Postharvest disinfestation of mango (Mangifera indica cv. Manila) with controlled atmospheres

Dinora M. León¹, Dora A. Ortega², Héctor Cabrera², Javier De La Cruz¹, Kirk L. Parkin³ and Hugo S. García¹

¹ UNIDA, Instituto Tecnológico de Veracruz, Apdo. Postal 1420, Veracruz, Ver. 91860, México. Phone/Fax 01(2) 934-5701. ² Campo Experimental Cotaxtla, CIRGOC-INIFAP, SAGAR, México ³Dept. of Food Science, University of Wisconsin-Madison, Babcock Hall, Madison, WI 53706, USA. *Responsible author to whom all correspondence should be addressed. Email: hsgarcia@itver.edu.mx

Abstract

Manila mangoes were infested in the tree by allowing fertile Anastrepha obliqua female flies for oviposition on fruits contained inside cages. Infested mangoes were exposed to nine different controlled atmospheres (CA) containing combinations of 1, 3 or 5% O₂ and 30, 50 or 70% CO₂. Surviving larvae were enumerated after subjecting the mangoes to the CA for periods of 1 to 5 days. Selected compositional and physical parameters (weight loss, pH, titratable acidity, colour, soluble solids, reducing sugars and texture) were analyzed during post-treatment ripening. Fully ripened fruits were also subject to sensory evaluation using a non-structured hedonic scale and a trained panel. CA containing 1% O₂ and either 30% or 50% CO₂, effectively killed all the larvae present in treated fruits. These treatments did not alter the composition or sensory characteristics of fully ripened mangoes. However, losses of 20 to 25% of fruits on the basis of sensory acceptability were attributed to the development of “spongy” tissue. CA's containing 70% CO₂ were also effective in disinfestation, but also affected compositional and sensory qualities of the fruits, and induced the “spongy” texture defect in 65% of the fruits.

Key words: Controlled atmosphere, disinfestation, mango, Anastrepha obliqua, larvae, post-treatment ripening, sensory evaluation, disinfestation, texture

Introduction

Mexican mangoes of the Florida varieties (Kent, Keitt, Haden and Tommy Atkins) are regularly exported to the U.S. market at an annual rate of over 100,000 tons. However the Manila variety, the one with the highest production in Mexico is only starting to get attention from the producers as a variety with potential for export. The main problem to solve for any exported variety is disinfestation of the mangoes from fruit fly larvae (Anastrepha sp.). Some of the alternatives for insect control are insecticide application, refrigerated storage (Hatton, 1991; Mitchell and Kader, 1992), irradiation (Windeguth, 1986; Mitchell and Kader, 1992; Toledo et al., 1992), and hot humid air (Balock and Starr, 1945; Heard et al., 1992). These treatments have not been entirely innocuous or injury-proof for the fruits. Some of their disadvantages are: possible carcinogenic residues (Coates et al., 1993), chilling injury, possible sterile larvae survival and spongy tissue. Since 1988, hydrothermal treatment is the only USDA-approved process for control of anthracnose and fruit fly disinfestation of mangoes destined for the U.S. market (Celedonio-Hurtado, 1992; Mitchell and Kader, 1992). Application of the procedure has resulted in several limitations, such as advanced ripening, weight reduction (Jagtiani et al., 1988), necrosis of defined areas in mangoes of small size (McIntyre et al., 1993), pitting in peduncular area (Rojas, 1993), scalding and off-flavours development as well as practical problems at large-scale packing plants (Celedonio-Hurtado, 1992).

The use of controlled atmospheres (CA) is a viable option for larval control at different maturity stages by exposing fruit to atmospheres containing low O₂ and high CO₂ concentrations for short times (Brandl et al., 1983; Soderstrom and Brandl, 1984, 1985; Lidster et al., 1981, 1984; Jay, 1984; Benschoter, 1987; Soderstrom et al., 1986, 1990; Yahia, 1998). Recent reports have indicated that exposure of Keitt mangoes to short periods of insecticidal CA did not affect normal ripening or sensory attributes of the fruits (Yahia and Vazquez-Moreno, 1992; Yahia, 1993; Yahia and Tiznado-Hernandez, 1993). These treatments were not tested on mangoes of the Manila variety. In this study we subjected Manila mangoes to CA with high CO₂ and low O₂ to evaluate their disinfestant ability. CA could be an alternative to hydrothermal treatment.

