peru. All these lots were HWT mangos, except for ‘Keitt’ mango used as a comparison. Days in transit for each lot was tracked to ensure mangos were not in transit longer than the typical commercial period. According to our previous one-year quality survey study Mexican, and Peruvian mangos take an approximate average of 15 d (SD = 5.0), and 31 d (SD = 4.7), respectively in transit from the packing house to US distribution-retail markets

(Velasquez et al., 2020). Mangos used in this test were selected free of mechanical, and insect damages, as well as no visual external disorders were obtained at arrival to distribution centers in the west coast of the US in 2019 season (Table 1). Upon arrival at the distribution centers lots were chosen based on their skin color, flesh color, shape, smell, and firmness by gently pressing cheeks and classified at different maturity ripeness categories. Ending with 'Kent' and 'Ataulfo' lots at the Mature-Soft, one 'Tommy Atkins' lot at the Mature-Firm, two HWT 'Tommy Atkins' lots and one NHWT 'Keitt' lot at the Immature-Firm ripeness categories were used in our studies (Table 2).

2.2 Treatments

Each mango lot was divided randomly into two groups of equal number of fruits. Both groups were placed into 330 L control atmosphere tanks at 20°C (68°F) for 24 hours; one group belonged to untreated treatment and did not receive any ethylene application. While the other group that was part of the ethylene treatment was exposed to exogenous ethylene at a concentration of 100 ppm at 20°C (68°F) for 24 hours following National Mango Board (NMB) guidelines (Kader et al., 2002; Brecht et al., 2014). Once ethylene treatment was completed, ethylene and untreated treatments were stored in environmental control rooms at 20°C (68°F) to simulate store display and allow for fruit ripening to initiate.

2.3 Fruit Evaluations

Initial quality evaluation took place upon arrival (day 0) in which, six to eight mangos per each of the six replications were used to measure flesh firmness, flesh color, ethylene production, soluble solids concentration, and dry matter were evaluated from less to more destructive measurements on individual mangos**.** For softening evolution assessment, six to eight mangos from each treatment per mango lot were used per evaluation day.

*Non-destructive measurements***:** Ethylene production rates were measured at arrival per each category lot. and results expressed as microliters per kilogram per hour (Burg and Burg, 1965; Crisosto et al., 1993). Individual fruits were weighed and placed in 0.705 L plastic containers, which were then sealed and held at 20°C (68°F) and 90% RH. Ethylene concentrations were determined using a gas chromatograph equipped with a packed alumina column operated at an isothermal oven temperature of 70°C (158°F), and peak detection was with a flame ionization detector (Carle AGC-211, EG&G Chandler Engineering, Tulsa, OK).

Destructive measurements: Flesh firmness was measured by removing a dime-sized piece of skin from two opposite cheek sides of six to eight individual mangos per each of the six replications per treatment with a mandolin peeler. Firmness was calculated in Newtons (N) force using a fruit texture analyzer (FTA) equipped with a 7.9 mm diameter tip (model GS-14, GÜSS, South Africa). Mangos were classified based on firmness into 'ready to eat', 'ready to transfer or buy', and 'ready to ripen' stages. Flesh visual color was determined by the authors working together according to the Mango Maturity, and Ripeness Guide (MMRG; NMB, 2019) consisting of five scores from stage 1 (immature mango flesh color) to stage 5 (over ripe flesh color). In addition, soluble solids concentration (SSC) was measured using a few drops of mango juice per each of the six replications per treatment on a temperature-compensated digital refractometer (model PR-32, Atago Co., Tokyo, Japan). Dry matter (DM) percentage is a parameter that has been correlated to consumer acceptance (Nassur et al., 2015; Whiley et al., 2006) and was measured during arrival quality evaluation. Thin slices of undamaged flesh from 6-10 mangos per each of the six replications per treatment were collected and dry for 24 hours period at 115°F using a FD-61 NESCO Food Dehydrator. After 24 hours, weight was recorded, and samples were dry for an additional hour. Weight was measured for a second time to ensure dry weight was consistent (Rodriguez-Bermejo and Crisosto, 2017). DM measurements were taken to evaluate potential consumer acceptance. The mango 'ready to eat' program proposes DM minimum quality indices (MQI) of 15% for 'Ataulfo' and 'Francis' mangos, 14 to 15% for 'Tommy Atkins', 'Haden' or 'Kent' mangos that will satisfy 75% of American consumers, according to in-store consumer surveys (Makani, 2013; Gonzalez-Moscoso, 2014; Nassur et al., 2015). Similar approach has been followed by the Australian Mango Industry Association (AMIA, 2016). After, this initial evaluation, the role of ethylene on fruit softening-ripening was evaluated as firmness changes over time by measuring flesh firmness of six to eight mangos per each of the six replications treatment every day or every other day depending on arrival firmness. Flesh firmness values of each mango were used to indicate percentages of mangos in the three ripening stages: ‘ready to eat’ (<17.8 N), 'ready to transfer or buy’ (17.8 – 35.6 N), and 'ready to ripen’ that varies depending on mango cultivar; ‘Kent’ (35.6 – 80.2 N), ‘Ataulfo’ (35.6 – 57.9 N), ‘Tommy Atkins’ (35.6 – 75.7 N), and ‘Keitt’ (35.6 – 66.8 N). Mangos that exceed the ‘ready to ripen’ range for each cultivar were classified as immature fruit. This classification allowed to assess firmness variability among treatments within each lot, and as ripening days advanced.

2.4 Statistical Analysis

In this completely randomized design using two treatments (ethylene and untreated), mango softening rates were used to evaluate the effectiveness of exogenous ethylene treatment in accelerating softening. Firmness data collected during ripening was used along with emmeans, lme4, and dplyr packages in R studio (R Core Team, 2022) to conduct a linear regression analysis. A linear regression analysis, and slope comparison were conducted for ethylene and untreated treatments within each mango lot. Slopes were compared for significant differences using a two-way variance analysis (ANOVA) LSD (p-value < 0.05) created by the implementation of car, and lme4 packages in R Studio. (R Core Team, 2022). In addition, coefficient of variation within every evaluation day was calculated to analyze ethylene effectiveness in reducing firmness variability compared to untreated mango (Table 4).

Table 1. Cultivars information on origin, transportation period, hot water treatment history and maturity ripeness category classification (a) Mature-Soft, (b) Mature-Firm and (c) Immature-Firm that were used for untreated and ethylene treatments in each mango lot.

Cultivar	Country of Origin	Days in Transit	Date of Arrival	Maturity Ripeness Category	Hot Water Treatment Application
‘Kent’	Peru	31	Jan 22	Mature-Soft	Yes
‘Ataulfo’	Mexico	18	Mar 13	Mature-Soft	Yes
‘Tommy Atkins’ (Lot 1)	Mexico	11	April 08	Mature-Firm	Yes

'Tommy Atkins' (Lot 2)	Mexico	9	Mar 13	Immature-Firm	Yes
'Tommy Atkins' (Lot 3)	Mexico	7	April 08	Immature-Firm	Yes
'Keitt'	Mexico	12	Sept 16	Immature-Firm	No

Table 2. Initial quality descriptors of imported US mangos upon arrival to distribution center or retail store of mangos used for untreated and ethylene treatments. Mean and standard deviation showed in parenthesis were calculated on ethylene production rate, firmness, soluble solids concentration (SSC), and dry matter (DM) measurements of each mango lot. Immature fruit percentage was calculated by the number of fruits in stage 1 of the color chart that is specifically for each cultivar in the Mango Maturity and Ripeness Guide (MMRG) from the National Mango Board (NMB, 2019).

Cultivar	**Ethylene Production Rate ($\mu l\ kg^{-1}\ hr^{-1}$)**	**Firmness Mean (N)**	**SSC (%)**	**DM (%)**	**Immature Fruit (%) NMB MMRG**
Mature-Soft Category (HWT)					
'Kent'	0.07 (0.02)	37.4 (22.7)	14.1 (1.2)	16.9 (1.9)	5.0
'Ataulfo'	0.15 (0.02)	29.4 (12.9)	13.7 (0.3)	18.5 (2.0)	0.0
Mature-Firm Category (HWT)					
'Tommy Atkins' (Lot 1)	0.14 (0.3)	48.6 (23.6)	12.7 (1.1)	15.2 (0.9)	8.0
Immature-Firm Category (HWT)					
'Tommy Atkins' (Lot 2)	0.03 (0.01)	126.5 (21.8)	11.2 (1.2)	15.8 (1.8)	80.0
'Tommy Atkins' (Lot 3)	0.04 (0.02)	134.1(8.9)	12.9 (1.1)	17.9 (0.8)	100.0
Immature-Firm Category (NHWT)					
'Keitt'	0.04 (0.02)	103.4(28.5)	7.7 (1.2)	17.4 (2.4)	51.9

3. Results

3.1 Mature-Soft Category (HWT)

Mature-Soft 'Kent' and 'Ataulfo' mangos arrived at retail stores after 31 and 18 days from packing-shipping in Peru and Mexico (Table 1). During the initial evaluation, 'Ataulfo' mango did not showed any immature fruit (0%) while 'Kent' showed only 5% of immature (Table 2) fruit that fell into stage 1 of MMRG color chart (NMB, 2019). Ideally, 90% of mangos in a lot should arrive at least on stage 2 of MMRG color chart (NMB, 2019) showing the initiation of flesh color development close to the seed. Mangos arrived with an average firmness of 37.4 N and 29.4 N for 'Kent' and 'Ataulfo' respectively: placing them into the mature-soft category (Table 2). 'Kent', and 'Ataulfo' mangos were producing 0.07 and 0.15 µl kg^{-1} $hr^{-1}$and displaying DM values of 16.9% and 18.5% respectively upon arrival that are above the proposed MQI for consumers acceptance (Nassur et al., 2015) indicating the high consumer quality potential at 'ready to eat' stage (Table 2).

The softening rates of untreated and ethylene treatments were compared using a linear regression in which 'Kent' and 'Ataulfo' softening rates did not show a significant statistical difference; p-value = 0.09 and 0.46 (Table 3). Coefficient of variation during ripening was also calculated to observe the effect of ethylene treatment on reducing firmness variability. 'Kent' treatments showed a slightly decrease of variability by the third day, and 'Ataulfo' treatments decreased variability constantly during ripening reducing it at least by half in both untreated and ethylene treatments (Table 4).

'Kent' mangos arrived predominantly in the 'ready to transfer or buy' stage with 50% of mangos in this category, and 11% 'ready to eat' (Figure 1). 'Ataulfo' mango lot was evenly split into the three ripening stages; 33% 'ready to eat', 33% 'ready to transfer or buy', and 33% 'ready to ripen' (Figure 1). Among both cultivars and treatments, mango softening occurred quickly during ripening; especially for 'Ataulfo' mango lot that reached 100% 'ready to eat' mangos in both treatments after two days of simulated display. In the case of 'Kent' mangos by the end of the test, between 86 and 94% of ethylene treated and untreated fruit was in 'ready to transfer or buy', and 'ready to eat' ripening stages.

