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Feasibility of Modified Atmosphere Packaging (MAP) Plus Ethylene Scrubbing for Extended International Shipping of Mangos

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The feasibility of incorporating modified atmosphere packaging (MAP) technology with or without ethylene scrubbing into existing mango handling systems was evaluated as a way to allow shipment of fully mature (tree-ripe) fruit from South America to the United States in order to improve the quality of mangos available to U.S. consumers. In preliminary research, the optimum atmospheres (low O_2; high CO_2) for slowing 'Tommy Atkins', 'Kent' and 'Keitt' mango ripening were determined using controlled atmosphere (CA) treatments. We then replicated the CA lab tests using Breatheway™ (BW) MAP with and without It's Fresh™ (IF) ethylene scrubbers that had been custom designed for us by the companies based on the results of the CA experiments. In November 2022, we conducted two simultaneous shipping trials from Brazil using 'Tommy Atkins' and 'Kent' mangos. The mangos were shipped by ocean from Brazil to New Jersey, then by refrigerated truck to the University of Florida Postharvest Lab in Gainesville, all at 9 °C/48 °F for a total of 28 days, followed by a 4-day shelf life period at 20 °C/68 °F, to evaluate a 4.5-kg carton-scale BW MAP +/– IF in commercial conditions. A third shipping trial with 'Kent' mangos from Peru to California was conducted in February 2023, with evaluations conducted in California by Hazel Technologies (Breatheway's parent company). The preliminary research established that tree-ripe mangos (Stage 3.5–4) can be stored as low as 7 °C/45 °F for 4 weeks without showing chilling injury symptoms or deterioration of fruit internal quality when held in minimum 4% O_2 + 5 to 10% CO_2 ('Kent' and Keitt') or minimum 6% O_2 + 5 to 10% CO_2 ('Tommy Atkins'). The shipping test results indicate that MAP with IF slowed ripening-related softening, color changes, and losses of sugars and acids, while sensory panelists rated the treated fruit as sweeter, less acidic, and with an overall higher eating quality than the untreated controls, which were overripe. Overall, our results lead us to recommend MAP plus ethylene scrubbing for long distance (4-week) shipping if the goal is to export/import tree-ripe mangos. A pallet-scale MAP that is available would facilitate better temperature management by allowing the packed fruit to be pre-cooled before application of the MAP plus ethylene scrubbers, promising even better results.

The long shipping distances from mango growing regions in South America to the export markets in the United States (U.S.) result in shipping durations that can exceed 3 weeks. This forces exporters in Ecuador, Peru, and Brazil to ship fruit of minimal maturity and at lower than recommended temperatures so as to manage the onset and progression of fruit ripening and thereby minimize arrival problems due to soft, ripe, or decayed fruit. However, these practices increase problems with hot water injury and chilling injury (CI), to which less mature mangos are more susceptible. Fruit harvested early are also more susceptible to damage from water loss and mechanical damage (abrasion). Even if symptoms of hot water injury or CI do not become visible in imported mangos, the mangos may still fail to ripen or ripen with poor sensory quality. We documented this situation in our previous National Mango Board-funded research project, "Monitoring and Evaluation of the Mango Supply Chain to Improve Mango Quality" as reported in our *Mango Postharvest Best Management Practices Manual* (Brecht et al., 2020). In that study, we identified shipment of immature mangos at low temperatures as the most important problem that reduces mango consumer quality in the U.S. market.

Mangos that are harvested too early (immature physiological state) are more sensitive to hot water injury and CI, causing failure to ripen or development of poor sensory quality when ripe, while those fruit harvested at too late a stage of maturity have reduced shelf life from over-ripening and increased susceptibility to mechanical damage (i.e., bruising) and postharvest diseases (Brecht et al., 2020; Sivakumar et al., 2011). Chilling injury susceptibility decreases as mango fruit develop, mature, and ripen. Thus, immature fruit are more susceptible to CI than