Materials and methods

Larval infestation. Manila mangoes were infested in the production fields of Cotaxtla Agricultural Experiment Station (INIFAP) with fertile Anastrepha obliqua flies grown on laboratory diets. An average of 10 fruits in the tree were placed inside plastic mesh cages and 30 fertile female and 10 male flies were introduced. Oviposition was allowed to proceed and fruits were harvested after 8 days, to insure that larvae were in the third stage of growth inside the fruit.

Disinfestant treatment. Preclimacteric infested mangoes were washed with a 0.1 g/l Benlate® (E.I. DuPONT de Nemours & Co. Inc.) solution. Fruits were sorted randomly and divided into
lots. One lot was used as control and set to ripen at 25 °C with no CA treatment. The other lots were differentially treated in a complete 3 x 3 design to CA with compositions of 1, 3 and 5% O₂ and 30, 50 and 70% CO₂. Certified gas mixtures were purchased from AGA gas. Twenty fruits were withdrawn at each sampling period and allowed to ripen at 25 °C for up to 12 days. Mangoes were exposed to CA for periods of 1 to 5 days at 25 °C in 0.45 m³ fiberglass chambers. Gas flow was 235 ml/min and humidified passing it through water before entering the chamber. At the end of the ripening period, mangoes were dissected for the purpose of counting larvae (dead or alive) and fruit damage was assessed visually. Efficacy of disinfestation was expressed as percentage of dead larvae found.

**Analyses:** Three mangoes were used for analysis at each treatment interval for each CA treatment. Physiological weight loss was measured by direct weighing. Textural hardness was measured with a fruit penetrometer fitted with a 6 mm conical probe and a gauge of 0-12 kg force. Colour reflectance changes in excised slices of the mesocarp were followed with a Minolta CR-200 colorimeter using the CIE L*a*b* scale. Mesocarp homogenates were employed to measure pH and titratable acidity by direct titration with 0.1 N NaOH. Soluble solids were monitored with a desktop Abbe refractometer, and reducing sugars with the 3,5 dinitrosalicylic acid procedure (Miller, 1959).

**Sensory Evaluation:** An hedonic test with a non-structured scale and 10 trained panelists was used to grade attributes of aroma, off-flavor, visual colour, sweetness, tartness and overall acceptance.

**Statistical Analysis:** Linear regression analysis was used to evaluate the relationship between time of exposure and the treatments. Physico-chemical as well as sensory evaluation data were analyzed by ANOVA and Tukey’s paired comparison test (α=0.05), employing the statistical software package MINITAB v. 10.1.

**Results and discussion**

The level of initial infestation obtained was 85 insects per 20 mangoes. Complete larval mortality was achieved within three days of treatment for CA of 1% O₂ and either 30 or 70% CO₂ (see Table 1). The CA containing 1% O₂ and 50% CO₂ required 5 days of treatment before 100% mortality was observed. According to the USDA, any treatment that could be considered for approval must assure a mortality of 99.99% of fly larvae and eggs (Landolt et al., 1984). Based on that premise, the treatments used in this study fulfill that criterion.

