タンゴール‘清見’果実のこはん症発生と品質変化に及ぼす包装内大気組成の影響

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Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat
Effect of MA Conditions on Occurrence of Kohansho and Quality of ‘Kiyomi’ Tangor

TECHAVISES Nutakorn*1 and HIKIDA Yoshio*2

*1 The United Graduate School of Agricultural Sciences, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566
*2 Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566

The effect of MA conditions under high humidity packaging on the occurrence of Kohansho and the quality attributes of stored ‘Kiyomi’ tangor (Citrus unshiu Marc. × C. sinensis Osb.) fruit was investigated. Modified atmosphere containing CO2 concentrations less than or equal to 12% and O2 concentrations more than or equal to 10% had no effect on the occurrence of Kohansho under high humidity conditions. However, the occurrence of Kohansho was accelerated when the CO2 level accumulated to 14%. Under the condition that CO2 levels are not too high and O2 levels are not too low, maintenance of a high relative humidity surrounding the fruit is considered to be the main factor for packaging design to prevent Kohansho. Furthermore, excessive CO2 levels (more than 8%) induced fermentative metabolism, resulting in high ethanol levels. MA conditions had no significant effect on the quality attributes, including firmness, TSS, TA, TSS/TA ratio, and AA contents under the high humidity condition. These qualities were preserved during the storage period, probably resulting from the maintenance of a high relative humidity inside the package. As recommended storage conditions and packaging design guidelines, ‘Kiyomi’ fruit should be stored particularly under a high relative humidity and should not be exposed to high CO2 levels (> 8%) and/or low O2 levels (< 5%) to prevent Kohansho and preserve high quality.

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Key words: modified atmosphere packaging, storage condition, O2 and CO2, Kohansho, ‘Kiyomi’ Tangor

‘Kiyomi’ tangor (Citrus unshiu Marc. × C. sinensis Osb.) is recognized as a high-quality late-season citrus fruit. ‘Kiyomi’ fruit as well as other citrus fruits such as Hassaku and Navel orange have been confronted with the serious problem of Kohansho, a non-chilling physiological rind disorder. Kohansho appears as small pitting and then develops into a brown-sunken area. This rind disorder is usually observed during storage, transportation, and marketing periods. The symptom diminishes only the external quality of the fruit while the internal quality of the fruit remains sound. However, once the disorder occurs, the fruit is typically rejected by the market. Preharvest factors have been found to be related to Kohansho. CHIKAZUMI reported that Kohansho in ‘Kiyomi’ fruit both held at 8°C and 20°C. Low temperature and high relative humidity storage was found to reduce the occurrence of rind injury in ‘Kiyomi’ fruit. The occurrence of Kohansho in ‘Kiyomi’ fruit was suppressed by reducing fruit weight loss during storage.

Modified atmosphere (MA) packaging is a well known means of storing fresh fruits and vegetables, because it preserves the high quality of produce by reducing produce respiration, retarding ethylene production and sensitivity, and decreasing the incidence of postharvest rind disorders. Wrapping fruit with PE bags not only maintains high relative humidity, but also modifies the atmosphere, generally increasing the CO2 level and decreasing the O2 level, inside the bag. In our previous study, the relationship between the occurrence of Kohansho and weight loss in ‘Kiyomi’ fruit during storage at ambient

§ Corresponding author. E-mail: hikida@ehimegw.dpc.ehime-u.ac.jp
atmospheric compositions was investigated. The results showed that the occurrence of Kohansho is suppressed by reducing fruit weight loss. The restriction of weight loss under high humidity conditions is essential for packaging design to prevent Kohansho. However, the effect of MA conditions (O₂ and CO₂ concentrations) on the occurrence of Kohansho is not clear when 'Kiyomi' fruit are packed under high humidity conditions. Therefore, in this study, the effect of MA conditions under high humidity packaging on the Kohansho disorder was investigated. Moreover, the effect of MA conditions on quality attributes of stored 'Kiyomi' fruit was also investigated to confirm the packaging design.