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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 (Medlicott et al., 1990; Mohammed & Brecht, 2002). Commonly, mango 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 mangos may ripen, but they are tasteless. Hydrocooling mangos after the quarantine hot water treatment was developed by mango handlers as a procedure to reduce incidence of hot water injury in low maturity fruit. The application of hydrocooling after hot water treatment is now proscribed by the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA–APHIS) protocol that specifies the allowed timing and water temperatures for both the hot water treatment and the hydrocooling (T102-a http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf). Another procedure that is used by many mango handlers to avoid damage due to the hot water treatment is to impose a period of "rest" at ambient temperatures following the hot water treatment and prior to grading and packing. The rest period is intended to allow symptoms of hot water injury to develop before the fruit are graded on the packing line so that damaged fruit can be removed. If tree-ripe mangos could be successfully shipped the necessary distances required for export from South America to the U.S., these issues with CI and hot water injury in low maturity mangos could be largely eliminated.

Many years of research have shown that controlled and modified atmosphere (CA and MA) technology can significantly extend the postharvest life of mangos and have led to well-recognized and accepted recommendations for the optimum O_2 and CO_2 concentrations for maintaining mango quality (Brecht and Yahia, 2009; Thompson, 2010). Commercial CA and MA systems have been developed and are in widespread use for storage, transport and marketing of many crops (Brecht et al., 2009; Yahia, 2009). Controlled and modified atmospheres reduce respiration (Kelany et al., 2010) and ethylene production, which is how they slow the ripening process and extend the storage life of the fruit. Research has been conducted demonstrating that elevated concentrations of CO_2 (5 to 10%) in the atmosphere also alleviate CI symptoms in mangos, but reduced O_2 concentration (5%) has no significant effect (O'Hare and Prasad, 1993). Our work at the University of Florida, showed that mangos could be shipped for 3 weeks in modified atmospheres at 8 °C (46 °F) for tree-ripe (Stage 4) fruit or 12 °C (54 °F) for mature-green (Stage 2) fruit without developing CI (Bender, et al., 2000 a, b). Selection of the atmosphere must be done with care, for extended exposure to levels of O_2 or CO_2 that are too extreme can induce physiological damage in the fruit (Brecht, 2018). However, while CA and MA systems are available for marine containers, the technology is quite expensive and the availability of CA-equipped containers is insufficient to handle mango export volumes.

Modified atmosphere packaging (MAP) is a low-tech application of CA that works by balancing the O_2 and CO_2 permeability of a sealed package with the respiration rate of the enclosed product, which consumes O_2 and releases CO_2 (Tasneem, 2004). The concentration of gases within the package depends on several factors, including the film's gas permeability properties, the relative area and volume of the packaging, and the quantity and temperature of the product (Oliveira et al., 2005). Thus, at equilibrium, the MAP headspace has an increased level of CO_2 and a reduced level of O_2 that is appropriate for the product, which must be designed into the package to match the tolerance of the fruit to those gases. Using MAP for mangos has until recently been hampered by the lack of availability of proper package materials to achieve optimum atmospheres for mangos. However, we identified the MAP provider Hazel Breatheway® as having developed a MAP system for mangos, and who had, along with TransFresh (Tectrol™), jointly developed a pallet-scale MAP system being used commercially for blueberries exported from Argentina to the United States. They provided a similar MAP designed specifically for mangos for our use in this research. Additionally, we have seen that many MAP systems (including Breatheway™) are relatively impermeable to ethylene gas, which can therefore accumulate to high levels inside the package. Therefore, since ripening mango fruit produce ethylene, we realized that scrubbing the ethylene may be an essential system component for maximum effectiveness of MAP for mangos.

The objective of this research was therefore to determine the feasibility of incorporating BW MAP technology with or without IF ethylene scrubbing into existing mango handling systems as a way to allow shipment of fully mature (tree-ripe) fruit from South America to the U.S. in order to improve the quality of mangos available to U.S. consumers. To accomplish this, we conducted laboratory CA studies to determine the optimum atmospheres (low O_2; high CO_2) for slowing 'Tommy Atkins', 'Kent', and 'Keitt' mango ripening. We then replicated the CA lab tests using Breatheway™ MAP with and without It's Fresh™ ethylene scrubbers that had been custom designed for us by the companies based on the results of the CA experiments. To evaluate BW MAP plus/minus IF ethylene scrubber filters in commercial conditions, we conducted shipping trials from Brazil to New Jersey, with 'Tommy Atkins' and 'Kent' mangos, with the mangos then shipped by refrigerated truck from New Jersey to Gainesville, FL for shelf life evaluation; and from Peru to California with 'Kent' mangos, with the shelf life evaluation performed at the Hazel Technologies lab in Fresno, CA.

