Time-temperature Combinations that Induce Chilling Injury of Mangos

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Investigators: Jeffrey K. Brecht and Maria Cecilia do Nascimento Nunes
Center for Food Distribution & Retailing
University of Florida

Fernando Maul
Universidad Del Valle, Guatemala

Introduction

Chilling injury (CI) decreases the quality of mangos and may be compromising their marketing in the U.S. and causing great economic losses to the mango industry members. This research project was initiated to establish the critical combinations of time and temperature and the associated chilling threshold temperature(s) for the most important varieties of mangos sold in the U.S. and the effect of other factors such as fruit maturity or ripeness and air circulation/cooling rate on susceptibility to and development of CI.

We held mangos at a range of potentially chilling temperatures for various times followed by transfer to a non-chilling temperature of 20°C to observe symptom development. The effect of air circulation on CI was tested by manipulating exposure of fruit to moving air in ways that changed the rates of fruit cooling and water loss. We evaluated CI based on visual, sensory and compositional analyses to quantify the effects of CI on mango appearance, flavor and.

The *overall objective* of this project was to determine the critical combinations of time and temperature and the associated chilling threshold temperature(s) for the most important varieties of mangos sold in the U.S.

The *specific objectives* of this research were to:

1. Quantify and evaluate the postharvest time-temperature combinations that induce the various symptoms of CI of major mango cultivars during two seasons in Florida and Guatemala.
2. Quantify and evaluate the effect of the circulation speed of air at a given temperature on CI symptom development.
3. Define future research needs, including methods to reduce chilling susceptibility, and continued testing using a standard protocol in order to evaluate possible variations in mango response to postharvest chilling conditions when grown in different regions and seasons.

Significance and Background

Mango fruit are susceptible to various physiological disorders that influence fruit quality. Among the most important of these is CI. In general, storage temperatures below about 10 to 13°C but above freezing have been reported to injure mature-green mangoes (Mukherjee, 1958; Akamine, 1963; Hatton et al., 1965; Musa, 1974; Couey, 1986). This problem limits the use of low storage temperature to manage postharvest ripening, and seriously affects the ability of handlers to store or transport mangos over long distances, because temperatures that are low enough to delay ripening, decay, and senescence may also be damaging to the fruit.

***Threshold temperature*.** An important concept with regard to CI is the *threshold temperature* for CI. Chilling injury occurs when a product is exposed to an injurious, low temperature for

sufficient time to initiate irreversible injury. The threshold temperature is the lowest temperature at which a susceptible fruit or vegetable can be held with no symptoms of CI ever developing. The product's shelf life or postharvest life will, of course, eventually end, but it will be due to some other event such as water loss (shriveling), overripeness, or decay – not CI. Thus, the critical information that must be found in order to avoid CI is the time temperature combinations that cause CI, along with the specific time temperature combinations involving the highest temperature at which CI can develop and the lowest temperature at which CI will not develop (i.e., the threshold temperature).

***Chilling injury symptoms*.** The symptoms of CI described for mango fruit include grayish, scald-like discoloration on the skin, followed by pitting, uneven ripening, and poor flavor and color development (Hatton et al., 1965; Medlicott et al., 1990). The latter two are especially important because loss of flavor due to chilling may occur without the development of the other, visual symptoms. The symptoms of chilling injury are also often not apparent while the fruit are at the low temperature, but develop later, when the fruit are brought to warmer temperatures for ripening or are displayed for sale. Chilling injury symptoms in mango fruit held at room temperature for 1 to 2 days after low temperature storage were described as discolored and pitted areas on the surface (Srivastava, 1967; Kane, 1977) followed by irregular ripening with poor color and flavor (Hatton et al., 1965) and increased susceptibility to microbial spoilage (Sadasivam et al., 1971; Subramanyam et al., 1975). Ketsa et al. (2000) reported that the mangoes stored at 4°C for 3 weeks developed blackened lenticels and grayish patches on the peel after the transfer to ambient temperatures.