Materials and Methods

1. Plant material

'Kiyomi' tangor fruit (size L: 7.3 to 8.0 cm in diameter) harvested on March 13, 2007 from Misaki, Ehime were stored in LDPE film packages at a farmer storage house for a week and then transported to the laboratory of Agricultural Process Engineering, Ehime University. The fruit with no Kohansho were used for the experiment on the following day (March 20, 2007).

2. Creation of MA conditions

MA packaging in the form of a "perforation-mediated package" was utilized to create various MA conditions by varying the diameters of a perforation (Diameters: 1, 1.5, 3, and 3.5 mm, designated as 1 P 1 M, 1 P1.5 M, 1 P 3 M, and 1 P 3.5 M, respectively) in the lid (2.5 mm thick) of a plastic box (6.01 × 10⁻³ m³). The condition of fifteen 2-mm-diameter perforations was used as the control to create ambient gas compositions. A set of three replicate experimental runs was conducted for each condition. All plastic boxes containing 10 'Kiyomi' fruit were stored at 15°C for 4 weeks, the average temperature and storage period in the farmer storage house. Relative humidity inside the plastic box was planned to be maintained at high levels (due to produce transpiration) throughout the storage period and monitored by a Thermo Recorder (model TR−72 U, T & D, Japan).

3. Evaluation of Kohansho

Kohansho was optically observed at the end of storage by measuring the diameter of affected areas and rated with the following Kohansho index: 0: no occurrence of Kohansho; 1: one spot of less than 5 mm; 2: one spot of 6–20 mm; 3: 2–3 spots of 20 mm; 4: over 4 spots of 20 mm. The results were expressed as a percentage and degree of Kohansho:

\[
\text{Percentage of Kohansho (\%) = } \left( \frac{\text{total number of fruit incurring Kohansho}}{\text{total number of fruit}} \right) \times 100
\]

\[
\text{Degree of Kohansho} = \left( \frac{\Sigma \text{Kohansho index \times number of fruit incurring Kohansho in each index}}{4 \times \text{total number of fruit}} \right) \times 100
\]

4. Determination of quality attributes

Fruit were weighed before and after storage to assess the percentage weight loss. Fruit firmness was determined with a fruit hardness tester, using a 1 kg weight. The total soluble solids (TSS) content in the juice was determined with a digital refractometer. The titratable acidity (TA) in the juice was determined by the indophenol titration method. Ascorbic acid (AA) in the juice was determined by the indophenol titration method. Ethanol content in the juice was determined following the incubation of 10 ml aliquots of juice in 50 ml glass vials sealed with an aluminum cap at 30°C for 30 min according to Schirra et al. After the incubation period, a 1 ml gas sample was withdrawn from the headspace and analyzed with a gas chromatograph (GC) (model GC-8A, Shimadzu, Japan) coupled with a TIF and a 200 cm × 0.2 cm (internal diameter) stainless steel column containing 15% Carbowax and Chromosorb W 80/100 mesh. The temperatures of the injector, column, and detector were 100°C, 90°C, and 100°C, respectively. Nitrogen gas was used as the carrier gas. Firmness, TSS, TA, AA, and ethanol were determined on day 0 (fresh) and on day 28 (end of storage).

5. Atmosphere analysis

A gas sample of 0.5 ml was taken from each box at 3-day intervals until the end of storage, and analyzed with GC (model GC-8A, Shimadzu, Japan) coupled with a TCD and a stainless steel column containing molecular sieve 5A (60~80 mesh) for determining O₂ and CO₂ concentrations. The temperatures of the injector, column, and detector were 80°C, 50°C, and 80°C, respectively. Helium gas was used as the carrier gas.

6. Respiration rate measurement

Respiration rate, expressed as CO₂ production, was measured using the closed system method. A single fruit was placed in an air-tight plastic box (9.60 × 10⁻³ m³) and incubated at 15°C for 4 h. After the
incubation period (the CO₂ concentration had changed by about 1% from its normal atmospheric concentration), a gas sample of 0.5 ml from inside the box was taken and analyzed with GC (model GC-8 A, Shimadzu, Japan) coupled with a TCD (same as the measurement of CO₂ concentrations mentioned above). Respiration rate was determined on day 0 (fresh) and on day 28 (end of storage). The respiration rate was then calculated at 15°C and atmospheric pressure.

7. Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) with SPSS software for Windows version 11.5 (SPSS Inc., IL, USA). The significance of differences between means was determined by the Scheffe test at $P < 0.05$.

Results and Discussion

A perforation-mediated package system was used to create various MA conditions. As the fruit respires, O₂ is consumed and CO₂ is produced, resulting in modified atmosphere inside the plastic box. By varying perforation diameters, various MA conditions can be created. Changes in O₂ and CO₂ concentrations during the storage period are shown in Fig. 1. Smaller perforation diameters created lower O₂ levels and higher CO₂ levels. It could be inferred that the respiration rate of produce did not change significantly during the storage period as O₂ and CO₂ levels were rather constant. The average O₂ and CO₂ concentrations throughout the storage period are shown in Table 1. As we expected, the relative humidity inside all plastic boxes was more than 95% throughout the storage period (data not shown).

According to previous literature, MA conditions can prevent, induce or have no effect on various non-chilling rind disorders in citrus fruits, depending on the concentration of modified atmosphere. Subjecting white grapefruit to low O₂ (4%) induced non-chilling pitting, but high CO₂ (8%) had no effect during CA storage. However, higher CO₂ conditions, like 8~10%, increased the incidence of superficial flavedo necrosis (noxan) in ‘Shamouti’ orange packed inside the plastic liner. Reduced O₂ (17.8%) and elevated CO₂ (2.4%) levels in combination with a high relative humidity reduced various postharvest rind disorders in citrus fruits. From these studies, it seems that MA conditions may benefit the control of non-chilling rind disorders when the CO₂ level is not too high and/or the O₂ level is not too low. Conversely, CO₂ levels higher than 8% and/or O₂ levels lower than 4% may induce non-chilling rind disorders.

To investigate the effect of MA conditions on the occurrence of Kohansho disorder, the percentage and degree of Kohansho that occurred at different MA conditions were plotted (Fig. 2). The results showed that low O₂ levels ranging from 10 to 18%
and high CO₂ levels ranging from 4 to 12%, as in the conditions of 1 P 1.5 M, 1 P 3 M, and 1 P 3.5 M, have no effect on the percentage and degree of Kohansho as compared with the control. However, very high CO₂ levels (14%), as in the condition of 1 P 1 M, tended to induce the occurrence of Kohansho as the percentage and degree of Kohansho were much higher than those in the control. BEN-YEHOSHUA et al. reported that high CO₂ levels (8–10%) induced the incidence of noxan and had off-flavors in ‘Shamouti’ orange packed under high relative humidity (99.6%) inside the plastic liner. O₂ and/or CO₂ levels beyond the limit tolerated by the commodity can induce physiological disorders. In the case of ‘Kiyomi’ fruit stored under high humidity, CO₂ concentrations less than or equal to 12% are recommended for the packaging design to prevent Kohansho. Weight loss was not significantly different among the MA conditions and was lower for all MA conditions (range from 0.70 ~ 0.80%) compared with the control (1.80%) (Table 1). This was due to a higher area of perforation in the control than in the other MA conditions. In general, MA packaging not only maintains a high relative humidity, but also modifies the atmosphere inside the bag. In present and previous studies, it has been concluded that the benefit of using MA packaging for reducing the occurrence of Kohansho in ‘Kiyomi’ fruit has mainly been a result of the prevention of water loss (transpiration) via the maintenance of the high relative humidity of the atmosphere inside the package, rather than the modification of the atmosphere. This finding reinforces the important role of maintaining a high humidity surrounding the fruit for packaging design to prevent Kohansho in ‘Kiyomi’ fruit. BEN-YEHOSHUA et al. also concluded that the main effect of packaging fruit in plastic bags or plastic liners on the reduction of noxan incidence is related to the saturated humidity of the micro-atmosphere of the fruit.