The interrelationships between CA parameters and larval mortality were modeled by linear regression analysis, which generated the following equation:

\[ \log_{10} Y = 0.913 - 0.426X_1 + 0.594X_2 - 0.861X_3 - 0.168X_1X_2 \\
+ 0.237X_1X_3 + 0.583X_2X_3 - 0.802X_1X_2 - 0.211X_2X_3 - 0.117X_1X_3X_2 \]

where \( X_1 \) is the % of CO₂, \( X_2 \) is the %O₂ in the CA, and \( X_3 \) is the number of days of exposure. The analysis indicated that the statistically significant factors which affected larval mortality were the main effects of % O₂, % CO₂, days of exposure, and the interaction between % O₂ and days of exposure. The interactions between %O₂ and %CO₂ or days of exposure, as well as the multiple interaction between the same variables were not significant. It was clear that greater larvae death rates were obtained at lower O₂ concentrations (Fig. 1). The statistical model was not adequate to explain the trends of CA containing 70% CO₂ (Fig. 2), since some treatments achieved 100% larval mortality within the first day of exposure. This fact reduced the correlation coefficient (\( r^2 \)) to 0.62.

**Table 1. Percentage mortality of Anastrepha obliqua larvae in Manila mangoes subjected to controlled atmospheres at 25 °C**

<table>
<thead>
<tr>
<th>CO₂ (%)</th>
<th>O₂ (%)</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>62</td>
</tr>
<tr>
<td>50</td>
<td>73</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>57</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>75</td>
</tr>
<tr>
<td>70</td>
<td>72</td>
<td>95</td>
</tr>
</tbody>
</table>

**Table 2. Spoilage observed in treated mangoes, expressed as percentage of fruits with injury symptoms, mainly internal breakdown**

<table>
<thead>
<tr>
<th>Days</th>
<th>CO₂ (%)</th>
<th>O₂ (%)</th>
<th>Spoilage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>30</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>1</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>1</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>1</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>1</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>1</td>
<td>75</td>
</tr>
</tbody>
</table>

Colour of the mesocarp in treated fruits did not show any change during the first three days and exhibited lesser color development compared to control mangoes (Fig. 3). It has been proposed that CA may inhibit chlorophyll degradation and carotenoid synthesis (Weichmann, 1986). Reducing sugars in mango juice was not affected by the treatments (Fig. 4). The rather constant levels of reducing sugars suggests that ripening was delayed because of the exposure to the CA, instead of increasing, which is consistent with the report of Krishnamurthy and Subramanyam (1973).

Treated mangoes lost less weight than control fruit probably due to the humidification of the CA during treatment. (Fig. 5). Relative humidity inside the chamber was ca. 90%, compared to 70-72% outside, this is likely responsible for the CA treated mangoes suffering less shrinkage, lack of juiciness and fewer overall appearance defects as compared to control fruit as previously found by Kader (1986).

The pattern of soluble solids changes in mangoes treated with 1% O₂ and either 30 or 50% CO₂ were not different from the control fruits during the five days of CA exposure (Fig. 6). However, CA containing 70% CO₂ produced statistical
Fig. 1. Survival of *Anastrepha obliqua* larvae after been exposed inside the fruits to gas mixtures containing 30% CO$_2$ and 1% (circles), 3% (squares) or 5% (diamonds) O$_2$.

Fig. 2. Survival of *Anastrepha obliqua* larvae after been exposed inside the fruits to gas mixtures containing 70% CO$_2$ and 1% (circles), 3% (squares) or 5% (diamonds) O$_2$.

Fig. 3. Hue angle of mango mesocarp exposed to gas mixtures containing 70% CO$_2$ and 1% (circles), 3% (squares) or 5% (diamonds) O$_2$.

Fig. 4. Reducing sugars in mangoes treated with gas mixtures containing 70% CO$_2$ and 1% (circles), 3% (squares) or 5% (diamonds) O$_2$.

Fig. 5. Physiological weight loss in mangoes exposed to atmospheres containing 30% CO$_2$ and 1% (circles), 3% (squares) or 5% (diamonds) O$_2$.

Fig. 6. Soluble solids (°Brix) of mangoes stored in atmospheres containing 1% O$_2$ and 30% (circles), 50% (squares) or 70% (diamonds) CO$_2$.

Fig. 7. Changes in mesocarp pH of mangoes exposed to atmospheres containing 1% O$_2$ and 30% (circles), 50% (squares) or 70% (diamonds) CO$_2$.