Materials and Methods

Laboratory determination of optimum atmospheres and evaluation of Breatheway™ MAP and It's Fresh™ ethylene filters. For experiments to evaluate CA +/– ethylene scrubbing, imported mango fruit at maturity stage 3.5 on a 1 to 5 flesh color development scale from 1 = no yellow color to 5 = 100% yellow or orange color were obtained from Central American Produce (CAPCO) in Pompano Beach, FL, on the day of arrival from the exporting country (Mexico, Brazil, Ecuador, or Peru for 'Tommy Atkins', 'Kent', and 'Keitt', respectively). For experiments to evaluate MAP +/– ethylene scrubbing, imported 'Tommy Atkins' mango fruit at maturity stage 3.5–4 were obtained from CAPCO on the day of arrival from Mexico or Ecuador.

The fruit were transported in an air-conditioned vehicle to the Postharvest Horticulture Laboratory at UF, Gainesville (approx. 4 hours) and stored at 7 °C and 85% relative humidity (RH) for 28 days, then transferred to 20 °C and 95% RH for up to 6 days. The CA treatments were established using a system employing needle valve flowmeters and manifolds to mix nitrogen, air and CO_2 delivered at equal pressure and the resulting gas mixtures were humidified by bubbling through water. The Breatheway™ MAP bags were large enough to hold 8 or 9 mangos weighing 4.5 kg and were designed based on results from previous respiration measurements to establish an atmosphere of 4 to 6% O_2 plus 10 to 15% CO_2 at 7 °C. Similarly, the It's Fresh™ filters were designed with sufficient capacity to adsorb the ethylene

produced by the mangos for the entire duration of the storage plus shelf-life periods (types A5 and A6 had different areas for ethylene adsorption).

There were three replicates for each treatment on each timepoint, and each replicate consisted of a carton of eight or nine fruit. Fruit evaluations were done on Days 0 and 28 at 7 °C; also on Days 2, 4, and 6 at 20 °C for shelf-life evaluation. The MAP bags were kept sealed during the first 4 days of the shelf-life period since the Breatheway™ MAP adjusts its permeability to accommodate temperature changes. A completely randomized experimental design was used for all studies and data were analyzed using one-way analysis of variance (ANOVA) on each time point.

The following controlled atmosphere (CA) conditions were applied:

CA1 = Air control

CA2 = 4 kPa O_2 + 5 kPa CO_2 CA5 = 6 kPa O_2 + 5 kPa CO_2

CA3 = 4 kPa O_2 + 10 kPa CO_2 CA6 = 6 kPa O_2 + 10 kPa CO_2

CA4 = 4 kPa O_2 + 15 kPa CO_2 CA7 = 6 kPa O_2 + 15 kPa CO_2

The following Breatheway™ MAP and It's Fresh™ (ethylene scrubbing) treatments were applied:

T1 = Air control + No It's Fresh™ T6 = Breatheway™ MAP + No It's Fresh™

T2 = Air control + A5 It's Fresh™ T11 = Breatheway™ MAP + A5 It's Fresh™

T3 = Air control + A6 It's Fresh™ T12 = Breatheway™ MAP + A6 It's Fresh™

Evaluations of treatment conditions and fruit physicochemical and sensory quality. Gas concentrations (O_2, CO_2, and ethylene) were measured on a weekly basis during CA and MAP storage at 7 °C and on alternate days in MAP at 20 °C using gas chromatography. The gas concentrations were also used to determine the respiration and ethylene production rates by placing fruit in sealed jars for 1 hour. Fruit susceptibility to CI, decay incidence, lenticel discoloration, and CO_2 injury were also scored based on the skin area affected by each disorder. Rating scales of 1 to 5 were used: 1 = 0%; 2 = < 5%; 3 = 5 to 10%; 4 = 10 to 25%; and 5 = > 25% of the affected fruit area. Fruit firmness as resistance to compression and puncture forces were measured on both sides of fruit using a Texture Analyzer (Texture Technologies) with a flat plate and 8-mm diameter convex tip probe, respectively, with the average value from both readings for each fruit used for statistical analysis. Color attributes were determined (two readings per fruit on opposite sides): skin ground color; subepidermal flesh color; at the stone color; the measured L*, a*, and b* values were converted to hue (shade of color); and chroma (color purity). The total soluble solids (TSS), titratable acidity (TA), TSS/TA, and pH of combined fruit juice were measured using refractometry and titration. Sensory evaluation was done during shelf life after 4 days at 20 °C once the fruit were ripe/ready to eat. Attributes evaluated were mango aroma, texture, juiciness, sweetness, acidity, overall eating quality and ripeness appearance. Photographs were taken on both sides of the fruit skin, the subepidermal flesh, and at surface of the stone on each timepoint.

Shipping tests. Brazilian-grown 'Tommy Atkins' and 'Kent' mango fruit with dry matter (DM) content of >13% ('Tommy Atkins') or 15% ('Kent') were selected at AgroBras, Petrolina, and imported by Amazon Produce, Vineland, NJ, while Peruvian-grown 'Kent' mango fruit with dry matter content of >15% were selected at Sunshine Export, Piura, and imported by Amazon Produce, Oxnard, CA. The DM values were measured using a NIR spectrometer (F-750, Felix Instruments, Camas, WA) utilizing a previously developed algorithm (Tonetto de Freitas et al., 2022).

The following treatments were applied:

T1 = Air control - No Breatheway™ and no It's Fresh™ filters (Control)

T2 = Breatheway™ + No It's Fresh™ filter (BW)

T3 = Breatheway™ + It's Fresh™ filter (BW+IF)

Mangos in 4.5-kg cartons containing 7 or 8 fruit were collected from the packinglines at AgroBras and Sunshine and either used as is or re-packed with the Breatheway™ bags used as a carton liner, either with or without an It's Fresh™ filter inserted, sealed by twisting the opening, folding over, and affixing a rubber band. The Brazilian fruit were transported by ship at 9 °C to Philadelphia, PA/Vineland, NJ, for 22 days, and from there by truck for 2 days at 9 °C to UF, Gainesville, FL, and held at 9 °C and 85% RH. The Peruvian fruit were transported by ship at 9 °C to Port Hueneme/Oxnard, CA, for 20 days, held in cold storage for 4 days at 9 °C, and from there taken by air-conditioned car to Hazel Technology, Fresno, CA, and held at 9 °C and 85% RH. In both New Jersey and California, the MAP gas concentrations were measured at the importer upon arrival from the port using a portable MAP Gas Analyzer (Bridge Analyzers, Inc., Bedford Heights, OH). When 28 days since the start of each experiment had elapsed, all fruit were transferred to 20 °C and 95% RH. Fruit evaluations were done as previously described after 28 days at 9 °C and after 2 and 4 days at 20 °C. An additional sensory evaluation of the Brazilian fruit was conducted after 10 days of shelf-life at 20 °C, when the T2 (Breatheway™ + No It's Fresh™ filter) and T3 (Breatheway™ + It's Fresh™ filter) fruit were ripe/ready to eat (Day 10). The bags were kept sealed during the initial 4-day shelf-life period since the Breatheway™ MAP adjusts its permeability to accommodate temperature changes. The bags were then opened for the next 6 days before the final sensory evaluation. A completely randomized design was used for this study. There were 21 or 24 replicates (three, 7-, or 8-fruit cartons) for each treatment at all timepoints, and each fruit was considered as one replicate.