***Other chilling injury effects*.** Chilling injury has other effects on mango fruit quality besides visual injury symptoms and flavor loss. Chilling injury induced in mango fruit stored at 4°C accelerated the softening of the fruit after they were transferred to 20°C; humidification of the ambient atmosphere reduced the symptoms (Kane et al., 1982). Krishnamurthy and Joshi (1989) reported disruption of mesocarp cells and inhibition in carotene development after 4 weeks in fruit stored at 7°C. These fruit failed to ripen evenly after holding for up to 5 weeks at room temperature. There is a significant decrease in soluble sugars and reduction of starch breakdown in chill-injured mango fruit, possibly due to increased invertase and decreased amylase enzyme activities (Chhaptar et al., 1971). Chilling injured mango fruit showed decreased accumulation of ascorbic acid, but increased accumulation of minerals in the chill-injured peel (Chhatpar et al., 1971; Chhaptar and Modi, 1974). Kane and Marcellin (1978) reported that the induction of CI in mango fruit stored at 4 or 8°C for up to 10 days was accompanied by a decreased capacity of succinate oxidation in mitochondria. Alcohols and aldehydes were reported to be formed as fermentative decarboxylation products in chilling-injured mangoes, particularly in an excessively high CO_2 atmosphere (Lakshminarayana and Subramanyam, 1970). Peroxidase and cellulase enzyme activities in the peel increased during CI development compared to their levels in non-chilled fruit (Zauberman et al., 1988).

Ways to reduce chilling injury. Elevated concentrations of CO_2 (5-10 kPa) in the atmosphere were reported to alleviate CI symptoms in 'Kensington' mangoes, but reduced O_2 concentration (5 kPa) had no significant effect (O'Hare and Prasad, 1993). Tolerance of 'Keitt' mango fruit to CI

was reported to increase after pre-storage heat treatments (McCollum et al., 1993). In our work at the University of Florida, we have shown that the APHIS hot water quarantine treatment and other time-temperature combinations reduced the susceptibility of Tommy Atkins and Keitt mangoes to CI (Brecht et al., 2000). We have also shown that mangos could be shipped for 2 to 3 weeks in controlled atmospheres at 8°C for tree-ripe fruit or 12°C for mature-green fruit without developing CI (Bender, et al., 2000a,b).

***Fruit maturity affects CI susceptibility*.** For fruit in general, CI susceptibility decreases as the fruit develop, mature, and ripen. Thus, immature fruit are more susceptible to CI than mature fruit, and fruit that are mature but have not yet begun to ripen are more susceptible to CI than fruit that are undergoing ripening. Commonly, fruit that are exposed to chilling temperatures before they have begun ripening are never able to ripen normally. Often this involves ripening without the development of normal flavor and aroma – in other words, chilled fruit may ripen, but they are tasteless. While this relationship between fruit maturation and CI susceptibility has been demonstrated for many fruits, it has not been as clearly recognized for mango. Most mango cultivars show injury below 10°C if fruit have just reached full maturity (i.e., "mature-green"), but there have been only a couple of examples of research demonstrating that mango tolerance to CI increases during ripening (Medlicott et al., 1990; Mohamed and Brecht, 2002).

***Mango cultivars differ in chilling susceptibility*.** Mango chilling susceptibility also varies with cultivar (Farooqui et al., 1985); `Haden` and `Keitt` are reported to be particularly susceptible. `Sensation` mangoes developed more skin symptoms than 'Sammar Bahisht' mangos (Farooqui et al., 1985). While CI has generally been reported to occur in mango fruit at temperatures below about 10-13°C (discussed above), some cultivars ('Dasheri', 'Langara') were reported to be safely stored at 7-8°C for up to 25 days (Mann and Singh, 1976), but the maturity stage of those fruit was unclear.

Thus, the variability among reports with regard to the lowest safe temperature to store mangos without danger of CI may be due to differences in cultivar susceptibility as well as differences in the stage of fruit maturity or ripeness in different experiments. However, up to now there has never been a research project undertaken to systematically compare the response of different major mango cultivars at different maturity and ripeness stages to a range of potentially chilling time-temperature combinations. Knowing the critical combinations of time-temperature and the associated chilling threshold temperature(s) for the most important varieties of mangos sold in the U.S. would provide basic information to decrease the incidences of CI and to deliver better quality, especially better tasting, mangos to the consumer.

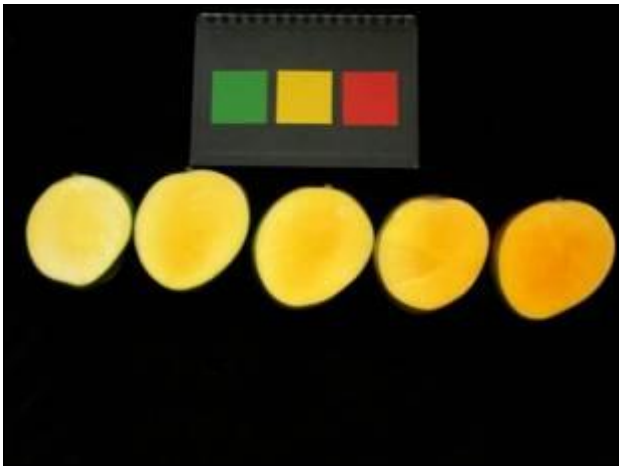
Materials & Methods

Obj. 1 – Time-temperature responses (Florida & Guatemala). This part of the research project was conducted during the 2008 & 2009 seasons in Florida and during the 2009 season in Guatemala. The following mango cultivars were included in this part of the study:

- Ataulfo (Guatemala)

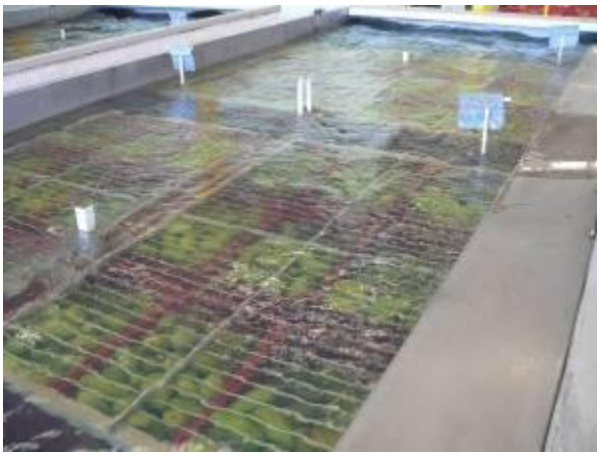
- Francis (Florida)
- Keitt (Florida and Guatemala)
- Kent (Florida and Guatemala)
- Tommy Atkins (Guatemala)


Maturity & ripeness: The harvest maturity stage distribution in each fruit lot was determined by determining the internal flesh color development of an initial 25-fruit subsample. Subjective pulp color ratings were based on the 5-point classification system, with 1 corresponding to white, 2 corresponding to ¼ yellow flesh; 3 corresponding to ½ yellow flesh, 4 corresponding to ¾ yellow flesh, and 5 corresponding to complete yellow flesh development. Our intent in following this procedure was to be able to relate results of CI incidence and symptom severity to initial maturity of the fruit.



Hot water treatment: All of the mangos were heat treated according to the USDA APHIS protocol, either at commercial facilities in Guatemala or with an experimental unit in Florida (Model HWH-2, Gaffney Eng., Gainesville, Fla.) that is the same as the apparatus that was used by USDA ARS scientists in developing the current hot water immersion treatment. Up to 32 kg of fruit can be heated to up to 55°C in each of two treatment tanks with incoming water

temperature controlled within +/- 0.1°C.



Time-temperature storage treatments: For application of the different time-temperature combination treatments, mangos were held at 5, 7.5, 10 or 12.5°C for up to 4 weeks, with weekly transfers to ambient temperature of 20-23°C for 5-6 days.

Measurements: After each storage period and after 5 or 6 days at 20-23°C, 25-fruit samples were taken for measurements. Nondestructive measurements included visual chilling injury symptom ratings, defect and decay ratings, skin color, and mango aroma according to methods we have used in our previous research (Nunes et al., 2007). Destructive measurements included flesh color (subjective & objective), flesh firmness, and % soluble solids content (°Brix), pH and titratable acidity of the juice. Flesh dry matter content was measured in Guatemala only.

The visual CI symptoms (lenticel darkening, skin pitting, scalding, uneven ripening) of each individual mango were assessed before and after transfer from the putative chilling temperatures to ambient temperature using a rating scale in which 1 = severe, >50% of the

fruit's surface showing damage; 2 = moderate, 25–50% chilling damage; 3 = slight, up to 25% pitting and/or scalding; 4 = trace (small pits), 2–5% of the total fruit surface damaged; 5 = no visible symptoms of injury.

Fruit firmness was assessed on each individual mango by gentle hand pressure using a rating scale in which 1 = fully soft, 2 = advanced softness, 3 = first softening, 4 = firm to the touch, 5 = very firm to the touch.

The shriveling of each individual mango was assessed using a visual rating scale in which 1 = extremely shriveled, wrinkled and dry, not acceptable under normal conditions; 2 = severe shriveling, definitely, objectionable; 3 = moderate, shriveling evident, becoming objectionable; 4 = slight, minor signs of shriveling, not objectionable; 5 = none, field fresh, no signs of shriveling.

The decay of each individual mango was assessed using a modified visual rating scale from Horsfall and Barratt (1945) where 1 = 76–100% decay, severe to extreme decay (the mango is either partially or completely rotten); 2 = 51–75% decay, moderate to severe decay; 3 = 26–50% decay, slight to moderate decay (spots with decay and some mycelium growth); 4 = 1–25% decay, probable decay (brownish/grayish sunken minor spots); 5 = 0%, no decay.