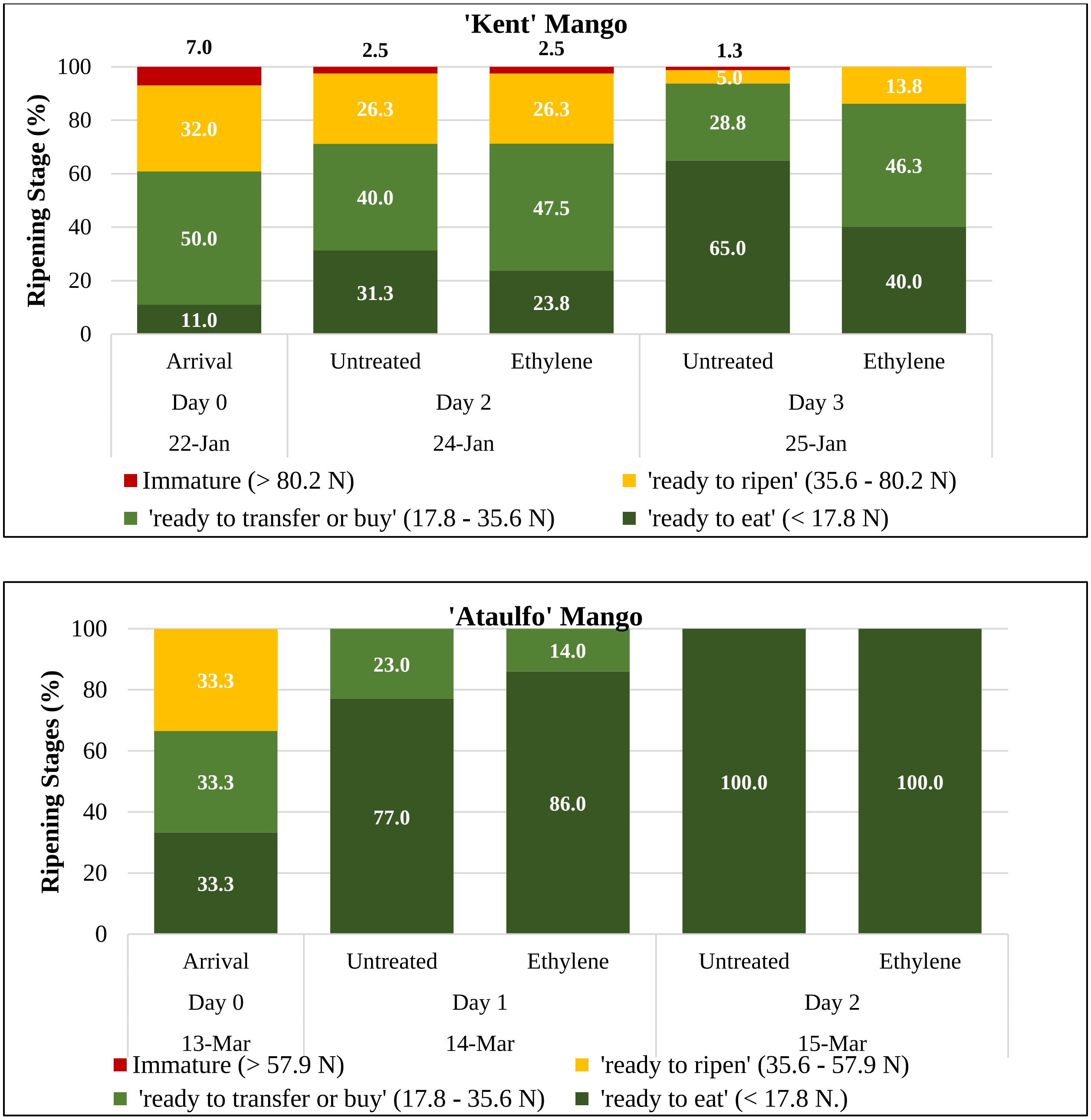


Figure 1. Softening evolution of untreated and ethylene treated mangos express as percentage distribution of mango firmness into three ‘ripening stages’ (1) ‘ready to eat’, (2) ‘ready to transfer or buy’ and (3) ‘ready to ripen’, and immature stage of ‘Kent’ (Jan 22) mangos from Peru and ‘Ataulfo’ (Mar 13) mangos from Mexico belonging to the mature-soft category. Cheek firmness was recorded for two days of ripening at 20°C (68°F) as 100% of mangos reached the ‘ready to eat’ ripening stage.

Table 3. Mango softening evolution of untreated, and ethylene treated mangos; expressed as a linear equation, and statistical comparison of softening rates of both treatments on different mango cultivars, and maturity ripeness categories imported to the United States.

Cultivar	**Treatment**	**Linear Equation**	R^2	**Slope Comparison (p-value)**
Mature-Soft Category (HWT)				
'Kent'	Untreated	y = -7.1x + 41.4	0.15	0.09
	Ethylene	y = -4.0x + 36.1	0.06	
'Ataulfo'	Untreated	y = -7.0x + 23.6	0.40	0.46
	Ethylene	y = -8.6x + 23.6	0.42	
Mature-Firm Category (HWT)				
'Tommy Atkins' (Lot 1)	Untreated	y = -11.1x + 52.1	0.50	0.83
	Ethylene	y = -11.6x + 51.2	0.54	
Immature-Firm Category (HWT)				
'Tommy Atkins' (Lot 2)	Untreated	y = -21.8x + 119.8	0.53	0.17
	Ethylene	y = -26.7x + 124.7	0.78	
'Tommy Atkins' (Lot 3)	Untreated	y = -19.6x + 108.2	0.37	0.86
	Ethylene	y = -20.1x + 91.3	0.60	
Immature-Firm Category (NHWT)				
'Keitt'	Untreated	y = -16.04x + 101.6	0.48	0.002**
	Ethylene	y = -28.9x + 94.4	0.62	

Table 4. Firmness coefficient of variation during mango softening of untreated and ethylene treated mangos classified into maturity ripeness categories and cultivars imported to the United States.

Cultivar	**Treatment**	**Coefficient of Variation during Ripening**					
		Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Mature-Soft Category (HWT)							
'Kent'	Untreated	65.8		61.2	51.2		
	Ethylene	50.6		65.1	48.5		
'Ataulfo'	Untreated	42.2	32.2	21.0			
	Ethylene	49.3	30.6	14.7			
Mature-Firm Category (HWT)							
'Tommy Atkins' (Lot 1)	Untreated	45.6	62.2	46.8	43.7	34.9	
	Ethylene	61.3	59.6	37.1	28.0	20.2	
Immature-Firm Category (HWT)							
'Tommy Atkins' (Lot 2)	Untreated	9.6	48.5	104.6	87.4	138.5	144.5
	Ethylene	8.8	48.2	55.1	45.4	17.6	46.7
'Tommy Atkins' (Lot 3)	Untreated	15.8	32.2	56.4	65.6	108.7	108.1
	Ethylene	10.3	15.0	39.9	46.5	87.0	44.7
Immature-Firm Category (NHWT)							
'Keitt'	Untreated	22.3	51.0	52.3	73.4	87.2	116.8
	Ethylene	32.9	42.6	60.2	67.8		

3.2 Mature-Firm Category (HWT)

Mature-Firm 'Tommy Atkins' mangos spent 11 days in transit from country of origin (Mexico) to retail in the US. This lot had 8% of fruit falling into stage 1 of MMRG color chart considering this 8% immature stage (Table 1). However, the average firmness of the lot was 48.6 N suggesting the dominance of 'ready to ripen' stage; 62% of mango were part of this ripening stage upon arrival (Figure 2). The initial quality evaluation of 'Tommy Atkins' mangos showed endogenous ethylene production (0.14 $\mu l\ kg^{-1}\ hr^{-1}$) to promote ripening, and fruit average DM (15.2%) above MQI (Table 2). In this lot of mature-firm mangos, softening progressed quickly and the relationship between firmness changes and days of ripening was represented using linear regression equations (Table 3). Ethylene-treated and untreated mangos softened at a rate of ~ 11.4 N per day in a uniform pattern, regardless of ethylene treatment. The slope comparison did not show a statistical difference among treatments (p-value = 0.83). Firmness variability measure as coefficient of variability during ripening slowing decrease for both treatments but specially for ethylene treatment as fruits were moving intro 'ready to transfer or buy' and 'ready to eat' ripening stages (Table 4).

Upon arrival, mature-firm 'Tommy Atkins' (April 08) displayed 25% of fruit at the 'ready to eat' and 'ready to buy or transfer' stage. The next day, approximately 36% mangos reached the 'ready to eat' and 'ready to transfer or buy' stage (Figure 2). After two days of ripening, almost 85% of mangos were less than 35.6 N, thus, within the 'ready to buy'. There were not differences on number of mangos in these categories between treatments. By day 4 of ripening, all untreated and treated mangos were below 17.8 N reaching 'ready to eat' stage.