Results and Discussion

Laboratory tests. The results (Figs. 1 and 2) showed that tree-ripe mangos (stage 3.5–4) can be stored at low temperature [7 to 8 °C (45 to 46 °F)] for 4 weeks without showing CI symptoms and deterioration of fruit internal quality. Air control fruit were generally unmarketable by the second day of shelf life at 20 °C. With respect to O_2 tolerance, tree-ripe 'Tommy Atkins' did perform better in CA at 6% O_2, developing a small amount of internal discoloration in CA at 4% O_2, while 'Kent' and 'Keitt' mangos both benefited more from 4% O_2. With respect to CO_2 tolerance, tree-ripe 'Tommy Atkins', 'Kent' and 'Keitt' mangos all benefited from CA with both 5 and 10% CO_2, however, CA with 15% CO_2 had deleterious effects on fruit quality. Based on fruit marketability, 'Tommy Atkins' had more marketable fruit when stored in CA with 6% O_2 + 5 or 10% CO_2 while 'Kent' and 'Keitt' did better when stored in CA with 4% O_2 + 5 or 10% CO_2. The mangos in Breatheway™ MAP developed atmospheres

at 7 °C of about 3 to 5% O_2 + 8 to 12% CO_2; the atmospheres became quite extreme during the shelf life period at 20 °C, with the O_2 reaching <2% and the CO_2 reaching >30%; however, the fruit did not show any indication of injury from those extreme atmospheres within the short time frame of the shelf-life testing. Including an It's Fresh™ ethylene filter in the MAP bags reduced ethylene concentrations by about 75% to almost 100%, with the A6 filter being more effective than the A5. The CA and MAP treatments reduced lenticel discoloration, CI and decay, while retaining firmer fruit with less color development (i.e., less ripe). Scrubbing ethylene in Breatheway™ MAP slightly further reduced softening and color changes (ripening).

Overall, our results lead us to recommend atmosphere concentrations of 4 to 6% O_2 plus 5 to 10% CO_2 for long distance (4 week) shipping of tree-ripe mangos, with or without ethylene scrubbing.

Shipping tests. The results (Fig. 3) showed that tree-ripe mangos (stage 3.5–4) can be transported or stored at low temperature [9 °C (48 °F)] for 4 weeks without showing CI symptoms and deterioration of fruit internal quality. The results suggest that Breatheway™ MAP protects fruit from stress as expressed by lower lenticel discoloration scoring and may also protect from CI. Fruit in Breatheway™ MAP treatments, especially with It's Fresh™, retained their firmness compared to the non-MAP control fruit by slowing ripening related fruit softening. Results from colorimetric measurements of flesh color showed that these areas were more green and less yellow in Breatheway™ MAP treatments compared to the non-MAP control. The combination of Breatheway™ MAP and It's Fresh™ delayed the ripening processes that consume acids and sugars compared to the other 2 treatments. Fruit sensory quality panelists on shelf life day 4 rated the Brazilian 'Tommy Atkins' and 'Kent' mangos from the T2 (Breatheway™ + No It's Fresh™ filter) and T3 (Breatheway™ + It's Fresh™ filter) treatments as sweeter, less acidic, and with an overall higher eating quality; on shelf life day 10, the T2