Ethanol, which is the dominant fermentative metabolite, could be used to indicate fermentative metabolism for mandarin fruit. Ethanol was lowest in the fresh sample (measured on day 0) and accumulated after 4 weeks of storage at higher levels under 1 P 1 M and 1 P 1.5 M conditions, indicating fermentation, than under the other MA conditions and the control (Fig.3). High CO₂ levels (12~14%), as in the conditions of 1 P 1 M and 1 P 1.5 M, probably activate pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) enzymes, which are two major enzymes in the fermentation. As found in sweet potato roots, under a high CO₂ atmosphere there was a stimulation of PDC and ADH activities, ultimately resulting in ethanol production. When the CO₂ level is higher than the CO₂ injury limit and/or the O₂ level is lower than the lower oxygen limit (LOL), fermentative metabolism induces accumulation of acetaldehyde and ethanol, which can lead to the development of off-flavors.
thus impairing fruit quality. Moreover, CO₂ levels that are too high can lead to detrimental effects, such as initiation of certain physiological disorders, off-flavors due to fermentative metabolism, susceptibility to decay, and irregular ripening. Therefore, it is necessary to prevent the ‘Kiyomi’ fruit from being exposed to high CO₂ levels (> 8 %) throughout storage and distribution periods. As O₂ concentrations created under all MA conditions, as shown in Table 1, were higher than the general LOL for citrus fruits (5 % O₂), in this study, we were not able to identify the LOL for ‘Kiyomi’ fruit. However, it could be considered that ‘Kiyomi’ fruit should not be stored under O₂ levels less than 5 % in order to prevent anaerobic respiration.

An increase in the perforation area tended to reduce firmness (Table 2). However, there was no significant difference in firmness measured after 4 weeks of storage between the MA conditions and control, as well as no significant difference from fresh fruit (measured on day 0). BEN-YEHOSHUA et al. also mentioned that the high humidity of the micro-atmosphere of the fruit created inside the MA package maintains turgidity and markedly inhibits the loss of water and softening of the sealed citrus fruits. With ‘Kiyomi’ fruit in this study, there was no significant difference in nutritional qualities, including TSS, TA, TSS/TA ratio, or AA contents, between the MA conditions and control (Table 2). Additionally, it was also found that all the MA conditions and control preserved all these qualities even after 4 weeks of storage as compared with fresh fruit (measured on day 0). The effect of MA packaging on quality preservation is probably a result of maintaining a high relative humidity inside the plastic box, rather than as a result of MA conditions. TSS, TA, and AA contents of ‘Valencia’ orange were not significantly influenced by either the low O₂ or the high CO₂ treatments. Conditions favorable to prevention of water loss (ex. wrapping) after harvest resulted in a reduction in vitamin C loss. From this present study, it was found that MA conditions had no significant effect on the quality attributes under the high humidity condition. The quality of ‘Kiyomi’ fruit can be preserved as long as the fruit is packed under the high humidity condition.

Under aerobic conditions, elevated CO₂ during storage can stimulate, inhibit or have no effect on the respiratory metabolism of harvested plants, depending on the commodity and the CO₂ level. CO₂ production measured after 4 weeks of storage tended to be highest under the condition of 1 P 1 M, which was probably a response to O₂ injury (Table 2). However, there was no significant difference in CO₂ production between the MA conditions and control, as well as with fresh fruit.

**Conclusions**

The beneficial effect of MA packaging on the reduction of Kohansho and on the preservation of quality in ‘Kiyomi’ fruit is mainly due to the maintenance of a high humidity surrounding the fruit, rather than MA conditions. However, CO₂ levels that are too high could induce the occurrence of Kohansho and also trigger the fermentative metabolism, thus reducing fruit quality. CO₂ concentrations less than or equal to 8 % and O₂ concentrations more than or equal to 5 % are proposed as acceptable CO₂ and O₂ concentrations for the packaging design of ‘Kiyomi’ fruit. As long as water loss from ‘Kiyomi’ fruit is effectively restricted under the high humidity condition, O₂ and CO₂ concentrations are within the acceptable levels, Kohansho can be prevented and quality can be preserved.