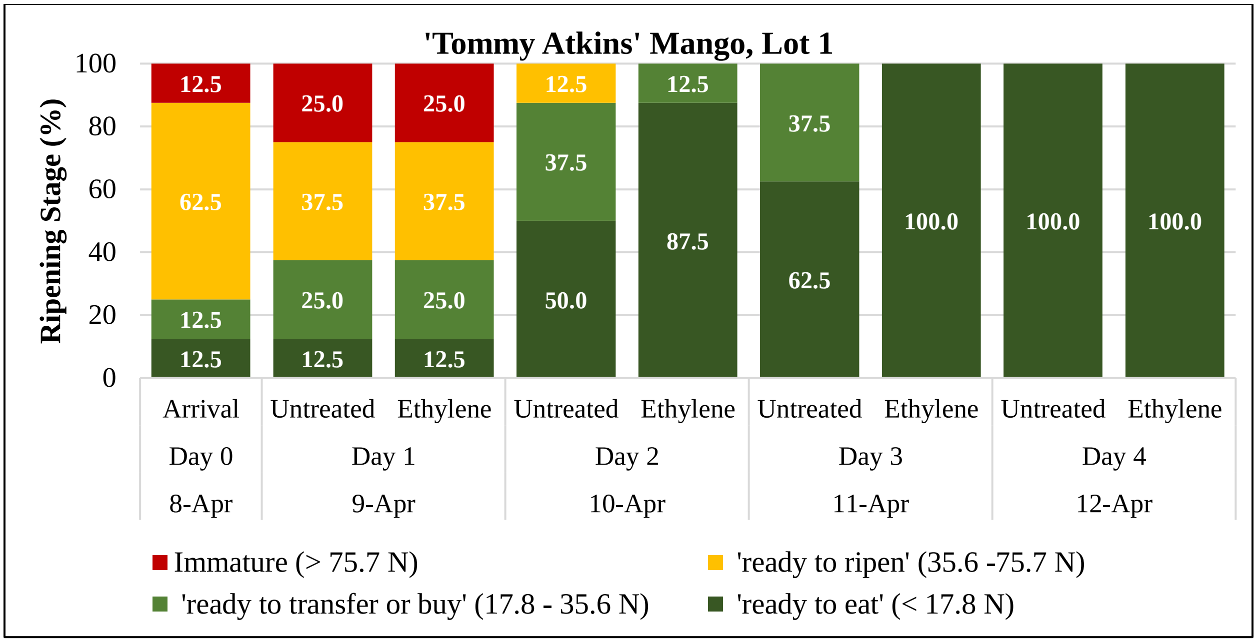


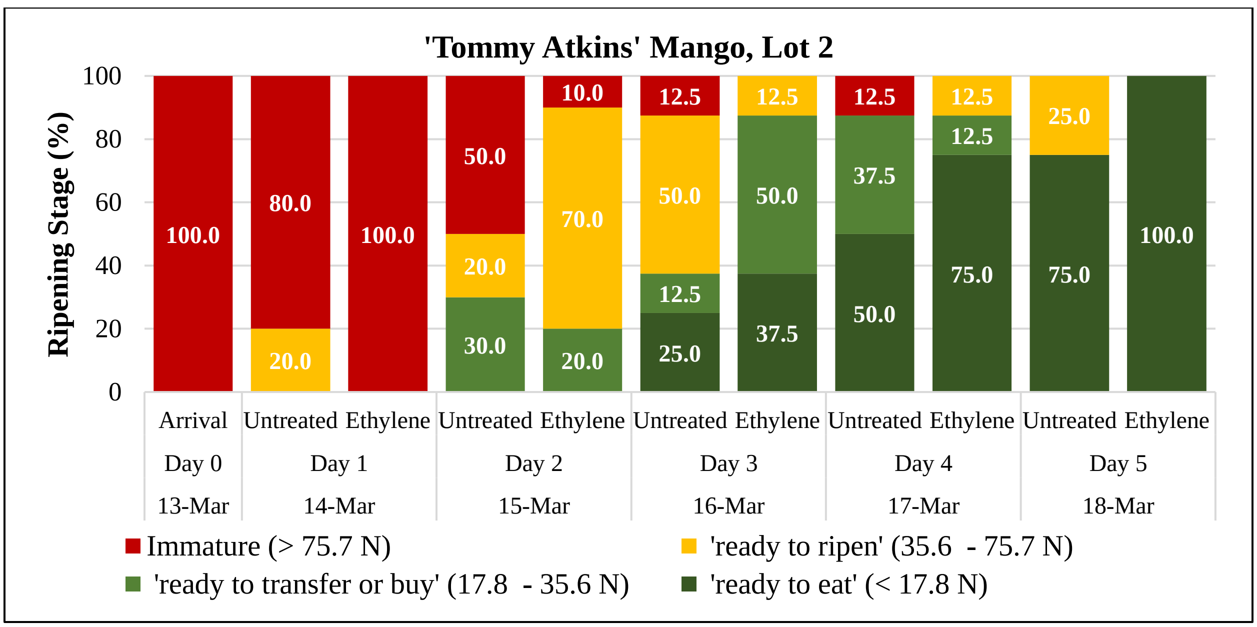
Figure 2. Softening evolution of untreated and ethylene treated mangos express as percentage distribution of mango firmness into three 'ripening stages' (1) 'ready to eat', (2) 'ready to transfer or buy' and (3) 'ready to ripen', and immature stage of 'Tommy Atkins' (Lot 1) (April 08) from Mexico belonging to the mature-firm category. Cheek firmness was recorded for four days of ripening at 20°C (68°F) until 100% of mangos reached 'ready to eat' ripening stage in both treatments.

3.3 Immature-Firm Category (HWT)

Two 'Tommy Atkins' mango lots (Mar 13, and April 08) from Mexico arrived at retail store within 7 to 9 days from packing house (Table 1). The initial evaluation showed 80 and 100 % immature fruit based on MMRG color chart. Average firmness of both lots was high; lot 2 (126.5 N), and lot 3 (134.1 N) confirming these lots belong in the immature-firm category (Table 2). Immature fruit was still dominant in the first day of ripening with 80 and 62% of immature fruit (Figure 3) even though they were producing small amounts of ethylene (0.03 and 0.04 µl kg^{-1} hr^{-1}) upon arrival. These two mangos lots also displayed DM of 15.8%, and 17.9% above MQI (Table 2).

The rate of softening for both treatments were very similar within each mango lot and there was not statistically different (p-value = 0.17 and 0.86) (Table 3). Firmness variability measured as coefficient of variability in these lots increased as ripening days advanced since most mangos were in the immature stage and moving progressively to the three ripening stages. Untreated fruit had a higher increase from ~ 12.7 to 126.3 on average when compared to ethylene treatment (~ 10.0 to 45.7) which is caused by the inability of immature fruit to undergo ripening without the exposure of exogenous ethylene (Table 4).

Mango softening became visual after two days of ripening, ~ 90% of ethylene-treated and 40% untreated ‘Tommy Atkins' mangos were at or below the ‘ready to transfer or buy’ stage. After three days of ripening, all treated mangos, but only 50% of untreated mangos, were below the ‘ready to transfer or buy’ stage. The percentage of soft mangos (firmness ≤ 17.8 N) was greater for treated (75%) than untreated (40%) mangos and the population of soft fruit increased after four days of ripening, reaching 100% and 75% for treated and untreated, respectively. Softening remained similar on day 5; however, 8% of immature mangos did not reach the ‘ready to eat’ stage without exogenous ethylene application in one of the lots (Figure 3). Immature fruit that did not soften was evaluated for SSC at this stage displaying 11.6%.



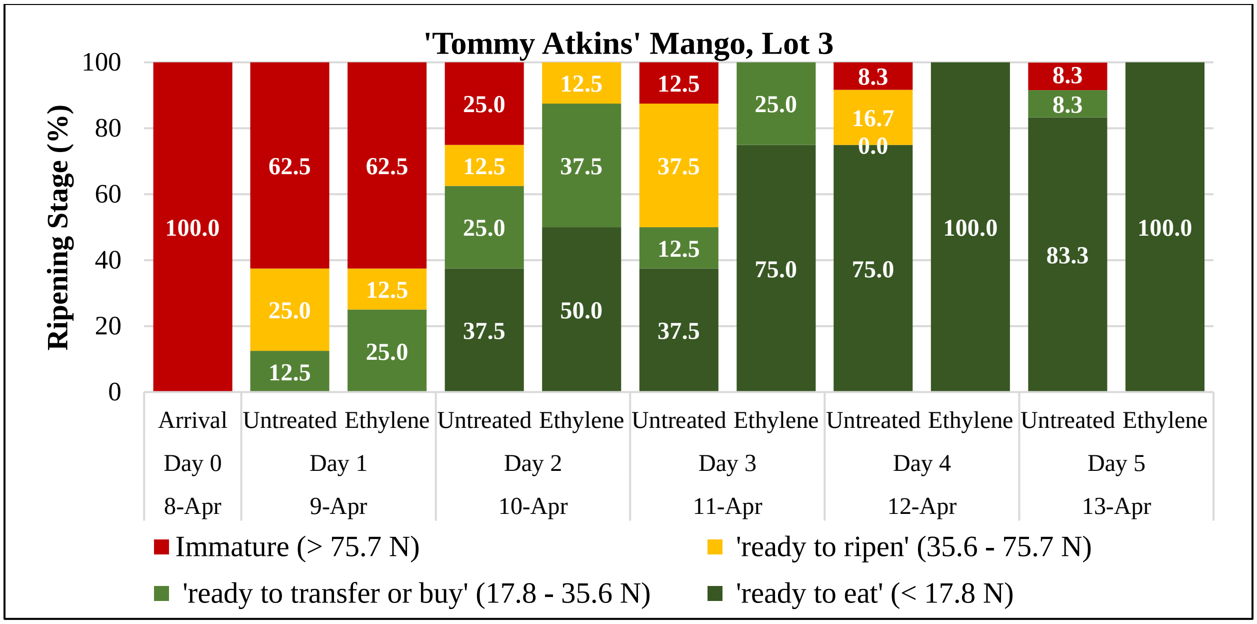


Figure 3. Softening evolution of untreated and ethylene treated mangos express as percentage distribution of mango firmness into three 'ripening stages' (1) 'ready to eat', (2) 'ready to transfer or buy' and (3) 'ready to ripen', and immature stage of two lots of 'Tommy Atkins' (Lot 2 and 3) (Mar 13 and April 08) from Mexico belonging to the immature-firm category. Cheek firmness was recorded for five days of ripening at 20°C (68°F) until 100% of ethylene treated mangos reached 'ready to eat' ripening stage for two consecutive days.

3.4 Immature-Firm Category (NHWT)

'Keitt' mango lot was coming from Culiacan, Mexico a free-fly zone therefore mango lot did not require hot water treatment. Mango arrived at retail after 12 days in transit (Table 1). At arrival, about 52% of 'Keitt' mango was classified as immature fruit based on flesh color development, and an overall average firmness of 103.4 N (Table 2). The combination of firmness above 'ready to ripen' (< 66.8 N) stage, and stage 1 color development place this mango lot on the immature-firm category. This lot of fruit as well as the other immature-firm mango lots was producing very small amounts of ethylene (0.04 µl kg^{-1} hr^{-1}) upon arrival. DM percentage was 17.4% which is higher than the proposed MQI however soluble solids concentration was very low (7.7%) compared to the average SSC (12.3%) of ‘Keitt’ mango during 2019 season (Velasquez et al., 2020).

The rate of softening of ethylene treated mango expressed in the linear equation was 28.9 N per day while untreated mangos softening rate was 16.0 N (Figure 5). The slope comparison (p-value = 0.002) confirmed the statistical difference among treatments (Table 3). During the ripening period, coefficient of variation increases since mangos firmness were changing from a predominant immature stage into the three ripening stages. However, ethylene treated fruit showed a lower coefficient of variance compared to untreated fruit suggesting that ethylene was effective in reducing firmness variability and enhancing an even and faster ripening of this mango lot (Table 4).

At arrival, 98% of NHWT ‘Keitt’ mango lot required application of ethylene to undergo ripening. After one day, 90% of mangos still required ripening. By day 2, only 12% of the ethylene-treated fruit needed more ripening and 88% were at the 'ready to transfer or buy' or lower stages, while only 25% of the untreated mangos were 'ready to transfer or buy’. By day 3, all ethylene-treated mangos had reached at least the ‘ready to transfer or buy’ stage. After 3 days, ethylene treated mangos had an average firmness of 16.9 N while ‘Keitt’ untreated mangos did not soften below 30.3 N even after five days of the ripening study (Figure 4). At the end of the ripening period, 90% of untreated mangos were at the ‘ready to transfer or buy' stage, only 50% reached the 'ready to eat' stage, and 12% of immature mangos did not ripen. These immature fruits displayed a low SSC of 9.4% on average.

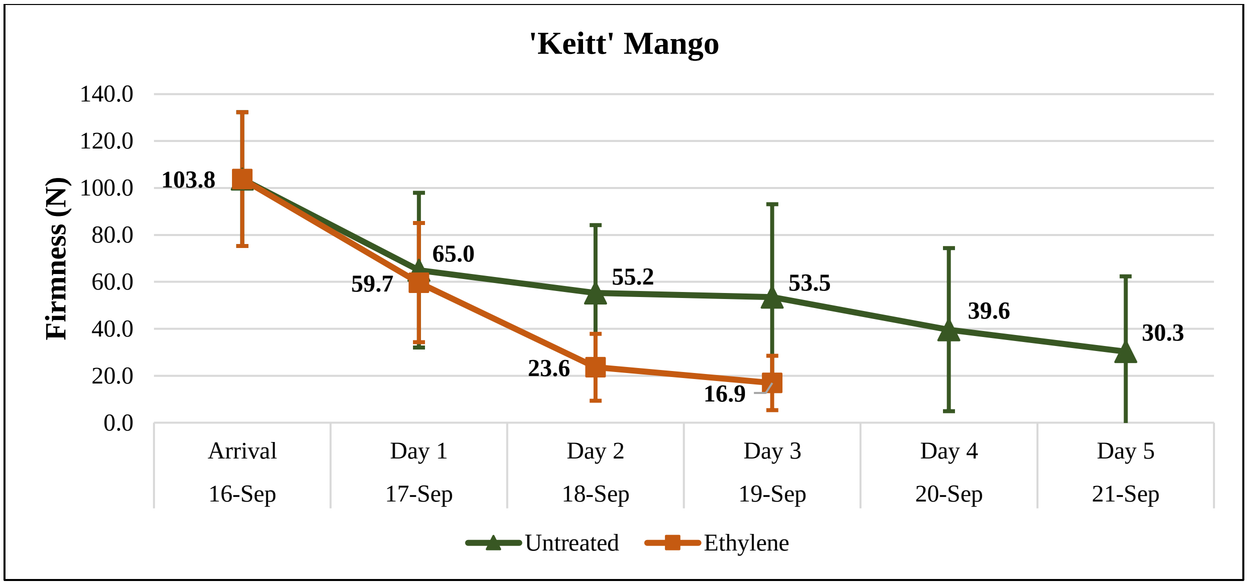


Figure 4. Softening evolution of untreated and ethylene treated 'Keitt' mangos belonging to mature-firm category from Culiacan, Mexico (fly-free zone) where hot water treatment was not required for this mango lot. Cheek firmness was recorded during ripening period at 20°C (68°F) for both treatments; ethylene treated mangos soften (16.9 N) after three days from ethylene application while untreated mangos softening was followed for six days reaching an average of 26.7 N by the end of the test.

