

EFFECTS OF PRESTORAGE DRY AND HUMID HOT AIR TREATMENTS ON THE QUALITY, TRIGLYCERIDES AND TOCOPHEROL CONTENTS IN 'HASS' AVOCADO FRUIT

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ABSTRACT

'Hass' avocado fruit were heated with dry (50% RH) or moist (95% RH) forced air at 38C for 6 h and then stored at 5C and 85% RH for up to 8 weeks. Fruit were evaluated weekly for quality and for the content of three triglycerides and three tocopherols. Heated fruit had higher weight loss. The nonheated fruit and those heated with dry air displayed the best external quality. Fruit heated with dry air exhibited the best internal quality and the lowest chilling injury incidence. The respiration rate was more intense in fruit heated with moist air. Fruit firmness immediately after harvest was 51 N, but decreased to less than 20 N at the end of the storage period in the three treatments. The analysis of triglycerides and tocopherols showed that the 1,2-Dilinoleil-3-Oleil-Glycerol and α -tocopherol were the most abundant compounds. Therefore, postharvest treatment with dry forced hot air before storage or transport reduces the incidence of chilling injury, and decreases quality deterioration in 'Hass' avocado fruit.

INTRODUCTION

Avocado (*Persea Americana* Mill.) fruit in Mexico has a great importance from the nutritional and economic point of view. Avocado fruit is characterized by unsaturated fatty acids (Gaydou *et al.* 1987), fat and water soluble vitamins (Slater *et al.* 1975). Avocado fruit contains vitamin E, which is represented by α -, β -, γ and δ -tocopherols as well as by the α -, β -, γ and δ -tocotrienol (Lozano *et al.* 1993). These structurally related antioxidant compounds have been isolated from vegetable oils and other plant materials, and whose deficiencies in humans have been associated with the risk of suffering from degenerative diseases (Bramley *et al.* 2000).

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The avocado is economically important in Mexico due to its high production, consumption, national and international commercialization. Mexico is the biggest avocado producer in the world, and its production is concentrated in regions that are distant from the final consumer, which entails an excessive handling of the fruit for its distribution and sale (FAO 2002), a process during which the fruit can ripen easily. The process of ripening of avocado fruit is strongly bound to an increase in its respiratory intensity, which is strongly affected by temperature and ethylene concentration (Eaks 1978). Thus, refrigeration is important to extend the postharvest life of the fruit, but temperatures below 7°C may cause chilling injury (CI) (Zauberman *et al.* 1977). CI is a physiological disorder distinguished by the appearance of gray, black or dark regions in the pulp, hardening and darkening of the vascular strands and in severe cases the formation of off flavors (Florissen *et al.* 1996), symptoms that reduce the quality of the fruit and can cause significant losses. Therefore, it is necessary to develop complementary techniques to refrigeration, that can slow-down the process of ripening without negatively affecting the quality of the fruit.

Heat treatments (HTs) with water or air have been proposed to control insects, diseases, alleviate CI, and control ripening in some fruits and vegetables (Yahia *et al.* 1997; Yahia and Ortega 2000). However, HTs can alter several components of fruit quality such as firmness, chemical composition, color, respiration rate, and ethylene production (Florissen *et al.* 1996). It has been reported that treatments with dry air reduces CI in avocado (Woolf *et al.* 1995; Florissen *et al.* 1996), affect the composition of some lipid fractions (Román and Yahia 2000), nevertheless the effect on vitamin E has not been studied in heated avocados. The objective of this study was to investigate the effects of post-harvest dry and moist forced hot air treatments on the quality, CI, triglyceride and tocopherol contents in 'Hass' avocado fruit.

MATERIALS AND METHODS

'Hass' avocado fruit were harvested from a single orchard in Uruapan, Michoacán, México. In the laboratory, fruit were selected for uniformity and freedom from defects, weighed and grouped into three lots of 81 fruits each. In addition, 75 fruits were used for initial evaluation. Fruits at harvest had an average weight of 247.8 (\pm 33.1) g for the control, 252.9 (\pm 34.1) g for the lot heated with dry air and 251.4 (\pm 31.8) g for the lot heated with moist air. The average dry matter content of harvested fruit was 27.4%.

Two lots of 81 fruits each were treated with forced air at 38°C for 6 h, in one the relative humidity (RH) of the air was 50% (dry), and in the other it was 95% (moist). The third lot of 81 fruit was not heated (control lot). After heat

treatment, fruits were cooled with water at ambient temperature for 30 min, and then stored at 5C and 85% RH for up to 8 weeks.