Utilizing a Konica Minolta portable colorimeter(Minolta, Ramsey, N.J.) with an 8 mm aperture, external peel color and internal pulp color measurements were taken using the CIE lab, L, a*, b* and converted to hue angle and chroma. Pulp samples from each pulp color stage were analyzed using a Minolta digital colorimeter to document objective color coordinates expressed as Hue Angle, Chroma and Lightness coefficients. Measurements were taken on two sides of the peel and pulp for each mango sample analyzed.

Pulp firmness was measured using a handheld manual penetrometer with an 8-mm Magness-Taylor probe. Measurements were taken on two sides of each mango and the average value of those measurements was reported as a pulp firmness for each fruit. Ten mangos were analyzed for each color category and/or variety after every storage interval.


Subjective pulp aroma ratings were performed by a small panel of 3 to 4 experienced mango experts from our laboratories. We placed 8 to 10 sliced mango halves inside 1 gallon glass jars, which were then sealed with polyethylene bags to accumulate headspace aromas for 5 minutes. Once the 5-minute period expired, the lab personnel smelled inside the jar and provided a subjective 1 to 7 rating on the aroma (1= green mango aroma, 5= ripe mango aroma 7 = overripe/souring mango aroma).


Pulp soluble solids and pH were measured using individual mango pulp samples that were macerated and then centrifuged to separate water soluble and insoluble components of the pulp. Soluble solids and pH readings were taken from the centrifuged supernatant. Pulp dry matter content was measured using 50-gram samples of mango pulp obtained from horizontal fruit sections, which were dried inside a convection oven set at 90°C. Depending on the mango variety, sample drying required between 48 and 72 hours. Dry matter readings were recorded once the mass of the dry mango sample remained constant over a 12-hour period. The dry matter weight was expressed as a percentage of the mango fresh weight.

Obj. 2 – Cooling rate versus chilling injury (Florida). This part of the research project was conducted during the 2009 season in Florida. The following mango cultivars were included in

this part of the study:

- Keitt
- Tommy Atkins

Maturity/ripeness & hot water treatment: Same as for Obj. 1.

Treatments: Following hot water treatment, the fruit were placed in a 5°C room (chilling temperature) to be cooled at different rates, or placed in a 12.5°C room as the control treatment:

- Slow room cooling (shielded from room fans by microperforated film – 18 hours)
- Rapid room cooling (fully exposed to room fans – 6 hours)
- Forced air cooling (2 hours)
- Control room cooling at 12.5°C

Forced-air cooling was performed using a small scale forced air cooling unit that we developed in a previous research project to evaluate pressure ripening of tomato fruit. The unit was designed to cool a half-pallet of tomatoes (44, 25-lb cartons) from approximately 30°C to 20°C in 2 hours by forcing the air in a refrigerated room to flow through the carton.

After the cooling treatments, the fruit were all stored for 2 weeks at 12.5°C, then transferred to 20°C for 5 days.

Results

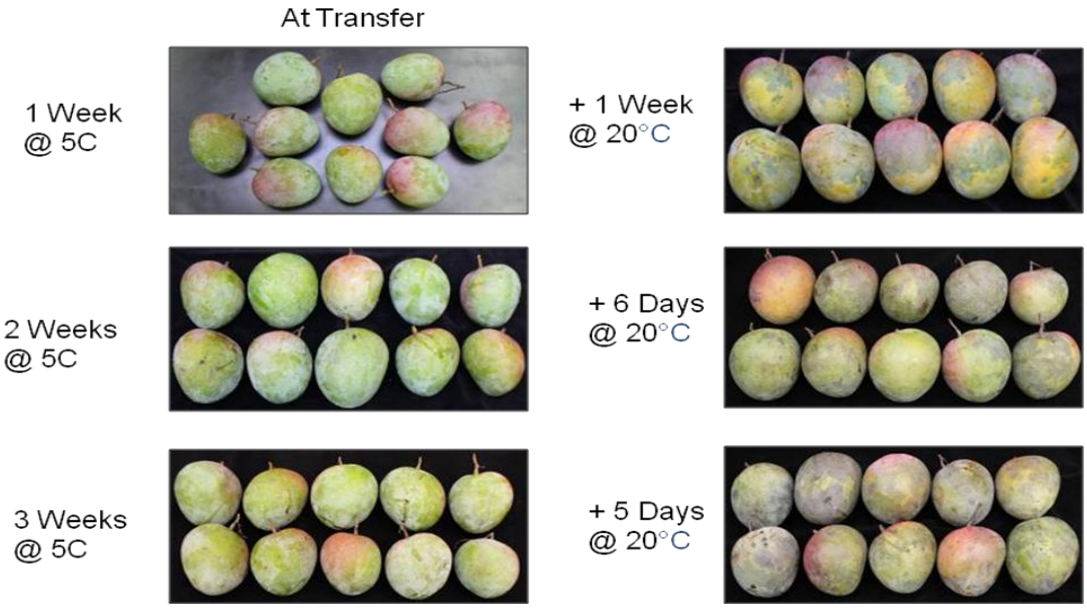
Obj. 1 – General Results and Key Observations