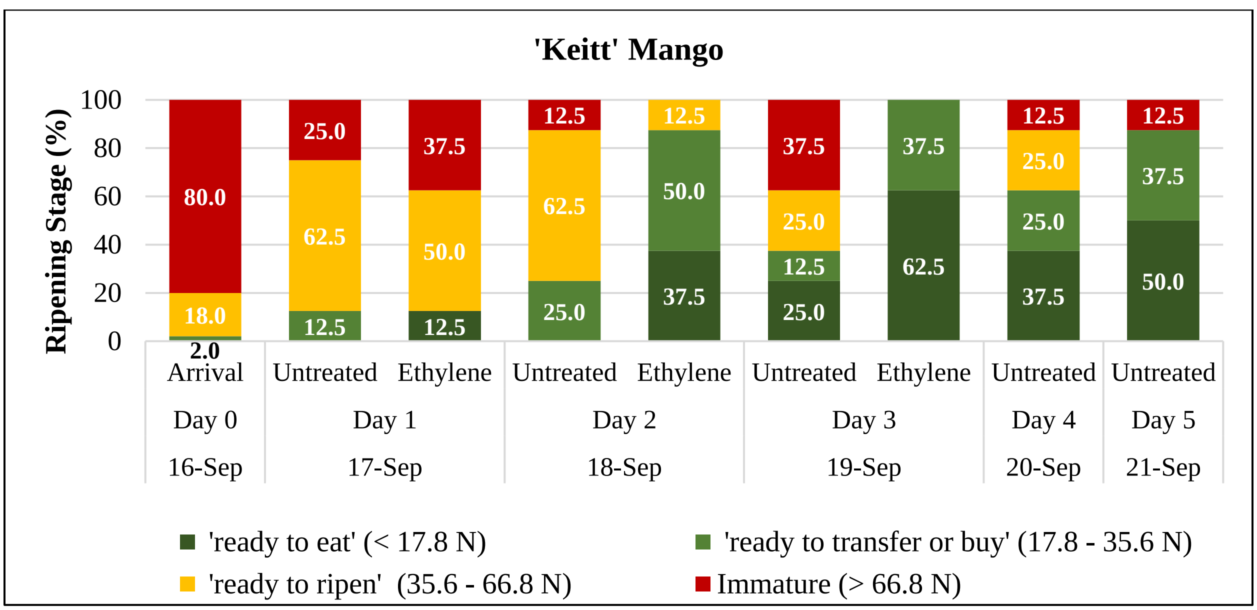


Figure 5. Softening evolution of untreated and ethylene treated mangos express as percentage distribution of mango firmness into three 'ripening stages' (1) 'ready to eat', (2) 'ready to transfer or buy' and (3) 'ready

to ripen', and immature stage of NHWT 'Keitt' (Sep 16) from Mexico belonging to the immature-firm category. Cheek firmness was recorded for three to five days during ripening at 20°C (68°F) for ethylene treated and untreated mangos, respectively. 100% of ethylene treated mangos reached 'ready to eat' and 'ready to transfer of buy' ripening stages by three days of ripening at 20°C (68°F) while untreated mangos were still displaying 12% of immature mango by the end of the test.

4. Discussion

4.1 Hot Water Treatment Triggers Production of Ethylene

Hot water treatment exposes mangos to 46.1°C (115°F) for 65 to 110 minutes depending on fruit shape, cultivar, and size (USDA-APHIS-PPQ Treatment Manual, 2022). This treatment represents an abiotic stress for this commodity and triggers the production of ethylene. For mature-soft, and mature-firm fruits, the production of ethylene is autocatalytic, and enhances proper ripening; referring to ethylene biosynthesis system 2 which operates during the ripening (Liu et al., 2015). On the other hand, immature fruit produces small quantities of ethylene (~ 0.04 µl kg^{-1} hr^{-1}) but do not have the ability to undergo ripening (Kader et al., 2002). Studies of immature tomato shows immature fruit operates ethylene biosynthesis system 1 in which ethylene is autoinhibiting and operates during fruit growth (Liu et al., 2015). Immature fruit has shown to soften after exogenous ethylene application but not showing other aspects of ripening (Lelièvre et al.,1997). Immature-firm 'Tommy Atkins' that were hot water treated and ethylene treated did not soften significantly faster than mangos that only were treated with hot water (Untreated). However, about ~10% immature 'Tommy Atkins' untreated mangos remained firm above 75.7 N while 100% of ethylene treated mangos soften during ripening reaching 'ready to eat' stage (Figure 3). In the case of immature 'Keitt' that did not receive hot water treatment or ethylene treatment, ~12% mangos remained firm by the end of the ripening period, and softening rate was statistically different (p-value = 0.002) suggesting exogenous ethylene was effective in speeding the ripening process. Coefficient of variation increased as ripening progresses; ethylene treatment CV increased up to 67.8 while untreated treatment CV increased up to 116.8 due to the 12% immature mangos that remained firm. Results from this test revealed the influence of hot water treatment on reducing or eliminating the need of exogenous ethylene application as part of the ripening protocol. The lack of immature fruit to soften without exposure to ethylene is an issue that can be mitigated by proper maturity selection at harvest, and orchard management (Anderson et al, 2017).

4.2 Physiological Maturity is a Key Factor for Completion of Ripening

Achieving physiological maturity before harvest provides several benefits to mango; mature mangos can produce ethylene and self-trigger the ripening process. During this experiment, there were three lots of mangos 'Kent', 'Ataulfo', and 'Tommy Atkins' (Lot 1) arriving with more than 90% mature fruit based on flesh color development and rounded shoulder (NMB, 2019) (Table 2). Mangos within mature-soft, and mature-firm categories had between 66 to 25% of mangos 'ready to transfer or buy', and/or 'ready to eat' (Figure 1, and 2). These mangos were ready to be placed at retail display for final purchase and consumption with the potential to express high sensory quality due to their ripening stage; ripe and partially ripe mangos have shown to be highly acceptable (~88%) by consumers (Nassur et al., 2015; Gonzalez-Moscoso, 2014). Mangos in mature-soft and mature-firm categories were already producing ethylene upon arrival two (0.07 µl kg^{-1} hr^{-1}) or tree folds (0.14 µl kg^{-1} hr^{-1}) more than immature mangos that were producing about 0.04 µl kg^{-1} hr^{-1}. These ethylene levels are already triggering ripening because mangos are highly sensitive to ethylene (Wills et al., 2001). Thus, mangos in the 'ready to ripe' could reach 'ready to eat' stage without receiving exogenous ethylene applications which is confirmed by the slope comparison (p-value) between untreated and ethylene treatments of each mango lot showing there is no statistical difference in the softening rate of mangos from both treatments. Ethylene and untreated mangos reached 'ready to eat', and 'ready to transfer or buy' within one to three days of simulated display. These ripening periods are shorter than other studies such as Montalvo et al. (2007) that found a ripening period for 'Ataulfo' to be reduced from 13 to 9 days due to the application of ethylene. Differences in ripening periods of this and other studies points out the influence of shipping period on the slow onset initiation of ripening even in cold temperatures. These mangos were in transit for 11 to 18 days, and 31 days from Mexico and Peru at around 13℃ ± 1 (55°F ±2) in which endogenous ethylene was produced and available in the atmosphere to trigger ripening.

Mature mangos stored at 12°C (54°F) have shown to increase SSC after 7 days in storage at this temperature and undergo flesh color changes after 14 days at 12°C (Medlicott et al., 1990) thus mangos arriving to the US might not need exogenous ethylene treatment upon arrival if harvested physiologically mature. In this study, mature-soft fruits arrived with ~ 60 % of mangos ‘ready to transfer or buy’ or ‘ready to eat’ which is ideal place them in display and satisfy consumers. The promotion of ‘ready to eat’ mangos sections have the potential to minimize fruit senescence, and loss at store and help consumers in the selection process.

Another benefit of mature mango selection is the higher tolerance to low temperatures (Brecht and Yahia, 2009). Chilling injury (CI) can be developed when mango is stored below 12°C (53.6°F), and it is expressed as skin color discoloration, lenticel damage, uneven ripening, poor color, and flavor (Sivankalyani et al., 2016). CI resistance is important to minimize lenticel damage (Rymbai et al., 2012) that can affect the permeability of gases such as endogenous ethylene (Paul et al., 2012), and therefore the ripening process. It is recommended to keep training pickers in fruit selection to obtain the benefits of mature fruit and deliver ‘ready to eat’ high quality mangos without the need of exogenous ethylene exposure. The application of this ripening treatment at these maturity ripeness categories can negatively impact fruit quality, and even cause fruit loss. Mangos in the ‘ready to eat’, and ‘ready to transfer or buy’ are close or below the critical bruising threshold (≤ 22.3 N) previously proposed (Baez et al., 2018b), therefore, the application of ethylene and extending ripening period on fruit that is already soft will only add handling that can caused impact or compression bruising. Mango delivery would be delayed by at least 24 hours which is the duration of treatment in which mangos might be overripe, physically damage and prone to decay.

5. Conclusions

Our data support the hypothesis that HWT mangos at United States arrival are already undergoing ripening confirming that exogenous ethylene did not speed ripening or reduce commercial firmness variability within the mango lots. In fact, HWT mango ripening is very fast and adding exogenous ethylene during the ripening protocol requires the use of expensive ripening chambers, extra equipment, and handling costs;

creating delays that may induce physical impact and compression soft fruit damage rather than improve fruit quality for consumers.

We recommend studies to detect and prevent chilling injury, packaging development, and online sorting technology to fast-sort mangos based on ripening categories to reduce firmness variability during ripening and potential bruising to improve the effectiveness of the ripening protocol. Maintaining effective 'ready to eat' programs for mangos and other tropical fruits is particularly challenging because of a long history of inconsistent quality in imported fruit, but crucial to increase consumption of heathy fruit by consumers.

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Chapter 2

Skin discoloration and softening are the primary factors impacting postharvest quality of hot water-treated mango (*Mangifera indica* L.).

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Abstract: About 85 to 90% of mangos imported to the United States from Mexico are hot water-treated (HWT). Mango chilling injury (CI) disorder limits prolonged low-temperature storage and affects the ability of shippers to transport mangos over long distances. Imported mangos display various CI symptoms that influence fruit postharvest quality, such as grayish, scald-like skin discoloration, lenticel spotting, skin pitting, loss of flesh color and flesh browning. There is limited information available on the interaction between harvest maturity, temperature and duration of exposure and their effects on postharvest life. We examined the relationship between cultivar, maturity stage at harvest, shipping temperature, and shipping duration of HWT mangos; the onset and severity of CI incidence, along with impact on consumer quality, and used the data to predict shipping-distribution postharvest life. Among the tested cultivars, ‘Tommy Atkins’ showed high incidence of skin damage (41%) after 10 days of cold storage, among all temperatures and maturity stages. Skin damage was the main barrier affecting postharvest quality. Within 24 days of simulated shipment, ‘Tommy Atkins’ showed a minimum of 11% incidence of flesh damage among temperatures and maturity stages. The onset of flesh damage occurred much later than skin damage and its incidence was low and manageable by selecting cultivar, harvest maturity and temperature during shipment to prolong postharvest life.