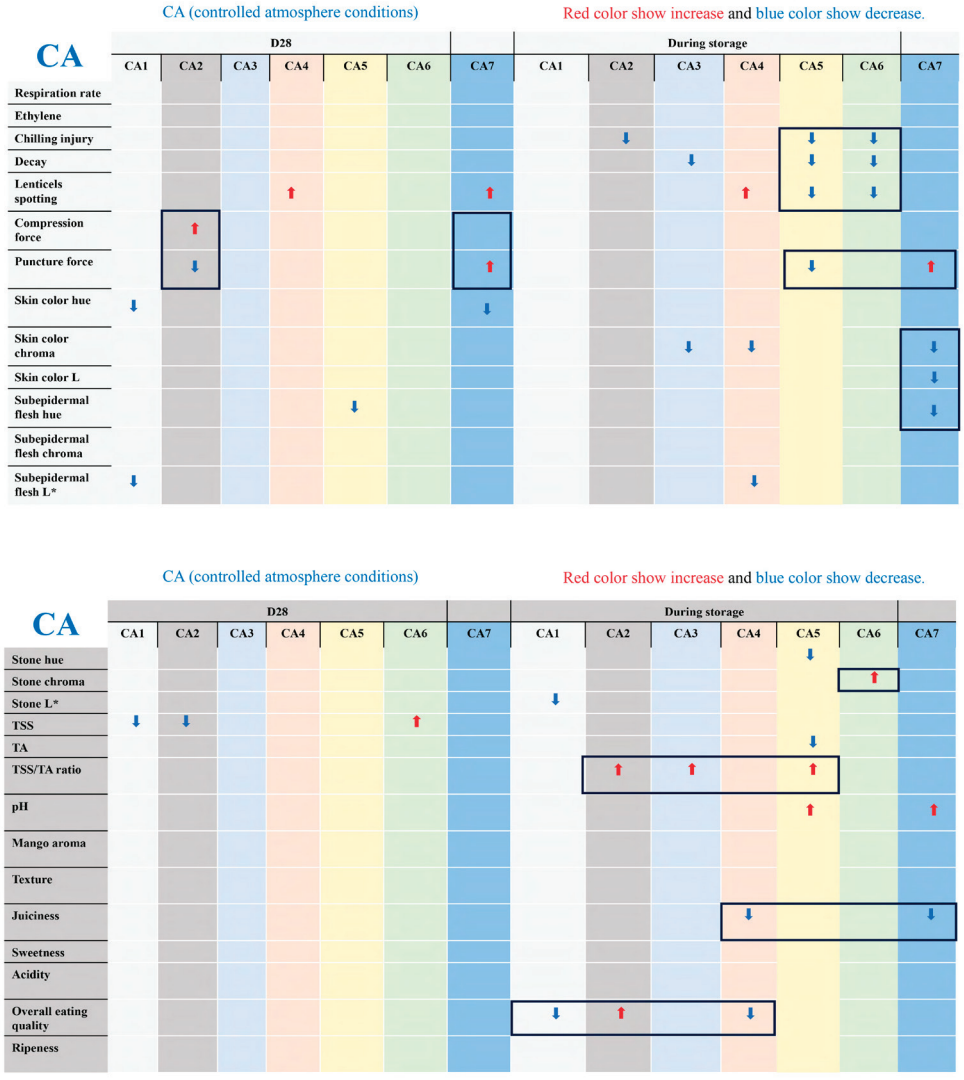

Fig. 1. Summary of laboratory controlled atmosphere (CA) test results showing the effects of treatments relative to the air control for tree-ripe 'Tommy Atkins', 'Kent', and 'Keitt' mangos. The treatments were: CA1 = Air control; CA2 = 4 kPa O_2 + 5 kPa CO_2; CA3 = 4 kPa O_2 + 10 kPa CO_2; CA4 = 4 kPa O_2 + 15 kPa CO_2; CA5 = 6 kPa O_2 + 5 kPa CO_2; CA6 = 6 kPa O_2 + 10 kPa CO_2; and CA7 = 6 kPa O_2 + 15 kPa CO_2. Red, upward-pointing arrows indicate increases and blue, downward-pointing arrows indicate decreases of a given factor relative to the air control treatment.