Heat treatments were conducted inside a purposely built gas-tight, temperature-controlled, and forced-air chamber (Yahia *et al.* 1997; Yahia and Ortega 2000). The chamber (156 cm high, 70 cm wide, and 132 cm deep) is constructed from stainless steel. Chamber temperature is elevated above room temperature by means of an electric strip heater energized using a time proportioned control technique, and temperature was maintained within ± 0.1 C in the range of 20 to 60C by automatically energizing four 1,000W finned, 230V electric heater elements as required. Humidity was provided through four atomizing nozzles, each has 2 ports; one for compressed gas and one for water. Air entrance to the chamber had a velocity of 4 m.s⁻¹ and air velocity inside the chamber averaged 2.5 m.s⁻¹. Length of treatment was measured from the time of sealing the chamber and turning-on of the controls.

During each week, nine fruits from each of the three treatments were evaluated for external color and external CI and quality. Of these nine fruits, three were evaluated for texture, and internal color and CI, another three fruits were used for the measurement of respiration rate, whereas the remaining three fruits were used for oil extraction. Respiration rate was measured at 20C using a respiratory flow board equipped with 3L glass jars for 5 days. Fruits were placed in the glass jars where CO₂-free air was continuously flowing at the rate of 120 mL/min. CO₂ production in the entrance and the exit of the jars was measured using a Nitec Model GA-20 portable O₂/CO₂ analyzer (Nitec Inc., Cincinnati, OH). The carbon dioxide sensor is a nondispersing infrared sensor with a range of 0-100 kPa and accuracy of ± 0.2 kPa of full scale. The rate of respiration was calculated according the following equation:

$$\text{mgCO}_2/\text{kg.h} = \frac{[(\text{CO}_{2e} - \text{CO}_{2i}) * (\text{F}) * (60)] * 1.84}{100\text{W}} \quad \text{where:}$$

F = Air flow (mL/h)

W = Fruit weight (kg)

CO_{2e} = CO₂ concentration (%) at the exit of the jar

CO_{2i} = CO₂ concentration (%) in the ambient air

The evaluation of the external and internal quality was carried out using a subjective scale of 0 to 3, where 0 indicates 0% of damaged surface and 3 indicates more than 90% of the surface is damaged. For the evaluation of internal quality, the fruits were cut longitudinally. For the evaluation of the external CI, the darkening not caused by maturation was considered, whereas

for the internal evaluation the darkening of the pulp and the vascular strands were considered. A subjective scale of 0 to 5 reported by Roman and Yahia (2000) was used, where 0 indicates 0% of damaged surface and 5 indicates a noticeable CI (presence of CI in more than 70% of the fruit surface). For the evaluation of the internal CI, fruits were cut longitudinally and the surface of each half was evaluated.

Weight loss was determined by weight difference, considering the weight of the fruit immediately after harvest and its weight during each evaluation.

External color was determined on three regions of the fruit (stem end, central region and apex) in longitudinal order. The determination of the internal color was done on the same region in which the external color was measured but after removing the exocarp. Color was measured with a colorimeter (Minolta model CM-2002, Minolta Co., Osaka, Japan), calibrated and zeroed with a white standard during each time a measurement was made.

Firmness measurements were done on the same three regions where the internal color was measured. The fruit was punctured at a depth of 8 mm with a 4 mm diameter stainless steel striker pin using a TA-HD Texture Analyzer (Stable Micro System, Haslemere, England). The maximum amount of force (in Newton) that was needed to puncture the avocado fruit was recorded.

Oil extraction was done according to the procedure of Bligh and Dyer (1959), but using instead a mixture of hexane-isopropyl alcohol (HIP) (2:1 v/v). The triglycerides (TAGs) analysis was carried out according to the procedure of Martín-Carratalá *et al.* (1999), by dissolving 10 μL of oil in 450 μL of tetrahydrofuran. Of this mixture, 10 μL were injected in a HPLC Hewlett Packard Series 1100, connected to a photodiodes array detector at 200 nm. The mobile phase was composed of 65 parts of acetone and 35 parts of acetonitrile. A Symmetry C18, 4.6 \times 150 mm column with a size particle of 3.5 μm was used at a temperature of 30°C and at a flow rate of 1 mL/min. The identification and quantification of trilinolein (LLL), 1,2-Dilinoleil-3-Oleil-Glycerol (LLO) and triolein (OOO) was calculated by comparing the retention time and the area under the curve of these triglycerides in the samples with those of triglycerides standard mixture (Supelco) that contained them in 2, 4 and 60%, respectively.