To avoid CI during transportation and assure high consumer quality, HWT mangos should be transported at ≥ 10.0 to 12.5°C. However, softening and decay incidence may become a problem during distribution at

the destination. Therefore, improved packaging to protect soft mangos during handling at the receiving end and consumer educational programs are critical.

Keywords: harvest maturity, shipping temperature-duration, lenticel spotting, flesh browning, commercial rejections.

1. Introduction

Mango (*Mangifera indica* L.) is consumed widely in tropical and subtropical regions and recently, consumption in temperate-zone countries has increased dramatically (Yahia, 2011). United States imports comprise 43% of the world total (120 million boxes) and have increased annually by an average of 12% in volume and 8% in value since 1994 (National Mango Board [NMB]; www.mango.org). The most frequently imported cultivars to the United States are 'Tommy Atkins', 'Ataulfo' and 'Kent', followed by 'Keitt' and 'Haden' (National Mango Board; www.mango.org). United States mango availability fluctuates by cultivar and source in a yearly cycle. According to the USDA, Foreign Agricultural Service 99% of imported mangos to the United States from 2018 to 2020, came from Mexico (64.6%), Peru (11.6%), Ecuador (9.9%), Brazil (7.9%), Guatemala (2.3%), and Haiti (1.7%). Mangos are transported in precooled-sanitized marine containers or truck trailers at recommended temperatures of 10.0 to 12.0℃ (Brecht et al., 2020); Ocean-refrigerated for long distance transportation is preferred over air freight shipment due to the cost difference involved. Mangos take between two to four weeks from packing house to United States retail stores (Velasquez, et al., 2020) therefore transportation timing is a major challenge, as on arrival, fruit can be overripe or have cold storage damage that becomes visual during retail distribution, affecting consumption. Low-temperature storage and shipment (0 to 10℃) is used commonly to delay fruit physiological deterioration, maintain quality and prolong postharvest life (Subramanyam et al., 1975). However, chilling injury (CI) is an important cause of deterioration in many subtropical and tropical fruits that are highly sensitive to low-temperature storage (> 10.0℃) (Farooqui et al., 1985; Couey 1986;

Krishnamurthy and Joshi, 1989; Sevillano et al., 2009; Lobo and Sidhu, 2017). Long exposure to cold can reduce the proportion of unsaturated/saturated fatty acids in cell membranes, leaving the membranes rigid and susceptible to peroxidation induced by ROS during cold stress, producing CI (Sevillano et al., 2009). Imported mango cultivars display several cold storage disorders that influence fruit quality, but the most important are CI symptoms. In general, storage temperatures below 10.0 to 13.0°C, but above freezing, can damage mature-green mangos, depending on cultivar (Hatton et al., 1965; Musa, 1974; Mann and Singh, 1976; Couey, 1986; Phakawatmongkol, et al. 2004.). Temperatures that are low enough to delay ripening, decay, and senescence may also damage fruit (Medlicott et al., 1990; Mohamed and Brecht, 2002).

This CI problem limits the utility of low-temperature storage and seriously affects the ability of handlers to transport mangos over long distances. Mango CI symptoms include uneven ripening and typical skin symptoms such as grayish, scald-like skin discoloration, lenticel spotting follow by skin pitting (Brecht, 2019). Flesh damage is characterized by increased susceptibility to decay, loss of flesh color, and, in severe cases, flesh browning. Jelly seed internal breakdown, soft nose internal breakdown, spongy tissue, stem end cavity and black flesh (corte negro) are other internal damages that can be observed in mango cultivars. (Hatton et al., 1965; Sadasivam et al., 1971; Subramanyam et al., 1975; Medlicott et al., 1990; and Brecht, 2019). The symptoms of CI are often not apparent while the fruits are at low temperature, but develop later, when the fruits are brought to warmer temperatures for ripening and display at retail stores and/or consumer homes (Chaplin et a., 1991; Medlicott et al., 1990; Phakawatmongkol et al., 2004; Brecht, 2019). There is limited information indicating that the onset and severity of CI depends on cultivar, fruit maturity and the duration of exposure to low temperature. Previous evaluations indicated that 'Haden' and 'Keitt' are more susceptible to CI than 'Tommy Atkins' (Brecht, 2019). Also, CI susceptibility decreases as fruit matures and ripens; immature mangos are more susceptible to CI than mature-green mangos, and these are more susceptible to CI than ripe mangos (Kader, 1997; Brecht et al., 2012). An important concept about CI is threshold temperature-exposure time. CI in other commodities occurs when a fruit is exposed to below-threshold temperatures for sufficient time to initiate irreversible injury (Crisosto et al., 1999; Brecht et al., 2012). The threshold temperature is the lowest temperature at which a susceptible commodity can be held with no symptoms of CI developing. In peach, the relationship between temperature and time of exposure is more important than temperature by itself; therefore, the safe temperature and exposure period have been determined (Crisosto et al.,1999). Knowing the critical time-temperature combinations for the most important mango cultivars imported to the United States would provide basic information to predict shipping-distribution postharvest life based on the onset and severity of CI. This would allow producers to decrease the incidence of CI, deliver higher-quality, better-tasting mangos, and gain consumer trust. Thus, our objective was to determine threshold temperature and duration for the most important mango cultivars and predict a postharvest life based on CI and softening.

2. Material and Methods

2.1 Cultivars and Harvest Maturity Stages

USDA-APHIS hot water phytosanitary-treated (HWT) ‘Tommy Atkins’, ‘Kent’ and ‘Ataulfo’ mangos were collected at a commercial packinghouse in Escuinapa, in Culiacan, Mexico. Medium sized ‘Tommy Atkins’, and ‘Kent’ mangos (550 – 700 g) were exposed to HWT at 46°C for 90 minutes. While ‘Ataulfo’ mangos (380 – 420 g) received HWT at 46°C for 74 minutes. Water temperature, and time of exposure was monitored during the treatment application to avoid any potential HWT damage. After HWT, sound mangos were carefully selected and segregated into two or three harvest maturity stages according to the National Mango Board Guide (NMB, 2009) based on visual parameters such as fruit shape, skin color, shoulder shape and location in relation to peduncle insertion, and skin texture. We did not select mangos at NMB Stage 1 (S-1, ‘*parcialmente sazones*’), as these mangos are easily identified and discarded because of color and shape. A nondestructive differential Absorbance (DA) meter cheek measurements were used to assist visual selection because it measures the absorption of chlorophyll ‘a’ in the mesocarp using the difference in absorbance between 670 and 720nm (index of absorbance difference, I_{AD}) thus providing an indication of maturity, and ripeness (McGlone and Kawano, 1998; Subedi et al., 2013; Jha et al., 2014; Spadoni et al, 2016; and

Rodriguez-Bermejo and Crisosto, 2017;). Eight to twelve mangos per maturity category for ‘Tommy Atkins’, ‘Kent’, and ‘Ataulfo’ were used to assess the accuracy of maturity segregation. Three harvest maturity stages of ‘Tommy Atkins’ [NMB Stage 2 (S-2; I_{AD} = 1.7 - 2.3), NMB Stage 3 (S-3; I_{AD} = 0.9 – 1.6) and NMB Stage 4(S-4; I_{AD} = 0.2 – 0.8)], and two maturity stages (NMB Stages 2 and 3) were found in ‘Kent’ [(S-2; I_{AD} = 1.9 - 2.4), (S-3; I_{AD} = 1.2 – 1.7) and ‘Ataulfo’ [(S-2; I_{AD} = 1.8 - 2.1), (S-3; I_{AD} = 1.3 – 1.7). These I_{AD} ranges aligned with I_{AD} values assigned in mango studies conducted by Kavitha (2015), and De Sousa Costa et al., (2021). Mangos at two or three harvest maturity stages were stored for up to 30 days in environmental chambers with 85% relative humidity at three temperatures (8.0°C, 10.0°C or 12.5°C), which were selected according to current CI temperature-cultivar information (Brecht, 2019; Brecht et al., 2020).

2.2 Fruit Harvest Initial Quality

Initial, destructive quality measurements were performed on unripe mangos harvested at different maturity stages prior to storage, to validate harvest maturity categories and describe the fruit material. Flesh firmness was measured by removing a dime-sized piece of skin from two opposite cheek sides of eight to twelve individual mangos with a mandolin peeler. Firmness was calculated in Newtons (N) force using a fruit texture analyzer (FTA) equipped with a 7.9 mm diameter tip (model GS-14, GÜSS, South Africa). Flesh visual color was determined by the authors working together according to the National Mango Board Guide (NMB, 2019) consisting of five scores from stage 1 (S-1, immature mango flesh color) to stage 5 (S-5, over ripe). In addition, quantitative colorimeter parameters expressed as Hue angle were taken using a Minolta colorimeter (McGlone and Kawano, 1998; Ja et al., 2014; Spadoni et al, 2016; and Rodriguez-Bermejo and Crisosto, 2017). Both types of color measurements were conducted on mangos after they were cut from stem to tip and assigned a flesh color maturity ripeness stage. Soluble solids concentration (SSC) was assessed using mango slices from eight to twelve individual mangos per maturity category to assess maturity category according to the NMB Maturity and Ripeness Guide. Cheese cloth grade 60 was used to collect juice and strain out pulp. A temperature-compensated digital refractometer (model PR-32α, Atago Co., Tokyo, Japan) was used to measure SSC (AOAC, 1984). Dry matter percentage (DM %) is correlated to consumer acceptance (Gonzales-Moscoso, 2013; Nassur et al., 2014; Nassur et al., 2015) and was measured during the initial quality evaluation. For this, thin slices of undamaged flesh from eight to twelve mangos were collected and dried for one day at 46.1°C using a FD-61 NESCO Food Dehydrator. After 24 hours, samples were weighted and dried for an additional hour. Slice weight was measured for a second time to ensure dry weight was consistent (AOAC, 1084; Rodriguez and Crisosto, 2017).

A) Lenticel spotting	B) Lenticel spotting and skin pitting	C) Scald-like skin discoloration	D) Grayish sunken skin discoloration

Figure 1. Severe skin damage observed in mangos exposed to low temperature shipment and storage (≤ 12.5°C). A) 'Tommy Atkins' mango displays severe lenticel spotting. B) 'Tommy Atkins' mango displays severe lenticel spotting along with skin pitting. C) 'Ataulfo' mango shows scald-like skin discoloration due to low temperature exposure and sapburn due to latex. D) 'Tommy Atkins' shows grayish sunken discolored areas. Photos taken by Ms. Andrea M. Velasquez.

2.3 Firmness Evaluation After Cold Storage

Firmness evaluations were conducted in 'Tommy Atkins', 'Kent', and 'Ataulfo' mangos after cold storage at day 0, 10, 17, 24 and 30 following the firmness protocol described previously. In this evaluation, seven individual mangos for 'Tommy Atkins', and 'Kent', and ten individual mangos for 'Ataulfo' were used per maturity-temperature combination. We paid close attention to the time required for firmness to decrease to 22.3 N or below for each treatment because prior studies have proposed 22.3 N as a critical bruising threshold (CBT) because fruits become highly susceptible to bruising at this firmness or below (Crisosto et al., 2001; Valero et al., 2007; and Gonzales-Moscoso, 2013; Nassur et al., 2014). Firmness evaluation was also important to describe softening rate which is defined by the rate of change in average mango firmness throughout evaluations dates (0, 10, 17, 24, and 30 days) in this study.

2.4 Ripe Mango Quality Evaluation After Cold Storage

As cold storage damage is well-expressed on ripe fruit (Chaplin et a., 1991; Phakawatmongkol et a; al, 2004. Brecht, 2019), internal and external quality was evaluated on ripe fruit. After 0, 10, 17, 24 and 30 days of cold storage, fruits were moved to 20ºC until they reached the 'ready to eat' stage of ~ 4.5 to 26.7 N (Nassur et al., 2015). External symptoms such as lenticel spotting, skin pitting, grayish, and scald-like skin discoloration (Figure 1) and internal symptoms such as increased susceptibility to decay, loss of flesh color, and, in severe cases, flesh browning (Figure 2) were determined.