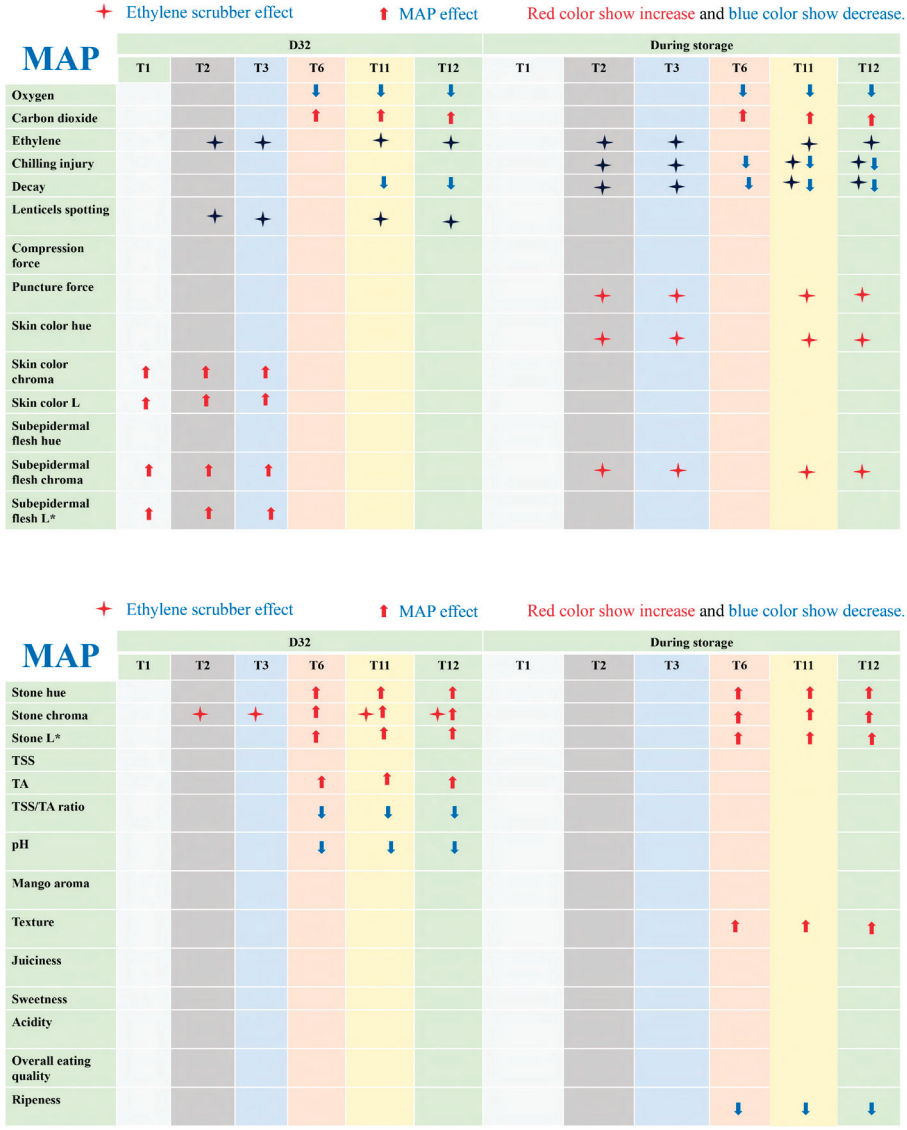

Fig. 2. Summary of laboratory modified atmosphere packaging (MAP) and ethylene scrubbing test results showing the effects of treatments relative to the air control for tree-ripe 'Tommy Atkins', 'Kent' and 'Keitt' mangos. The treatments were: T1 = Air control + No It's Fresh™; T2 = Air control + A5 It's Fresh™; T3 = Air control + A6 It's Fresh™; T6 = Breatheway™ MAP + No It's Fresh™ ; T11 = Breatheway™ MAP + A5 It's Fresh™; T12 = Breatheway™ MAP + A6 It's Fresh™. Red stars or upward-pointing arrows indicate increases and blue stars or downward-pointing arrows indicate decreases of a given factor relative to the air control treatment with stars indicating a response to ethylene scrubbing and arrows a response to MAP.

(BW, Breatheway™ + No It's Fresh™ filter) and T3 (BW/IF, Breatheway™ + It's Fresh™ filter) Brazilian fruit were rated as sweet and juicy, but there were no fruit from the control treatment available at that time for comparison.

Overall, our results lead us to recommend Breatheway™ MAP with It's Fresh™ filters for long distance (4-week) shipping if the goal is to export/import tree-ripe mangos.

Summary and Recommendations

Select fruit at about Stage 3–4 flesh color and/or >15% DM content. Use a Breatheway™ MAP system that establishes and maintains 4 to 6% O_2 + 5 to 10% CO_2. It's Fresh™ filter (ethylene scrubbing) adds additional protection over Breatheway™ MAP alone for delaying ripening, particularly fruit softening. The mangos will maintain about a week of shelf life after transfer to a ripening-conducive temperature. We did not make a comparison between low maturity versus high maturity fruit; however, we know from experience that there is a large quality difference between mangos harvested at Stage 1–2 versus 3–4 after hot water treatment and long-term cold storage.

Future Research Needs

- Pallet scale Breatheway™ MAP would be more economical than the carton-scale Breatheway™ MAP system used here and is worth testing.
- The benefits of It's Fresh™ filters that effectively remove ethylene should be evaluated as a standalone treatment.
- Hazel Technology 1-MCP release sachets placed within a Breatheway™ MAP is also worth testing.