A. Chilling injury symptoms common to all varieties

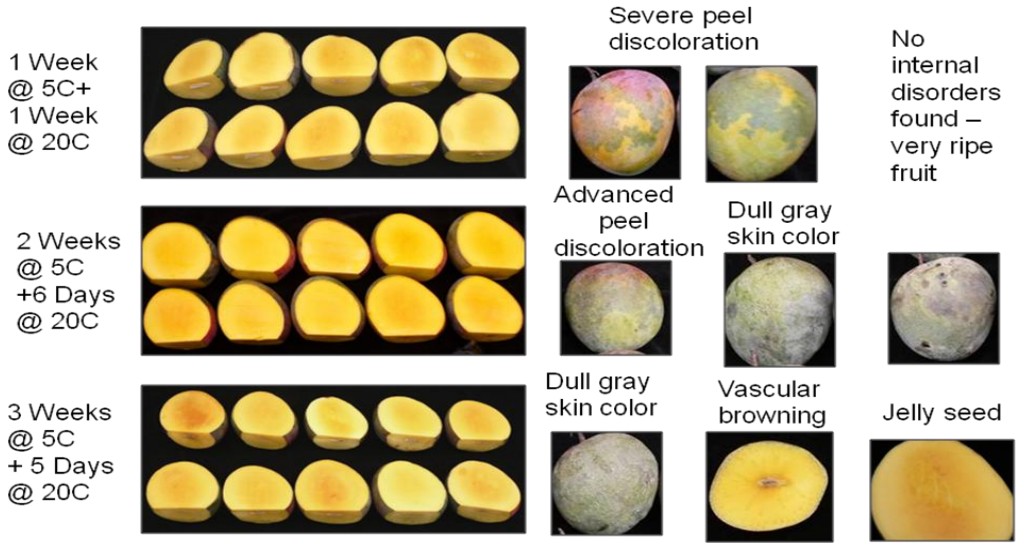
- **Loss of aroma** is the first symptom to develop, and chilled mangos do not recover normal aroma levels even after 5 days at ambient temperature.
- **Lenticel discoloration** is the earliest visual symptom: this symptom appears as soon as 1 week at 5°C or 2 weeks at 7.5°C.
- **Skin discoloration** (gray or brown appearance) and **vascular (internal) browning** are the next visual symptoms, occurring after 2 weeks at 5°C or 3 weeks at 7.5°C.
- **Scald**-like skin collapse appears last.