External symptoms will be presented as skin damage incidence percentage. It was measured using a visual scale of aggregated area measurements to assign the degree of damage. The categories were sound skin: no damage present; light skin damage: < 25% of fruit area damaged; moderate skin damage: 25 to 50% of fruit area has damage; and severe skin damage: > 50 % fruit area has damage (Nunes et al., 2007; Brecht et al., 2012). Internal symptoms will be presented as flesh damage incidence percentage in which we measured severity of flesh browning; the categories were sound: no damage present; slight damage: damage < 19.1 mm in diameter; moderate damage: 19.1 to 38.1 mm in diameter; and severe damage: > 38.1 mm in diameter. Evaluations were carried out by the authors, assisted by photo examples to ensure uniform and consistent measurements (Figures 1 and 2). For this study analysis, mangos with moderate to severe skin or flesh damage (Nunez et al., 2007) were counted as commercially damaged and, therefore, potential commercial rejections.

	
Sound (S) ‘Kent’ Mango	Slightly Brown (SL) ‘Tommy Atkins’ Mango
	
Moderate Brown (M) ‘Ataulfo’ Mango	Severe Brown (SE) ‘Kent’ Mango

Figure 2. Flesh browning injuries at different levels of severity expressed in ‘Tommy Atkins’, ‘Kent’ and ‘Ataulfo’ mangos that were used as examples throughout the evaluations conducted between 10 to 30 days of cold storage at three temperatures; 8.0°C, 10.0°C, and 12.5°C. Photos taken by Ms. Andrea M. Velasquez.

2.5 Statistical analysis

This is a factorial randomized trial that have three factors harvest maturity stage, storage temperature, and storage time creating 45 unique combinations for ‘Tommy Atkins’ (three harvest maturity stage, three storage temperatures, and five evaluation dates), and 30 unique combinations for ‘Kent’ and ‘Ataulfo’ individually (two harvest maturity stages, three storage temperatures, and five evaluation dates). Using eight to ten mangos as observations per treatment; Analysis of Variance (ANOVA), and Tukey test for instrumental and visual evaluation with a 95 confidence interval were carried our using Minitab version 17.0 software (https://www.minitab.com/en-us/products/minitab/).

3. Results and Discussion

Within each of three cultivars, the best destructive maturity physiological indicators based on the NMB guide (NMB, 2019), displayed different average values for each parameter per each harvest maturity stages within each cultivar, indicating that the DA-meter, skin color, shoulder shape visual observations that were used to create the two or three harvest maturity categories successfully separated these initial harvest maturity stages for our studies (Table 1). ‘Tommy Atkins’, ‘Kent’, and ‘Ataulfo’ NMB maturity stages express different value ranges of flesh color (hue), SSC, and firmness values at harvest that aligns with SSC and firmness values provided NMB Maturity Ripeness Guide. In general, within each cultivar, flesh color changed from green-yellow to yellow-orange and SSC increased from low (S-2) to high (S-4) maturity. ‘Tommy Atkins’ was firmer on average than ‘Ataulfo’ and ‘Kent’. ‘Tommy Atkins’ mangos harvested at S-2 NMB maturity had 168.4 N, compared to 142.6 or 116.7 N for S-3 or S-4, respectively. ‘Ataulfo’ harvested at S-2 was 79.7 N and declined to 61.5 N at S-3. Similarly, ‘Kent’ was 108.7 N at S-2 and dropped to 53.0 N when harvested at S-3. In all cases, DM % average values were greater than the proposed minimum consumer quality index (Gonzales-Moscoso S. 2013; Nassur et al., 2015), but these values vary among cultivars and harvest maturity stages.

Table 1. Mango quality evaluation conducted to assess quality at harvest of ‘Tommy Atkins’, ‘Ataulfo’, and ‘Kent’ cultivars at two or three different NMB maturity stages. Parameters are presented as the average value of eight to twelve individual ‘Tommy Atkins’, ‘Kent’ or ‘Ataulfo’ mangos. Flesh color represented as hue angle was measured to assess initiation of internal color development as an indicator of physiological maturity achievement prior to harvest. SSC %, and DM % were included in the evaluation because they

have shown to be good indicators of consumer acceptance (Moscoso-Gonzalez, 2014), and flesh firmness is highly correlated to ripening stage (Nassur et al., 2015; Brecht et al., 2020). Standard deviation is indicated in parenthesis next to the average value of the parameter presented.

Cultivar	**NMB maturity stage**	**Flesh color (Hue)**	**SSC (%)**	**Flesh firmness (N)**	**DM (%)**
'Tommy Atkins'	S-2	74.6 (1.7)	8.4 (0.6)	168.4 (13.5)	17.2 (1.7)
	S-3	73.5 (2.3)	8.6 (0.5)	142.6 (27.1)	17.7 (1.6)
	S-4	71.7 (6.9)	10.3 (3.2)	116.7 (21.2)	18.5 (1.8)
'Ataulfo'	S-2	71.4 (2.3)	11.6 (1.4)	79.7 (43.7)	18.9 (1.8)
	S-3	68.8 (2.9)	11.9(1.9)	61.5 (45.4)	19.4 (2.0)
'Kent'	S-2	73.7 (1.7)	7.5 (0.5)	108.7 (29.1)	21.2 (1.7)
	S-3	70.7 (4.0)	10.8 (0.5)	53.0 (36.5)	22.6 (2.8)

Table 2. Firmness collected throughout cold storage evaluations (Day 0, 10, 17, 24 and 30) was subjected to analysis of variance (ANOVA) using a 95% confidence interval to assess the significance of interactions between cold storage exposure time (days), Maturity stage (Mat); S-2, S-3, and S-4, and storage temperatures (Temp); 8.0, 10.0, 12.5°C in each cultivar in the study. This table presents the P-values corresponding to each factor, and interactions compared.

Source		**Cultivar**	
	'Tommy Atkins'	**'Kent'**	**'Ataulfo'**
Days	0.000	0.000	0.000
Maturity Stage (Mat)	0.000	0.005	0.000
Temperature (Temp)	0.000	0.000	0.000
Days x Mat	0.000	0.000	0.191

Days x Temp	0.001	0.000	0.031
Mat x Temp	0.025	0.001	0.007
Days x Mat x Temp	0.000	0.000	0.030

3.1 Rate of softening

For each mango cultivar; 'Tommy Atkins', 'Kent', and 'Ataulfo', firmness varied significantly over time (Days), P-value = 0.000, 0.000, 0.000 respectively (rate of softening). The interaction between maturity, time in cold storage (days), and temperature was significant for each cultivar; 'Tommy Atkins', 'Kent', and 'Ataulfo' P-values = 0.000, 0.000 and 0.030 respectively (Table 2). As a practical application of this information, we recorded the time require for each treatment to soften ≤ 22.3 N as the critical bruising threshold establish in previous studies on firmness bruising energy (Crisosto et al., 2001). Bruising energy during receiving-retail handling can trigger mechanical damage in mangos softer than 22.3 N (Crisosto et al., 2001; Valero et al., 2007; and Gonzales-Moscoso, 2013; Nassur et al., 2014). Using this ≤ 22.3 N critical firmness threshold, the three mango cultivars at their tested maturities did not soften to ≤ 22.3 N when stored at 8.0ºC, except for 'Kent' harvested at S-3, which reached values near 22.3 N after 17 to 24 days of storage at 8.0ºC (Table 3). Mangos stored at 10.0ºC behaved in a similar matter. 'Tommy Atkins' and 'Ataulfo' mangos harvested at different maturity stages did not soften below 22.3 N, except for 'Tommy Atkins' mangos harvested at S-3, which softened below 22.3 N after 24 days. 'Kent' mangos stored at 10.0ºC remained below 22.3 N for between 17 to 24 days if harvested at S-2 maturity and for 0 to 10 days if harvested at S-3 maturity. All three mango cultivars stored at 12.5ºC remained below this threshold during our simulated shipment period: 'Tommy Atkins' harvested at S-2 and S-3 for 24 days and late harvest (S-4) for 17 days; 'Ataulfo' for 30 or 24 days when harvested at S-2 or S-3, respectively (Table 3); while 'Kent' mangos reached below 22.3 N after 10 days if harvested at S-2 and between 0 to 10 days if harvested at S-3 (Table 3).

Table 3. Time (days) required in cold storage for mango firmness of 'Tommy Atkins', 'Ataulfo' and 'Kent' mangos harvested at different NMB maturity stages (S-2, S-3, and S-4) and stored at three temperatures (8.0, 10.0, and 12.5°C) to reach firmness ≤ 22.3 N. Firmness was measured at day 0, 10, 17, 24, and 30 of cold storage.

Cultivar	NMB Maturity Stage	Storage Temperature		
		8.0°C	**10.0°C**	**12.5°C**
'Tommy Atkins'	S-2	Not observed within this period	Not observed within this period	After 24days
	S-3	Not observed within this period	Not observed within this period	After 24days
	S-4	Not observed within this period	Between 24 and 30 days	After 17days
'Ataulfo'	S-2	Not observed within this period	Not observed within this period	At 30 days
	S-3	Not observed within this period	Not observed within this period	Between 24 and 30 days
'Kent'	S-2	Not observed within this period	Between 17 and 24 days	Between 10 and 17 days.
	S-3	Between 17 and 24 days	Between 0 and 10 days	Between 0 and 10 days

3.2 Fruit Skin Damage

In these three mango cultivars, skin damage, expressed as lenticel spotting, skin pitting, grayish, scald-like skin discoloration (Figure 1), was not significantly related to the interaction between harvest maturity, storage temperature, storage duration (P-value = 0.09). However, skin damage was significantly related to duration on ‘Tommy Atkins’ at 10 days, where only storage temperature had a significant effect on skin damage (P-value = 0.03) (Figure 3). Skin damage looks similar as hot water treatment damage (Luna et al., 2006; Osuna, 2015), However, in all cultivars, high skin damage incidence was not present prior to place HWT mangos under different temperatures and clearly detected at the first evaluation date (Day 0), thus this skin damage was evaluated as CI. For example, ‘Kent’ and ‘Ataulfo’ attained ~ 60 to 100% skin damage by 10 days across maturity and temperatures (data not shown), while ‘Tommy Atkins had near 41% damage across maturities (Figure 3). At 17 days, skin damage incidence increased to near 100% across treatments for ‘Tommy Atkins’ mangos. However, at 10 days, storage temperature significantly affected skin damage in ‘Tommy Atkins’ (P-value = 0.03). Mangos stored at 8.0°C had 69% of skin damage incidence while mangos stored at 10.0°C or 12.5°C had ~ 25% (Figure 3). Because the onset and peak of damage was observed early and reached similar high incidence across temperatures and harvest maturity stages, this skin damage is a main concern on cosmetic quality at retail. As skin damage is mainly cosmetic problem that does not affect flavor, consumer educational training should be developed as well as promote flesh cut programs.