**Table 2** Effect of different MA conditions on the quality and CO₂ production of ‘Kiyomi’ fruit

<table>
<thead>
<tr>
<th>Condition</th>
<th>Firmness (kg)</th>
<th>TSS (%)</th>
<th>TA (%)</th>
<th>TSS/TA ratio</th>
<th>AA (mg/100ml)</th>
<th>CO₂ production (μl/kg)</th>
</tr>
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<tbody>
<tr>
<td>Fresh</td>
<td>0.956±0.004</td>
<td>12.0±0.3</td>
<td>0.96±0.04</td>
<td>12.7±0.8</td>
<td>49.4±1.2</td>
<td>10.71±0.51</td>
</tr>
<tr>
<td>1 P 1 M</td>
<td>0.958±0.005</td>
<td>11.0±0.2</td>
<td>0.92±0.05</td>
<td>12.1±0.6</td>
<td>48.7±1.6</td>
<td>12.85±1.95</td>
</tr>
<tr>
<td>1 P 1.5 M</td>
<td>0.955±0.008</td>
<td>11.9±0.3</td>
<td>0.87±0.06</td>
<td>14.2±1.1</td>
<td>46.5±2.0</td>
<td>11.16±1.34</td>
</tr>
<tr>
<td>1 P 3 M</td>
<td>0.953±0.006</td>
<td>11.9±0.3</td>
<td>0.79±0.04</td>
<td>15.2±0.5</td>
<td>42.5±1.8</td>
<td>11.79±0.81</td>
</tr>
<tr>
<td>1 P 3.5 M</td>
<td>0.951±0.004</td>
<td>11.6±0.4</td>
<td>0.90±0.04</td>
<td>13.1±0.6</td>
<td>43.0±1.3</td>
<td>12.47±1.17</td>
</tr>
<tr>
<td>Control</td>
<td>0.944±0.005</td>
<td>10.9±0.3</td>
<td>0.94±0.07</td>
<td>12.3±1.1</td>
<td>47.0±1.7</td>
<td>8.00±0.46</td>
</tr>
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</table>

Data are means ± S.E. of 3 replicates of 3 independent measurements. Under the fresh condition, all parameters were determined on day 0 (initial day of experiment); while under other conditions, all parameters were determined on day 28 (end of storage). Means in each quality attribute and CO₂ production are not significantly different as determined by the Scheffe test at P<0.05 between MA conditions and control, as well as not significantly different from the fresh condition.
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タンゴール‘清見’果実のこはん症発生と品質変化に及ぼす包装内大気組成の影響

テチャ・ヴィセ ス ナッタコーン Nikon なはと田殿芳

*1 愛媛大学大学院農芸学研究科
〒790-8566 愛媛県松山市樽喰 3-5-7

*2 愛媛大学農学部
〒790-8566 愛媛県松山市樽喰 3-5-7

高湿度条件下の包装内大気組成（O₂およびCO₂濃度）が貯蔵中‘清見’果実のこはん症発生と品質変化に及ぼす影響について実験を行った。CO₂濃度が12％以下でO₂濃度が10％以上の大気組成ではこはん症の発生に影響は認められなかった。しかし、CO₂濃度が14％程度で高くなると発生が促進された。したがって、CO₂濃度が12％を超えないような大気組成では、包装内を高湿度で維持することがこはん症を防止するための主要な指針となるものと考えられた。8％以上のCO₂濃度は果実内に嫌気代謝を誘因し、その結果エタノールの蓄積が認められた。果実硬度、糖度（TSS）、クエン酸含量（TA）、TSS/TA比、アスコルビン酸含量においては、包装内の大気組成の違いによる有意な差異は認められなかった。これらの品質指標は貯蔵期間中に維持され、これは高湿度条件に依るものと考えられた。‘清見’果実の貯蔵や包装条件を設定するには、高湿度条件を保つことが必要であるに限ると考えられることから、こはん症防止と品質保持のために不可欠な条件である。

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