The following pictures illustrate these results for Kent mangos…

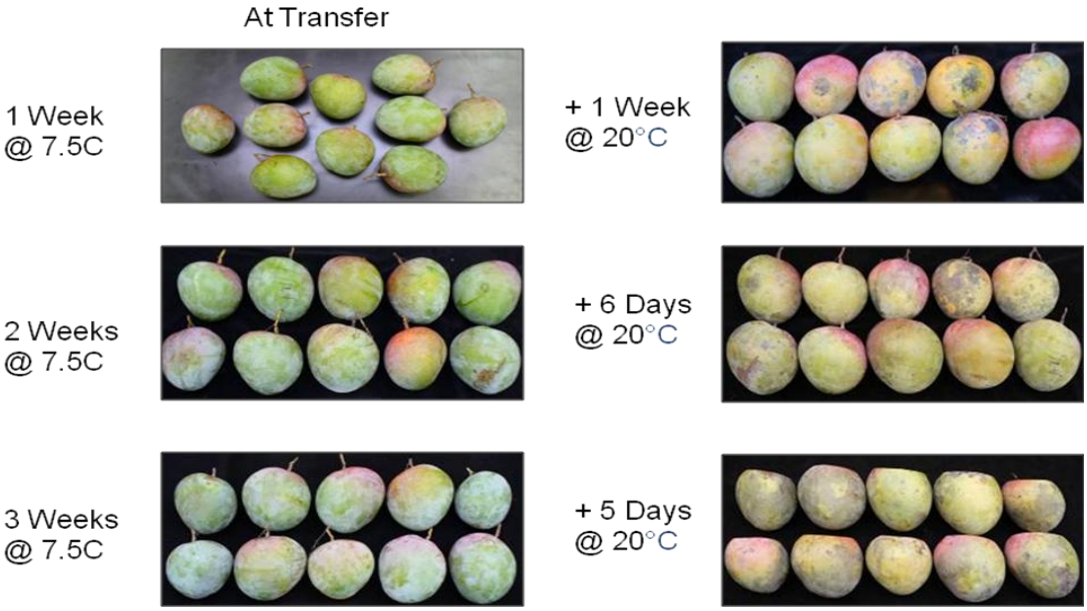
Kent 5°C



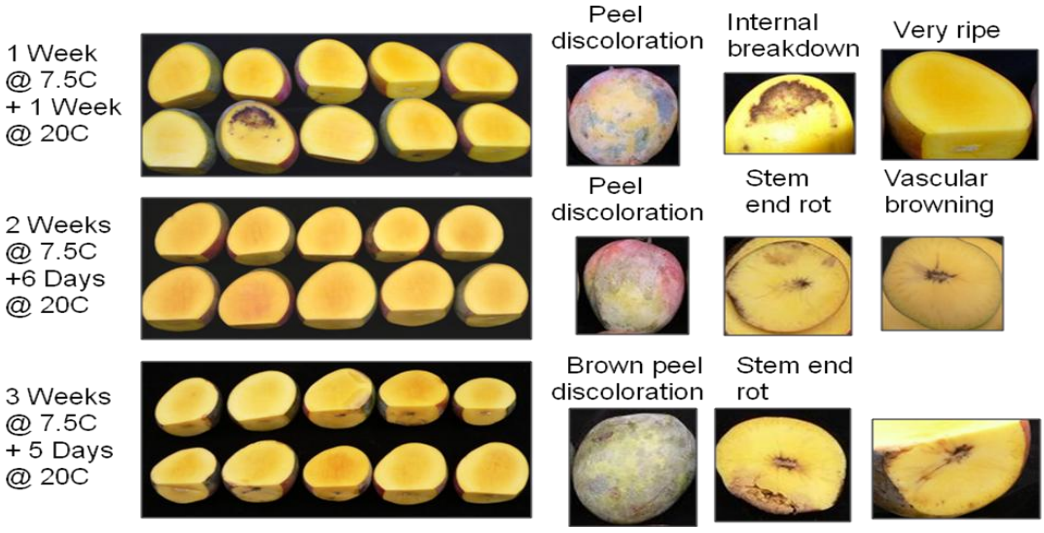
Observations



Kent 7.5°C

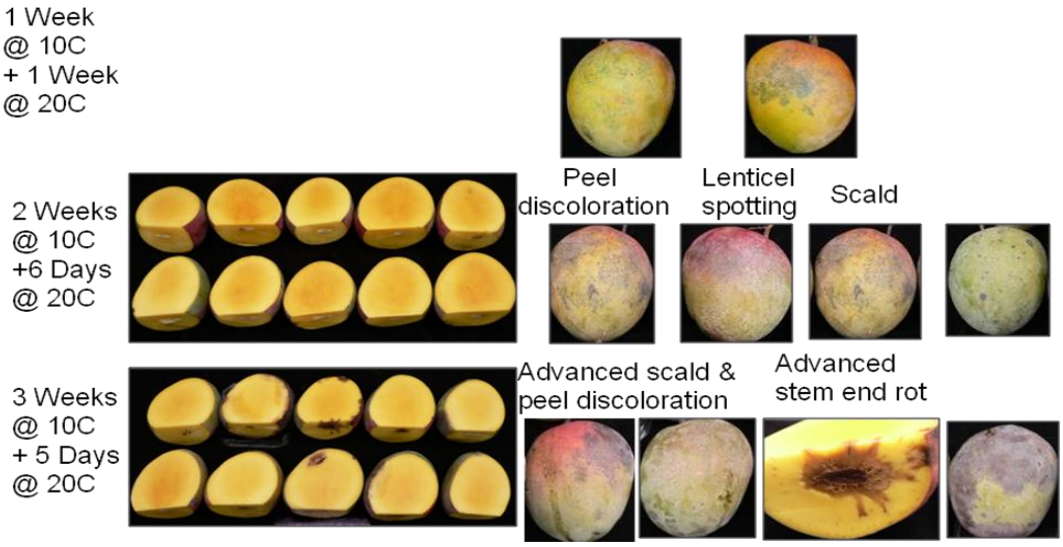


Observations



Kent 10°C

Observations



Kent 12.5C

At Transfer

1 Week @ 12.5C

+ 1 Week @ 20°C

2 Weeks @ 12.5C

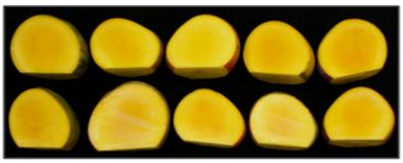
+ 6 Days @ 20°C

3 Weeks @ 12.5C

+ 5 Days @ 20°C

Observations

1 Week @ 12.5C + 1 Week @ 20C



Very little damage found

70% yellow in color

Mangos almost ripe

2 Weeks @ 12.5C +6 Days @ 20C

Dull skin color

Internal browning

3 Weeks @ 12.5C + 5 Days @ 20C

Dull skin color

Decay

B. Cultivar differences in CI susceptibility

- Ataulfo is the most sensitive variety. Flesh browning development occurred around the seed after 2 weeks at 5°C to 12.5°C, but 3 or 4 weeks at 10 and12.5°C resulted in the worst browning. Other CI symptoms for Ataulfo were:
    - Skin shriveling
    - Aroma loss (most sensitive of all the varieties)

- Kent developed a dull, bleached external appearance

C. Color, firmness and composition related to CI

- There were some effects of low temperatures on fruit softening (see Keitt results), but there were no major effects on fruit color, dry matter, soluble solids content (°Brix) or acidity.

D. Fruit harvest maturity related to CI

- The proportion of fruit that developed the most severe symptoms of CI after storage and ripening were roughly proportional to the proportion of least mature fruit in initial samples.
- Initial sample size (25 fruit) was probably not large enough to accurately estimate the maturity distribution.

Obj. 1 – Results by Variety

A. Ataulfo

- Ataulfo mangos were very susceptible to low storage temperatures in both experiments conducted.
- The initial stage of ripeness did not affect chilling injury response in the first experiment. Fruit with initial ripeness rated between 2 and 4 were all affected by low temperatures.
- Pulp browning symptoms were the most notable chilling injury symptom documented.
- Pulp browning symptoms became evident after 2 weeks of storage in all storage temperatures in both experiments.
- Severity of browning increased with storage duration.
- Severity of pulp browning was higher in fruit stored at either 10 or 12.5°C when compared to lower storage temperatures. Pulp browning was accompanied by a leathery/dry pulp texture, especially in pulp tissues close to the mango seed.
- Ataulfo pulp firmness was affected by storage temperature and duration. After 21 and 28 days of storage, Ataulfo mangoes were slightly firmer than firmness measurements after 8 and 15 days - probably due to changes in pulp texture occurring in conjunction with pulp browning.