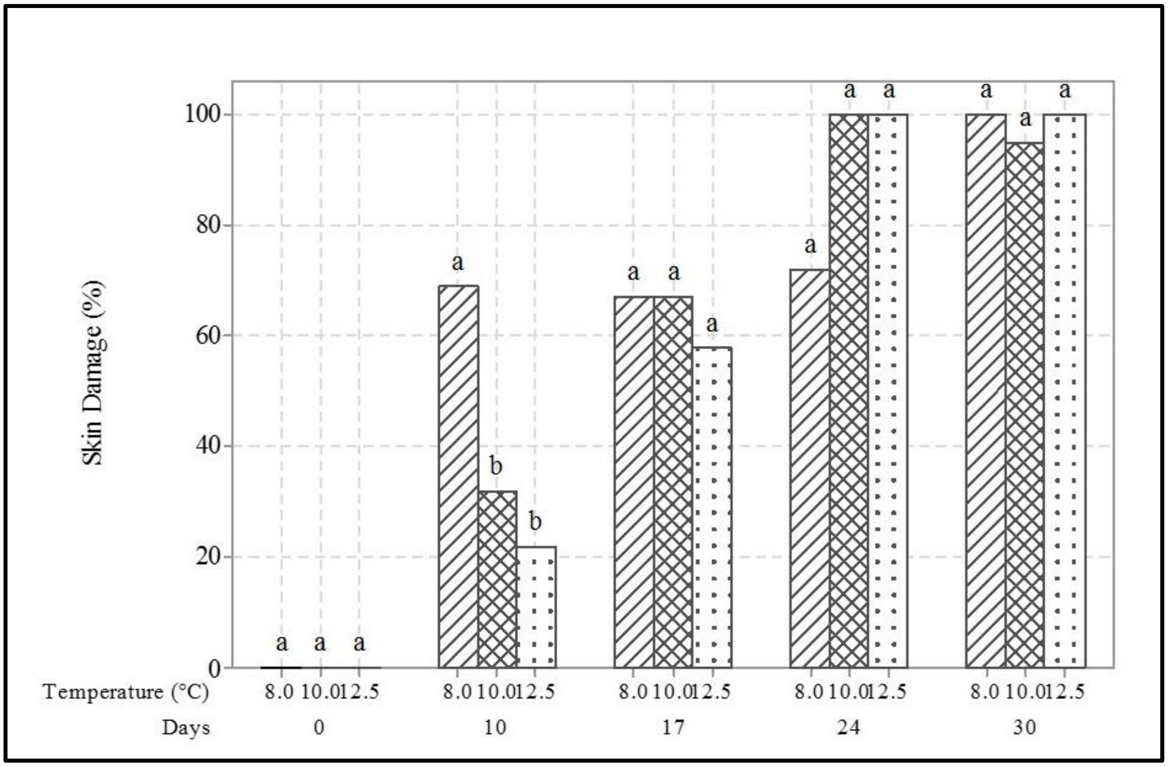

Figure 3. Skin damage incidence percentage includes lenticel spotting, skin pitting, grayish sunken skin discoloration, and scald-like skin discoloration in 'Tommy Atkins' mangos at different storage temperatures (8.0, 10.0, and 12.5°C) throughout storage time (days). Different letters across temperature treatments within each evaluation date indicate statistical difference (P-value < 0.05) among storage temperatures.

3.3 Fruit Cold Storage and Commercial Flesh Damage

Loss of flesh color and flesh browning were evaluated as flesh damage. For all three mango cultivars, commercial flesh damage (moderate and severe damage) depended on the interactions among maturity at harvest, storage temperature and length of cold storage. In addition, cultivars had different levels of flesh damage. Commercial flesh damage observed during cold storage ranged from 20 to 100% for 'Ataulfo', 11 to 100% for "Tommy Atkins', and 14 to 100% for 'Kent'.

In 'Tommy Atkins' mangos, the first symptoms of flesh damage (~12%) became visible by 10 days at

12.5°C storage temperature, but only on mangos harvested at S-3 (Table 4). By 24 days, 'Tommy Atkins' mangos harvested at S-3 reaching damage levels from 11 to 77%, while those harvested at S-4 only showed damage at 8.0°C (44.4%). By 30 days, 'Tommy Atkins' mangos harvested at S-2, and S-3 had 100.0% damage across all storage temperatures, with less flesh damage in mangos harvested at S-4 and stored at 10.0ºC or 12.5ºC. At all evaluations, mangos harvested at S-2 had more flesh damage symptoms than mangos harvested at S-3. S-4 showed less incidence during all the evaluations. The best combination was S-4 at 12.5°C throughout the entire simulated shipment (Table 4).

Table 4. Flesh damage incidence percentage on evaluation of ripe 'Tommy Atkins' mangos harvested at three maturity stages (S-2, S-3, S-4) during cold storage (Day 0, 10,17, 24, and 30) at three cold temperatures (8.0, 10.0, and 12.5°C).

Storage (days)	**Flesh Damage (%)**								
	8.0°C			**10.0°C**			**12.5°C**		
	S-2	**S-3**	**S-4**	**S-2**	**S-3**	**S-4**	**S-2**	**S-3**	**S-4**
0	0.0c[z]	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c
10	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	12.5c	0.0c
17	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c
24	0.0c	11.1c	44.4b	0.0c	25.0c	0.0c	0.0c	77.8a	0.0c
30	100.0a	100.0a	100.0a	77.8a	100.0a	50.0b	100.0a	100.0a	0.0a

[z]Different letters across treatment interactions indicate statistical differences (P-value < 0.05) between means according to Tukey's test.

In 'Ataulfo', all factors affected the onset of flesh damage. The first flesh damage symptoms were detected 17 days after storage at either 8.0 or 10.0ºC, reaching ~20.0% damage incidence (Table 5). At this evaluation date, flesh damage was greater on mangos picked at S-2 (~ 25.0% average) than at S-3(20.0%), but not in mangos stored at 8.0°C, where harvest maturity did not affect flesh damage. At 24 days, flesh damage increased to 75.0% for S-2 mangos and 33.3% for S-3 mangos stored at 8.0°C while there was no flesh damage on mangos harvested at S-3 and stored at 10.0°C or 12.5°C. Flesh damage incidence percentage also increased ~90% on average for S-2 mangos stored at 10.0 and 12.5°C.

Table 5. Flesh damage incidence percentage on ripe 'Ataulfo' mangos harvested at two maturity stages (S-2, S-3) for 24 days storage at three cold temperatures (8.0, 10.0, and 12.5°C).

Storage (days)	**Flesh damage (%)**					
	8.0°C		**10.0°C**		**12.5°C**	
	S-2	**S-3**	**S-2**	**S-3**	**S-2**	**S-3**
0	0.0c[z]	0.0c	0.0c	0.0c	0.0c	0.0c
10	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c
17	20.0c	20.0c	33.3b	0.0c	0.0c	0.0c
24	75.0a	33.3b	100.0a	0.0c	80.0a	0.0c

[z]Different letters across treatment interactions indicate statistical differences (P-value < 0.05) between means according to Tukey's test.

In 'Kent' mangos, flesh damage was not detected until 30 days. At 30 days, mangos stored at different temperature still had low incidence of flesh damage (~ 40%) across harvest maturities and storage temperatures, showing no significant effect of harvest maturity stage and/or storage temperature.

Our detailed postharvest evaluations using fresh-harvested HWT mangos indicated that mango global marketing is limited due mainly to external skin damage development and softening. The degree of these postharvest quality problems depends on cultivar, harvest maturity and shipping temperature duration.

Unfortunately, the onset of severe skin damage incidence was detected across cultivars at 10 days (first evaluation after storage) during the simulated transportation. This skin damage was also the top damage on our retail store survey (Velasquez et al., 2020) and consumer preferences may remain unfavorable because of this cosmetic problem. Additional studies to understand the impact of skin cosmetic damage on consumer preference and educate consumers should be carried out. Increasing quality and demand for fresh-cut mango should assist sale of mangos with potential skin cosmetic problems. Softening below the critical firmness threshold was also a potential limitation, particularly on 'Kent' mangos, where 30 days of postharvest handling was only accomplished on mangos harvested at S-2 and stored at 8.0°C. 'Tommy Atkins' and 'Ataulfo' softening became a concern on mangos harvested at S-3 and stored at 10.0°C and 12.5°C. Flesh damage symptoms such as loss of flesh color, and flesh browning were visible later (24 days), than skin damages (10 days) with the exception of 'Tommy Atkins' S-3 at 12.5°C that showed 12.5% of flesh damage incidence after 10 days. The onset of damage occurred at 30 days for 'Kent', 24 days for 'Ataulfo' and 24 days for 'Tommy Atkins'. The intensity of damage was greater in 'Ataulfo' than in other cultivars, and more frequent on mangos harvested at low maturity across storage temperatures. Hence, in general, greater maturity (S-3, S-4) at harvest and higher shipping temperature (10.0 to 12.5°C) trigger softening but protect from flesh CI, while low harvest maturity (S-2) and low shipping temperature (8.0°C) induced CI symptom development and potentially reduced consumer quality. All cultivars will benefit from a shipping container that protects fruit from physical abuse during postharvest handling, allowing soft mangos to arrive at retail without bruises. Currently, some shippers use low-maturity mangos (S-1 and S-2) to ensure arrival of firm fruit that will tolerate handling at the destination, since HWT mangos are imported over long distances. However, our previous quality survey revealed that mangos picked at low maturity (S-1 and S-2) and shipped at low temperatures (<10.0°C) have poor consumer acceptance due to low DM % (<15.0%, sensory quality), CI symptoms and other storage disorders (Velasquez et al., 2020). To overcome these barriers, attempts to control softening and CI development during transportation using controlled atmosphere (CA) at 12.5°C has been assessed. CA systems with elevated concentrations of atmospheric CO_2 (five to 20 kPa) and reduced O_2 (five kPa) have been tested over the years (Baez et al., 2018a). In non HWT 'Kensington' mangoes, CA did not produce significant benefits (O'Hare and Prasad, 1993). Studies conducted in Florida using HWT mangos concluded that HWT mangos could be shipped for two to three weeks in controlled atmospheres at 8.0°C for tree-ripe fruit or 12.5°C for mature-green fruit without developing CI (Bender et al., 2000). A 2018 CA study in Culiacan, Mexico, used 10 or 20% CO_2 combined with 5% O_2 to slow down softening of HWT mangos harvested at S-2 during simulated shipment at 12.5°C for 20 days. However, mangos exposed to oxygen concentrations < 5.0% acquired 'off flavor' due low anaerobic respiration during exposure (Baez et al., 2018a, 2018b). Thus, CA did not overcome the CI development and potential softening barriers for the primary mango cultivars.

4. Conclusions

The DA-meter reliably assisted visual observations to segregate mangos into different harvest maturity categories. DA-meter validations which should be developed into a practical commercial application since the DA-meter is both nondestructive and easy to apply.

The onset of severe skin damage and softening that we detected among the tested cultivars highlighted the main barriers to high-quality arrival after 30 days. Among the tested cultivars, onset of severe skin damage by 10 days, which was detected across treatment categories, was the primary barrier affecting postharvest quality. Flesh damage was both less abundant and occurred later during storage than skin damage. This flesh damage was reduced by selecting S-3 and S-4, allowing longer postharvest life. Harvest maturity stage and shipping temperature and duration limit mango quality in diverse ways depending on cultivar.

To avoid chilling damage during transportation and assure high consumer quality, HWT mangos should be transported at ≥ 10.0 to 12.5°C. However, mango softening and decay may become a problem during distribution at the destination. Therefore, improvement of packaging to protect softer mangos during handling at the receiving end is critical, as are consumer educational programs.