For tocopherol analysis, the sample was prepared by dissolving 0.5 g of oil of avocado in 3 mL of hexane. Of this mixture, 20 μL were injected in a HPLC Hewlett Packard Series 1100. The column used and the detector were the same as those used for the triglycerides, and the analysis was done at 294 nm. The mobile phase was 100% methanol, at a flow rate of 0.75 mL/min through the column. The identification and quantification of α -, γ - and δ -tocopherol was done by comparing the retention time and the area under the curve of the peaks of these vitamers in the samples with the peaks obtained with a standard of α -, γ - and δ -tocopherol (Sigma).

The results were analyzed by ANOVA (analysis of variance) followed by a Dunnett's multiple comparison test.

RESULTS

The three stages of the respiratory pattern (preclimacteric, climacteric maximum and postclimacteric) of the avocado fruit were noticeable throughout the storage period, however, the preclimacteric and the climacteric maximum disappeared during the last stages of fruit ripening. The respiration rate increased during the initial sampling carried out immediately after applying the HTs, but decreased after storage at 5C. Fruit heated with air at 38C and 95% HR had the most intense respiratory rate throughout the storage period. Fruit heated with dry air had an intermediate respiratory behavior between the control and the lot heated with moist air, but there were no significant differences between this lot and the control. In the last two weeks the respiration rate in the three lots increased, probably due to the presence of fungi.

After the first 14 days of storage, external quality started to decrease in the heat-treated fruits (Fig. 1A). Fruit heated with moist air had greater deterioration than the other fruits. The external quality of nonheated fruit started to deteriorate during the fifth week of storage. However, in the three lots the damage was never more than 20% of the surface of the fruit. External darkening (data not shown) was the only factor that caused the loss of external quality.

Internal quality (Fig. 1B) was very good throughout the storage period in the three treatments, however, nonheated fruits and those heated with moist air had lower internal quality than fruits heated with dry air.

Darkening of vascular strands (Fig. 1C) was the principal symptom of CI observed. Nonheated fruits had a noticeable vascular darkening compared with the heated fruits, although the disorder was very slight in fruit heated with dry air.

Internal darkening (data not shown) was very low and was only detected in the control until the seventh week of storage.

The tendencies of the parameters of external color (a^* , b^* and L^*) were very similar in the three treatment groups (data not shown). The values of a^* and b^* fell to the clear green region of a chromaticity diagram, approaching the center of this diagram where gray tonalities of little brightness predominate. The statistical analysis indicated that the control and the lot heated with dry air were different with respect to a^* and b^* values.