- Ataulfo aroma ratings were normal during first 21 days of storage. After 28 days of storage, Ataulfo mangos stored at 5°C were judged to have sour/overripe aroma, other temperature treatments had normal ripe/fruity aroma.
- Anthracnose decay became significant in fruit stored at 12.5°C for 21 to 28 days.
- Pulp browning symptoms did not relate to any external visual symptoms.
- There was no relationship between storage temperature and lenticel spotting.
- Ataulfo mangos are susceptible to CI at temperatures of 12.5°C or lower. Symptoms develop after 15 days plus 5 days at room temperature, with severity increasing with storage duration. In commercial shipments from South America this could be a relevant quality concern. It could be recommended to maintain refrigerated storage even at the supermarket to prevent pulp browning upon return to room temperature.

B. Francis

- A single experiment was conducted with Francis mangos, but the fruit had been transported by ocean from Haiti to Florida and were of too advanced maturity when delivered for the experiment to be considered valid.

C. Keitt

- After 21 days of storage, fruit stored at 5 or 7.5°C had a sour aroma while the other temperature treatments had normal aroma.
- Mangos stored at 12.5°C had consistently lower pulp firmness when compared to other storage treatments.
- Pulp browning symptoms were not apparent in Keitt mangos stored at any temperature.
- No relationship between storage temperature and lenticel spotting was evident.
- Decay in Guatemala experiments:
    - During the first storage experiment severe anthracnose decay symptoms developed after 15 days at all temperatures. The experiment was terminated after 21 days due to severity of decay.
    - During the second experiment, an additional heat treatment was effective in reducing the severity of anthracnose decay.
    - Anthracnose incidence had a strong relationship with pulp firmness. Increasing severity correlated with lower pulp firmness.
    - Pulp firmness dropped after refrigerated storage, even after 15 days storage, more dramatically than any other variety tested.
    - Anthracnose decay affected mangos stored at 12.5°C after 15 days of storage with severity increasing with storage duration.
    - Anthracnose symptoms were severe after 28 days of storage.
- Pulp browning was observed in 30 to 40% of mangos stored at 5 or 7.5°C for 28 days.
- Subjective aroma ratings showed no significant differences between temperature treatments during the first 21 days of storage. However, after 28 days of storage, mangos stored at 5 or 7.5°C were judged to have sour/overripe aroma.