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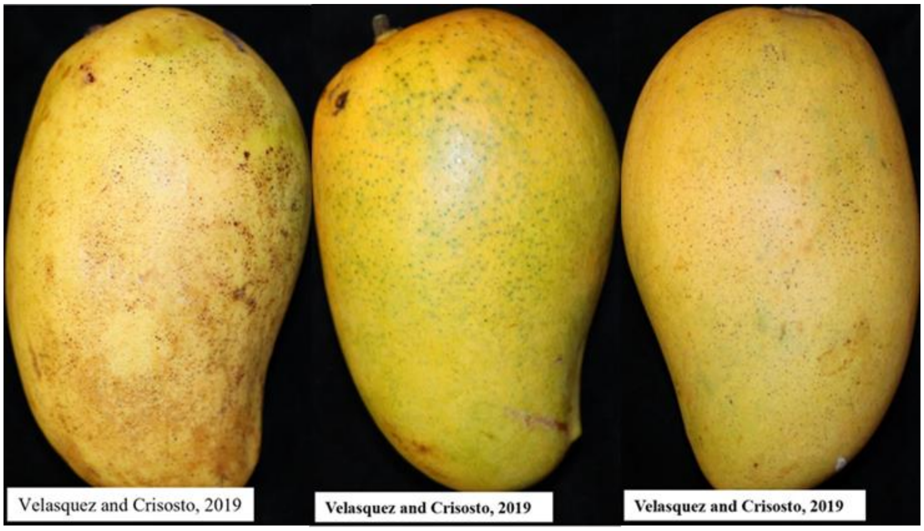
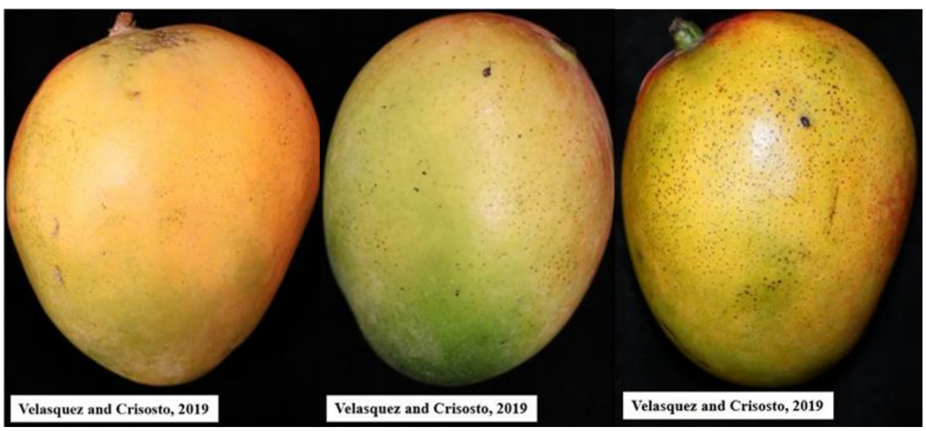
Appendix A:

External and Internal Mango Damages and Diseases Photos

1. External Visual Damage

a. Lenticel Spotting



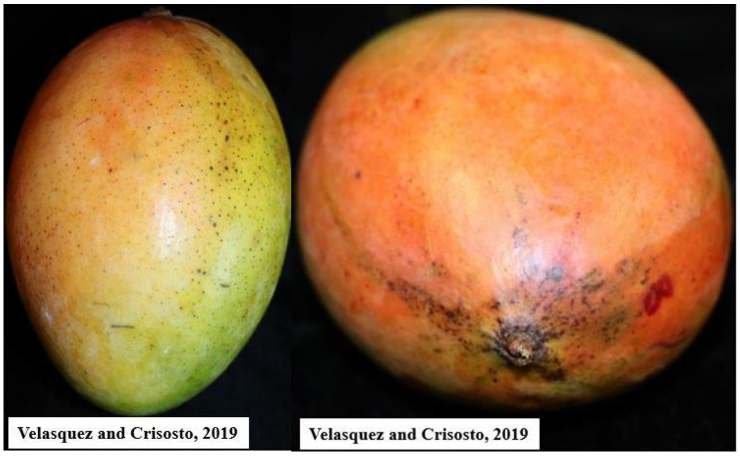


b. Skin Pitting

c. Scab

d. Mechanical Damage

e. Latex Damage



f. Water Loss

g. Skin Breaks and Cracks

h. *Insect Damage*

i. *Abrasion*

j. *Stem-End Rot (Lasiodiplodia theobromae)*

k. Sunken Shoulder Areas due to Heat Damage

l. Stem Not Well Trimmed

m. Compression Bruises

n. Anthracnose Decay (Colletotrichum gloeosporioides)

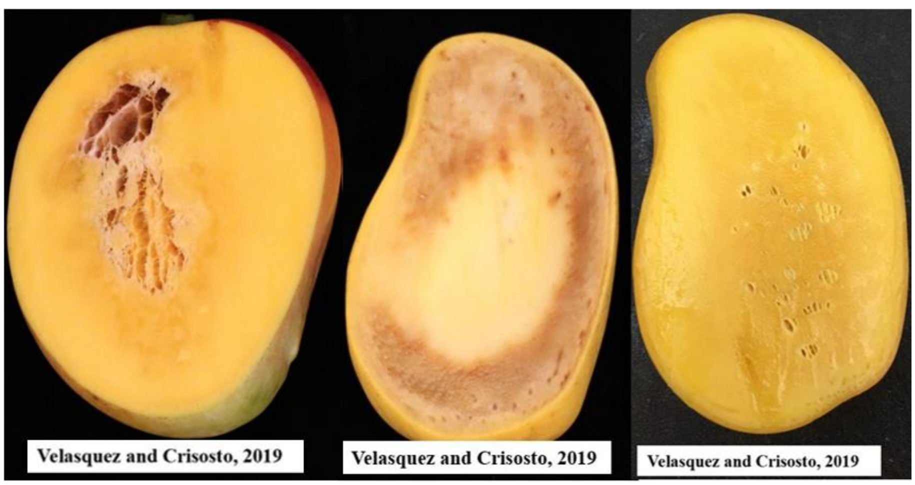
Velasquez and Crisosto, 2019

2. Internal Visual Damage

a. Pepita Negra

Velasquez and Crisosto, 2019

b. Hot Water Treatment Damage



Velasquez and Crisosto, 2019 Velasquez and Crisosto, 2019 Velasquez and Crisosto, 2019

c. Jelly Seed

d. Internal Browning

e. Stem End Cavity

Appendix B:

External an Internal Mango Damage and Diseases Incidence of US Imported Mangos During One Season

Table1. External defects incidence in major mango cultivars from different production areas arriving to the US. Defect severity was classified into two categories; light severity (L, ≤ 25% affected area), and rejections (R, > 25% affected area).

External Defects	**‘Tommy Atkins’**		**‘Keitt’**		**‘Kent’**		**‘Haden’**		**‘Ataulfo’**	
	L (%)	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**
Abrasion	1.5	0.6	5.6	3.8	2.3	0.9	0.0	0.0	1.7	0.4
Anthracnose	0.5	0.5	0.0	0.0	1.8	1.4	1.5	0.0	0.4	0.4
Compression Bruising	1.3	0.3	0.0	0.0	1.4	0.9	0.0	0.0	0.0	0.0
Impact Bruising	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Insect Damage	0.5	0.3	1.9	0.0	1.8	0.0	1.5	0.0	0.4	0.0
Latex Damage	5.8	1.5	24.1	1.9	15.2	1.8	4.4	10.3	35.3	13.7
Lenticel Damage	29.4	9.0	9.3	85.2	19.4	11.5	13.2	22.1	17.4	13.7
Mechanical Damage	0.5	0.0	1.9	1.9	0.0	0.5	0.0	0.0	0.8	0.0
Misshape	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0
Russeting	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Scab	3.8	0.5	3.7	5.6	4.6	1.4	2.9	0.0	4.6	2.1

Shriveling	0.8	0.0	0.0	0.0.	8.3	0.0	0.0	0.0	0.4	0.4
Skin Breaks and Cracks	2.3	0.3	7.4	0.0	4.6	0.5	1.5	2.9	1.7	0.4
Skin Pitting	0.8	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
Stem Not Well Trimmed	2.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2
Stem End Rot	0.0	0.3	0.0	0.0	0.5	1.4	0.0	0.0	0.0	0.0
Sunburn	0.0	0.0	0.0	0.0	0.5	0.9	0.0	0.0	0.0	0.0
Sunken Shoulder	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Uneven Ripening	1.0	0.5	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0

Table 2. Internal defects incidence in major mango cultivars from different production areas arriving to the US. Defect severity was classified into two categories; light severity (L, ≤ 25% affected area), and rejections (R, > 25% affected area).

Internal Defects	**'Tommy Atkins'**		**'Keitt'**		**'Kent'**		**'Haden'**		**'Ataulfo'**	
	L (%)	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**
Black Flesh (Corte Negro)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.2	0.0
HWT Damage	0.8	0.0	0.0	0.0	1.8	0.0	0.0	0.0	5.0	0.8
Internal Flesh Browning	0.0	0.5	1.9	0.0	0.0	0.0	0.0	0.0	1.7	2.1
Jelly Seed	0.0	0.8	0.0	0.0	0.9	0.5	0.0	0.0	0.0	0.0
Pepita Negra	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Stem End Cavity	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 3. External defects incidence in major mango cultivars from different production areas when ripening was completed. Defect severity was classified into two categories; light severity (L, ≤ 25% affected area), and rejections (R, > 25% affected area).

External Defects	**'Tommy Atkins'**		**'Keitt'**		**'Kent'**		**'Haden'**		**'Ataulfo'**	
	L (%)	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**
Abrasion	0.8	0.3	5.6	1.9	1.4	1.4	0.0	0.0	1.2	0.0
Anthracnose	2.3	1.0	1.9	0.0	1.0	3.8	0.0	0.0	1.2	1.2
Compression Bruising	1.8	0.5	1.9	0.0	4.8	0.5	0.0	0.0	0.4	0.0
Impact Bruising	1.6	0.0	1.9	1.9	1.9	1.0	0.0	0.0	0.0	0.4
Insect Damage	0.5	0.3	0.0	0.0	0.5	1.4	1.5	0.0	0.0	0.0
Latex Damage	4.2	1.8	20.4	3.7	13.9	3.8	5.9	11.8	29.0	11.2
Lenticel Damage	26.0	13.8	11.1	75.9	14.9	17.8	14.7	29.4	17.8	17.8
Mechanical Damage	0.5	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Misshape	0.0	0.3	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
Russeting	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Scab	1.8	0.5	13.0	1.9	1.4	0.5	0.0	2.9	3.3	1.7
Shriveling	6.5	1.8	7.4	0.0	7.7	4.3	0.0	1.5	7.5	7.1
Skin Breaks and Cracks	1.3	1.0	1.9	0.0	2.9	1.0	2.9	2.9	0.4	0.4
Skin Pitting	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Stem Not Well Trimmed	3.1	0.5	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0
Stem End Rot	1.3	1.3	1.9	0.0	1.4	1.9	0.0	1.5	0.4	1.2
Sunburn	1.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.4
Sunken Shoulder	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Uneven Ripening	0.3	0.3	0.0	0.0	0.0	0.0	1.5	7.4	0.8	0.4

Table 4. Internal defects incidence in major mango cultivars from different production areas when ripening was completed. Defect severity was classified into two categories; light severity (L, ≤ 25% affected area), and rejections (R, > 25% affected area).

Internal Defects	**'Tommy Atkins'**		**'Keitt'**		**'Kent'**		**'Haden'**		**'Ataulfo'**	
	L (%)	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**	**L (%)**	**R (%)**
Black Flesh (Corte Negro)	0.0	0.3	0.0	0.0	0.5	0.5	1.5	0.0	0.0	0.0
HWT Damage	1.3	1.0	0.0	0.0	1.9	0.0	0.0	0.0	5.4	1.7
Internal Flesh Browning	3.1	2.3	1.9	0.0	2.9	0.5	0.0	0.0	2.5	4.1
Jelly Seed	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0
Pepita Negra	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0
Stem End Cavity	0.8	0.3	0.0	0.0	1.9	0.0	0.0	0.0	0.0	0.0