Fig. 8. Fruit hardness measured as resistance to penetration of mangoes exposed to atmospheres containing 1% O$_2$ and 30% (circles), 50% (squares) or 70% (diamonds) CO$_2$. 
differences in soluble solids by the fifth day of treatment. It appears that elevated CO\textsubscript{2} concentrations interfere with the conversion of starch into simple sugars (Kader, 1986; Weichmann, 1986), which constitute the majority of the soluble solids in mango (Krishnamurthy and Subramanyam, 1973).

Mango pulp pH did not display any difference as a consequence of all treatments during the first four days of treatment, but changes occurred by the fifth day and pH of CA treated mangoes remained low, compared to control mangoes (Fig. 7). Kader (1986) reported that pH remains low in fruits stored under CA due to two factors: When metabolism is reduced, loss of acids from the Krebs cycle is reduced and these acids accumulate. Second, enhanced dissolved CO\textsubscript{2} diffuses from the environment into the fruit tissue is utilized for synthesis of organic acids, mainly malic acid.

Hardness of the fruits was measured to be 12 kgf/cm\textsuperscript{2} (Fig 8) upon arrival. The hardness decreased to 2.74 kgf/cm\textsuperscript{2} after 5 days of ripening. There was no statistical difference in textural patterns of softening between treated and control mangoes, which suggests that short-term exposure to CA had no deleterious effects on fruit firmness. There is good correlation between loss of firmness and increased polygalacturonase activity in mangoes (Gomez-Lim, 1993). According to Kader et al. (1989) polygalacturonase activity is depressed when fruits are exposed to low O\textsubscript{2} and high CO\textsubscript{2} levels. However, Lakshminarayana and Subramanyam (1970) reported softer texture in “Alphonso” mangoes that were exposed to atmospheres prepared with initial concentrations of 5, 10 or 15% CO\textsubscript{2} for 21 days. These sets of results can not be strictly compared because of the different CO\textsubscript{2} levels and times of treatment.

Sensory evaluation of fully ripened mangoes demonstrated the absence of non-typical aromas, and off-flavors or texture changes in mangoes treated with CA for three days with 1% O\textsubscript{2} and either 30, 50 or 70% CO\textsubscript{2}. There were no sensory differences in overall acceptance of mangoes subjected to CA with 1% O\textsubscript{2} and either 30 or 50% CO\textsubscript{2}, however, mangoes treated with CA containing 70% CO\textsubscript{2} were judged as neither liked nor disliked, or even unacceptable (Fig. 9).

Table 2 reports the incidence of mangoes that suffered injuries and were judged as unacceptable following exposure to CA. Symptoms of spoilage were necrotic spongy tissue, damage that is also known as internal breakdown. It is known that this symptom can develop during ripening due to nutritional deficiencies during growth in the tree (Krishnamurthy, 1981). It has also been reported (Esguerra et al., 1990; Yahia, 1998) that the same type of spoilage may be attributed to alterations of starch metabolism and a toxic effect by ethanol and acetaldehyde produced during anaerobic respiration, or even by hydrothermal treatments in Carabao mangoes.

From the results reported herein, it may be concluded that exposure for 3-5 days at 25 °C to the CA with 1% O\textsubscript{2} and either 30 or 50% CO\textsubscript{2} exerted an insecticidal effect without affecting the wholesomeness of the mangoes. However, 20-25% of the fruits showed signs of internal breakdown. The CA treatment with 1% O\textsubscript{2} and 70% CO\textsubscript{2} was also an effective disinfestant atmosphere, but affected the chemical composition and sensory attributes of the mangoes. Additionally, this treatment induced breakdown in 65% of fruits.

As Yahia and Paull recommended (1997), our results also suggest the possibility of utilizing CA to kill fruit fly larvae with little effect on fruit quality and should provide mango producers with an alternative treatment for exporting mangoes to foreign markets.

References