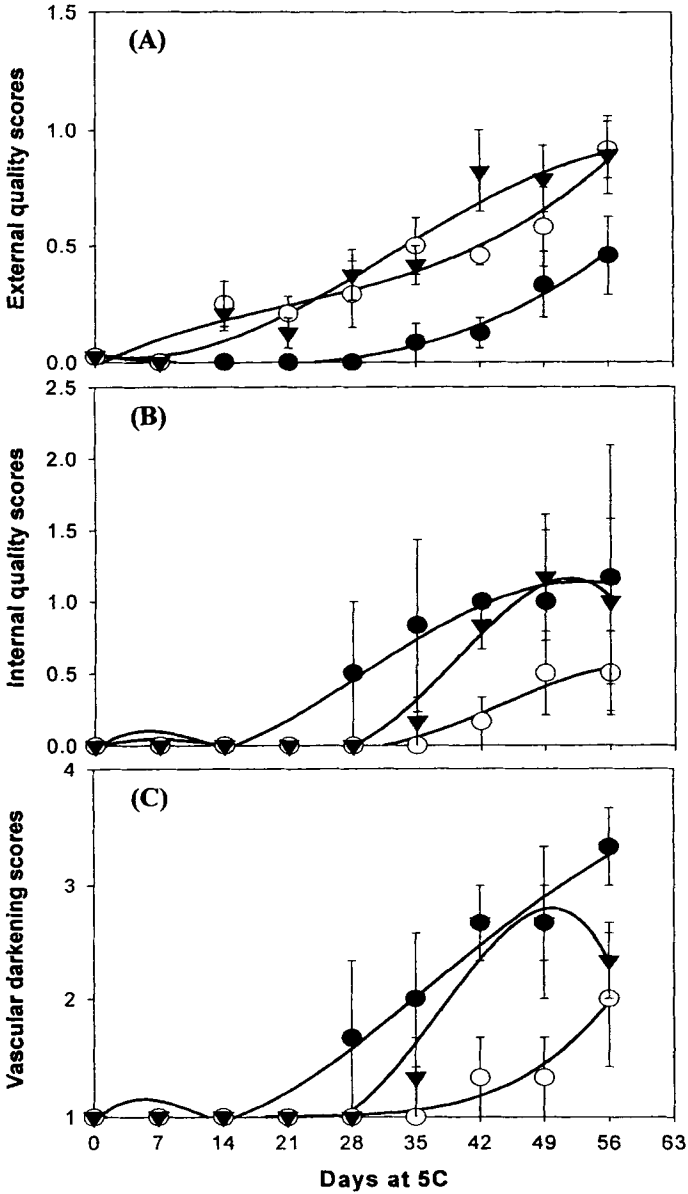


FIG. 1. EVALUATION OF EXTERNAL QUALITY (A), INTERNAL QUALITY (B) AND VASCULAR STRANDS DARKENING (C) IN 'HASS' AVOCADO FRUIT, NONHEATED (-●-), HEATED WITH DRY (-○-), OR HUMID (-▼-) AIR, AND STORED AT 5C AND 85% RH FOR UP TO EIGHT WEEKS

Vertical bars represent standard error of the mean.

The initial values of internal color (data not shown) had very few variations throughout the storage period, showing important changes in the control only until the eighth week of storage. Nonheated fruits and those heated with dry air were statistically different with respect to a^* , b^* and L^* values.

Fruit firmness decreased in heated and in nonheated fruits (Fig. 2). Fruit heated with moist air had greater firmness, however, differences were not very clear.

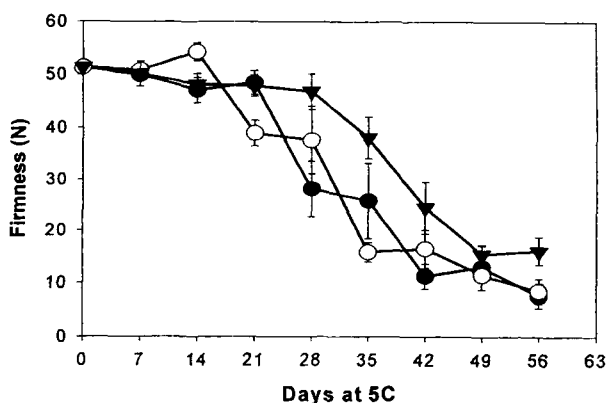


FIG. 2. FIRMNESS OF 'HASS' AVOCADO FRUIT, NONHEATED (●), HEATED WITH DRY (○), OR HUMID (▼) AIR, AND STORED AT 5C AND 85% RH FOR UP TO EIGHT WEEKS

Vertical bars represent standard error of the mean.

Weight loss increased in heated and in nonheated fruits (Fig. 3), and heated fruit had higher weight loss than those nonheated.

1,2-Dilinoleil-3-Oleil-Glycerol was the most abundant of the three triglycerides analyzed. The maximum amount of triglycerides analyzed was reached after 7 days of storage at 5C. However, there was no clear tendency, except for a slight decrease after one week of storage, nor were there clear differences between the three treatments. Fruits treated with moist air and those nonheated had different triolein content within the third and fifth weeks of storage at 5C.

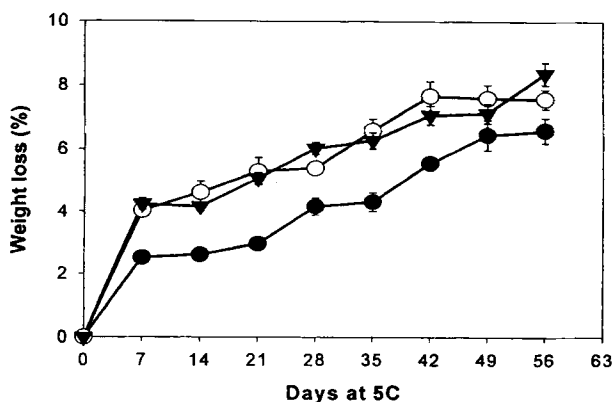


FIG. 3. WEIGHT LOSS (%) IN 'HASS' AVOCADO FRUIT, NONHEATED (-●-), HEATED WITH DRY (-○-), OR HUMID (-▼-) AIR, AND STORED AT 5C AND 85% RH FOR UP TO EIGHT WEEKS
Vertical bars represent standard error of the mean.