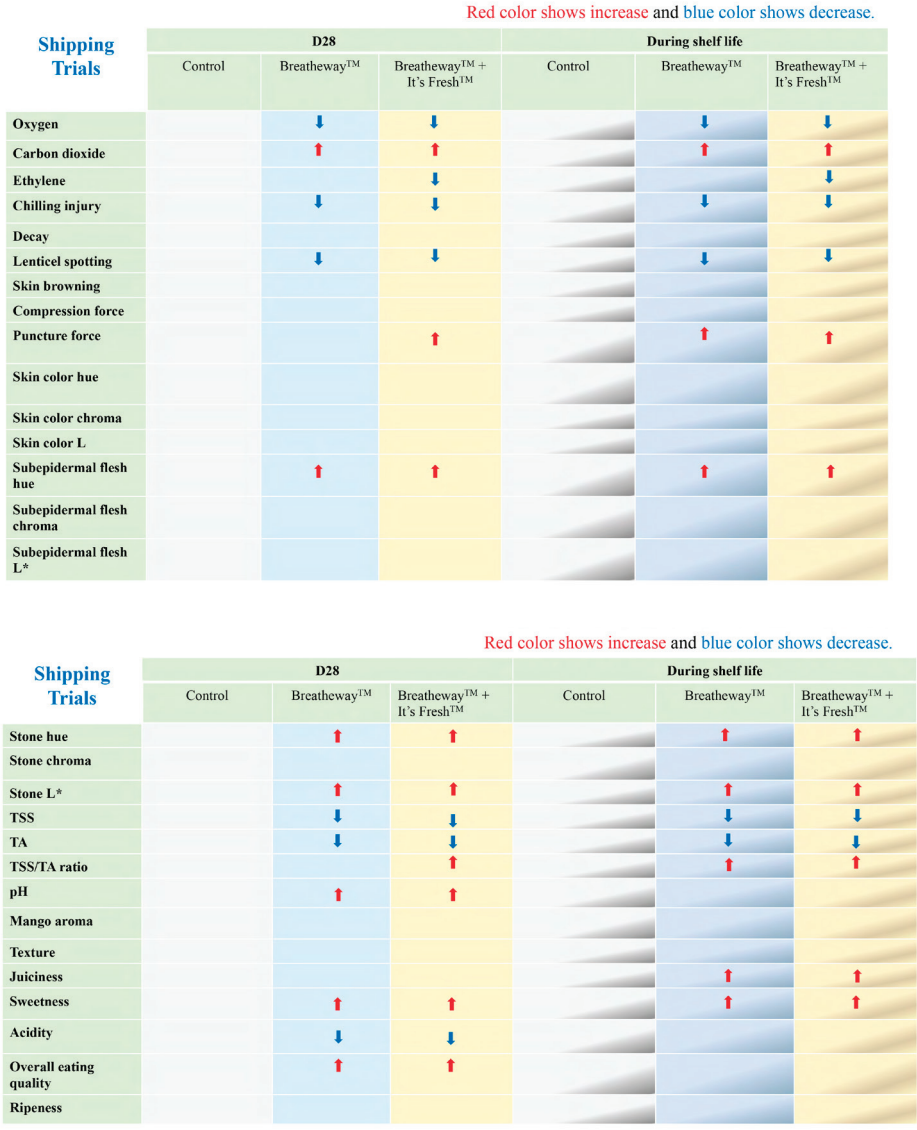

Fig. 3. Summary of shipping test results for tree-ripe 'Tommy Atkins' and 'Kent' mangos shipped by ocean from Brazil to New Jersey then by refrigerated truck to Florida, and tree-ripe 'Kent' mangos shipped by ocean from Peru to Oxnard, California then by air-conditioned car to Fresno, CA. The fruit were at 9 °C for 28 days, then at 20 °C for 4 days; for the Brazil–New Jersey tests, an additional 10-day shelf life evaluation was conducted [modified atmosphere packaging (MAP) and MAP plus ethylene filter treatments only]. The treatments were: 1) Control (unpackaged); 2) Breatheway™ MAP; and 3) Breatheway™ MAP plus It's Fresh™ filter. Red upward-pointing arrows indicate increases and blue downward-pointing arrows indicate decreases of a given factor relative to the air control treatment.

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