D. Kent

- After 21 days of storage, mangos stored at 5 or 7.5°C remained firmer and had lower subjective pulp color readings when compared to higher storage temperatures (i.e., ripening inhibition due to CI).
- Peel discoloration was evident in mangos stored below 10°C for at least 21 days.
- There was no relationship between storage temperature and lenticel spotting.

E. Tommy Atkins

- Tommy Atkins mangos appear to tolerate low temperature without major symptoms during the first 2 weeks of storage. No significant differences were observed between temperature treatments.
- Mango aroma was affected as a result of storage temperature. After 21 and 28 days of

storage, mangos subjected to 5 or 7.5°C lost their fruity/ripe aroma and instead were judged to have a sour/overripe aroma while other parameters such as pulp firmness or °Brix were not significantly different between treatments.

- Pulp browning symptoms were observed in 30 to 40% of the samples stored at either 5 or 7.5°C for 28 days in both storage experiments. No browning symptoms were observed with shorter storage periods.
- There would be an increased risk for pulp browning and aroma changes if Tommy Atkins mangos are stored at 7.5°C with no significant benefits in other ripening parameters.
- Tommy Atkins mangos stored at 12.5°C had more severe symptoms of stem-end rot when compared to fruit stored at lower temperatures, especially with storage times greater than 21 days.
- Anthracnose decay was not a significant quality problem with Tommy Atkins mangoes.
- It is important to note that pulp firmness, °Brix and subjective pulp color were comparable between mangos stored at either 7.5 or 10°C. This would suggest that storing mangos at 7.5°C will probably have no better effect in retarding mango ripening or extending shelf life, when compared to the recommended 10°C storage temperature.
- There was no evident relationship between storage temperature and lenticel spotting.
- Tommy Atkins mangoes retained their pulp firmness well after 5 days at room temperature following low temperature storage. Other commercial varieties such as Kent and Keitt lose firmness more dramatically during this ripening period.

Obj. 2 – General Results and Key Observations

A. Forced-air Cooling *versus* CI

- We found no evidence that forced-air cooling increases CI.

We speculate that there may be situations in which forced-air cooled mangos that are shipped long distances in marine containers at 7-8°C (i.e., chilling temperature) may develop CI, whereas, the same mangos 'stuffed hot' and shipped at 7-8°C may not develop CI. This would be because the 'hot' mangos would cool very slowly, if at all, in a container and would thus be maintained above the chilling range during transport

Future Research Needs

Recommended future research needs related to understanding and alleviating CI of mangos are:

- Determine CI susceptibility for the same mango varieties:
    - grown in other regions
    - grown in different seasons with different environmental conditions
    - harvested on different dates from the same orchard

- Test additional varieties (e.g., Francis and Haden)

- Test the effect of treatments that may alleviate CI, such as coatings or MAP

Recommended Standard Protocol for Testing Mango CI Susceptibility

The recommended standard protocol for testing CI susceptibility of mangos, for use by industry and others:

1. Collect samples of mangos after carton filling, or after precooling (if performed).
    a. Use the most common size in the lot of fruit.
    b. Cut a sample of at least 100 fruit to determine the distribution of maturity stages using the 5-point classification system.
2. Place samples at 5°C for up to 4 weeks, with four, evenly spaced, evaluations performed within that time frame.
    a. Remove 100-fruit samples for immediate evaluation after each storage period.
    b. Transfer additional 100-fruit samples to 23-25°C after each storage period and observe the visual appearance daily for 5 days.
    c. Evaluate fruit for external and internal appearance after 5 days at 23-25°C.

CI evaluation procedure:

1. **External appearance**
    a. Score fruit for lenticel discoloration and scald using 1 to 5 scales.
    b. Document decay incidence and severity.
    c. Observe color development, and note any gray or brown discoloration or blotchy color development
2. **Internal condition**
    a. Cut fruit as for maturity determination and score the internal color using the 1 to 5 scale.
    b. Note if the aroma is normal or if unripe or off odors are present.
    c. Measure the fruit firmness and °Brix.

This standard protocol can be used to determine CI susceptibility for the same mango varieties grown in other regions and in different seasons with different environmental conditions, to test additional varieties, to test the effect of treatments that may alleviate CI, such as coatings or MAP, and can be used as part of a quality control (QC) or quality assurance (QA) program during actual transport and handling operations.

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