The total content of α -, γ - and δ -tocopherol is shown in (Fig. 4). The α -tocopherol was the most abundant vitamer. All the tocopherols analyzed decreased during storage. The initial concentration of α -, γ - and δ -tocopherol was 0.023, 0.013 and 0.016 mg/g oil, whereas the final content was of 0.013, 0.005 and 0.002 mg/g oil in the nonheated fruits, 0.002, 0.007 and 0.0004 mg/g oil in fruits heated with dry air, and 0.003, 0.005 and 0.0005 in fruits heated with humid air. Statistical analysis indicated that the nonheated fruits and those heated with dry air had different content of α -tocopherol.

DISCUSSION

The premature deterioration of the external quality and the behavior of the parameters of external color (a^* and b^* values) in heated fruits during the first three weeks, support the idea that the tissue was injured by heat. It is possible that the high relative humidity in the air used in one of the treatments increased the efficiency of the heat transfer process in the fruit, causing possible heat injury, mainly external. The highest weight loss in avocado fruit was due to heating with moist air, while heating with dry air caused less weight loss and might have conferred some protection to the fruit against dehydration. The increase in respiration in fruits heated with moist air might have been the result of stress by water loss (Adato and Gazit 1974). However, in spite of having

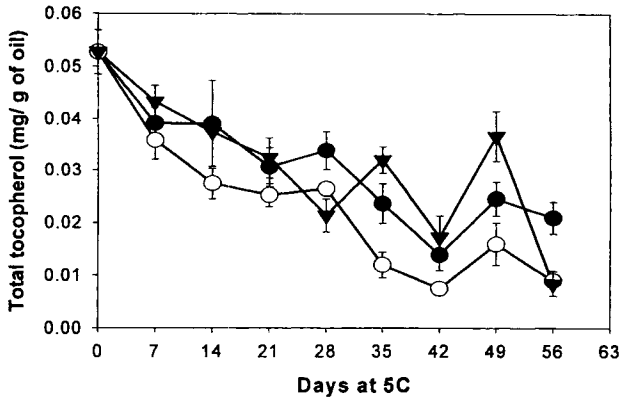


FIG. 4. THE TOTAL CONTENT OF α -, γ - AND δ -TOCOPHEROL IN 'HASS' AVOCADO FRUIT, NONHEATED (●), HEATED WITH DRY (○), OR HUMID (▲) AIR, AND STORED AT 5C AND 85% RH FOR UP TO EIGHT WEEKS
Vertical bars represent standard error of the mean.

exhibited higher respiration rate, fruit heated with humid air had greater firmness, probably due to the inactivation of softening enzymes, as it has been reported in other fruits (Obenland and Carroll 2000). Dry heat caused a reduction of CI, probably due to the induction of heat shock proteins (Florissen *et al.* 1996; Woolf *et al.* 1995). HTs can promote fruit ripening by increasing the respiration rate (Paull and Chen 2000). The oxidative processes triggered by ripening require antioxidant compounds such as α -tocopherol and that involved in glutation (Hariyadi and Parkin 1991), which might explain the reduction in the amount of tocopherols evaluated. The lowest levels of tocopherol was present in fruits heated with dry air, probably because these fruits had different types of oxidative stress such as heat, CI and ripening. During the development of avocado fruit under normal conditions, it has been observed that the main fatty acids were oleic and linoleic, whereas other acids such stearic are commonly present in trace amounts (Gaydou *et al.* 1987). Therefore, one should expect important amounts of triglycerides with high oleic and linoleic acid content, but it has been observed that oleic acid content decreases while stearic acid content increases under cold storage conditions (Cajuste 1995). This could explain the reason behind the decrease of the three triglycerides analyzed. HTs have been attributed to cause an increase in unsaturated fatty acids such as linoleic and linolenic acids, and in the activity of the desaturase enzyme (Harris and James 1969). Experiments done with heat-treated soybean (Cheesebrough

1990) and in mutant arabidopsis plants (Ohlogge and Browse 1995) indicated that high and low temperatures activate desaturases as a mechanism for protection. Therefore, the increase in the content of the evaluated triglycerides during the first week of storage as nonheated avocado fruit and in fruit heated with humid air could be due to an increase in the activity of desaturase enzyme by low temperature.

CONCLUSIONS

Postharvest treatment with dry air at 38C and 50% RH for 6 h resulted in a better internal fruit quality, reduced the incidence of CI, resulted in a lower reduction in the content of three triglycerides, but caused a slight heat injury, which was masked by the ripening process.

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