Effect of Cold Storage and Harvest Ripeness on the Quality and Chemical Composition of Loquat Fruits

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Loquat fruits (cv. Mogi) were harvested at three stages of ripeness (less-ripe, ripe, and full-ripe) and stored at 5°C to determine the optimal harvest ripeness for storage. During low-temperature storage, the weight of loquats at all three ripeness stages was decreased, whereas the weight losses showed no significant difference with ripeness. The decay of less-ripe fruit was worse than that of ripe and full-ripe fruits. Loquats underwent a significant decrease in titratable acidity (TA), malic acid and sucrose contents, while total sugars (TS), polyphenols, citric acid, succinic acid and fumaric acid contents did not vary greatly and resulted in a significant increase in TS/TA ratios during storage at 5°C. Development of skin color and carotenoids occurred progressively during the first 30 days of storage at 5°C and then did not vary significantly. Fructose and glucose contents of loquats increased during the first 30 days but after that appeared to decrease slightly. The sorbitol content of loquats increased steadily, while galactose decreased to a trace level during storage. Based on the data from this study, harvest ripeness greatly affected the quality of loquats at harvest as well as during storage. Ripe fruit showed superior storage capacity compared to fruits harvested at less-ripe and full-ripe stages.

Keywords: loquat, Eriobotrya japonica LINDL., harvest ripeness, cold storage, quality, fruit composition

The quality of loquats is highly dependent on the degree of ripeness (Uchino et al., 1994). Loquats harvested in the fully ripe stage have the optimum quality; therefore, they are left on the tree until fully ripe unless they are to be used in jellies or for cooking (Shaw, 1980). However, in commercial situations where transport and shelf-life are involved, loquats are generally harvested at the eating-ripe stage before becoming fully ripe, which may result in reduced quality of the fruit. The effects of storage temperature on different loquat cultivars have been reported (Mukerjee, 1958; Shaw, 1980; Shinborti & Nakai, 1991). Cold storage of loquats will retard senescence and lengthen storage life. However, there was no indication regarding at what stage of ripeness loquats should be harvested to maintain high quality after storage.

The appearance of skin color and contents of polyphenols, sugars and organic acids are the key components that contribute to a high quality of fresh loquat. However, little information is available in the literature on those parameters during low-temperature storage. The objective of this study was to characterize the postharvest physical and chemical changes in loquat fruits in relation to ripeness at harvest.

Materials and Methods

Plant materials The loquat (Eriobotrya japonica LINDL. cv. Mogi) fruits were obtained from the affiliated teaching farm of the College of Agriculture, Osaka Prefecture University, Osaka, Japan. The fruits were hand-picked and sorted into less-ripe, ripe and full-ripe stages (Table 1) according to fruit surface color and the Hunter "a" values of the skin, and were stored at 5°C packaged in perforated polyethylene bags. During the storage period, samples were collected at 15-day intervals and 20–30 loquats were analyzed each time. Before and after 5°C storage, the fruits were peeled by hand, the pulp was cut into small pieces, and the composite fruit samples ranging from 10–20 g of pulp were weighed and frozen in liquid nitrogen, then stored at -38°C until analyzed.

Determinations of skin color, respiration rate, total soluble solids and titratable acidity Twenty loquats of each ripeness stage were weighed. Surface color was measured at opposite locations along the equatorial diameter of each fruit using a Color Difference Meter (1001 DP, Nippon Denshoku Kogyo Co., Ltd., Tokyo). CO₂ production was determined by taking 1-ml gaseous samples from the container kept at 20°C on a gas chromatograph equipped with a Porapak P column (50-80 mesh, 2 m×3 mm, 60°C) and a thermal conductivity detector (TCD). Total soluble solids (TSS) was measured by a refractometer (PIKA, Tokyo). Titratable acidity (TA) was determined with 0.1 N NaOH, and the acidity is expressed as grams of malic acid per 100 g FW of loquat pulp.

Extraction and determination of carotenoids About 10 g of pulp was mixed in a chilled homogenizer with 80 ml of cold 40% aqueous methanol containing a small amount of MgCO₃ and butylated hydroxytoluene (BHT). The mixture was filtered through a 1-cm Celite 545 layer. The filtrate was discarded and the residue was extracted with acetone until the extract became colorless. The extraction and saponification of carotenoids were carried out according to the method of Kon and Shimba (1988). The extracts were run through an HPLC condition introduced by Hamauzu et al. (1991). Chromatographic peaks were identified by comparing both the retention time and absorbance spectra obtained at each peak maximum with those found in the literature, and the concen-
tration was determined from published 1% absorptivity coefficients (Davis, 1976).

**Extraction and determination of phenolics** Phenolic compounds were extracted with methanol, separated with a C<sub>18</sub> Sep-Pak (Waters, Milford, MA) according to the method of Jaworski and Lee (1987). Total phenolic content was determined with Folin-Ciocalteu phenol reagent (Julkunen-Titto, 1985). Chlorogenic acid content was identified and determined with HPLC (Shimadzu LC-9A pump, 6PD-6VA UV-vis spectrophotometric detector, and R6A Chromatopac recorder). Separation of phenolics was carried out on a 4 mm x 250 mm GL Sciences Inertsil ODS-2 column at room temperature (23°C) and detected at 280 nm. The elution curve was linear within 50 min to reach 50% of the solvent acetic acid-acetonitrile-water (5 : 80 : 15, v/v) in the initial acetic acid-water solvent (5 : 95, v/v) at a flow rate of 1.0 ml/min. A standard curve was prepared to quantify the chlorogenic acid.

**Determination of sugars and organic acids** Twenty grams of frozen pulp was immediately homogenized in 80 ml of cold MeOH (95%) for 1 min and shaken for 10 min. The homogenate was filtered and the residue was extracted twice with 80% cold aqueous MeOH. The combined extracts were evaporated in vacuum at 35°C until the MeOH was removed, and finally the volume was made up to 20 or 100 ml with water. The resulting extraction was used for the assay of sugars and organic acids.

A 5-ml extraction was passed through a Sep-Pak C<sub>18</sub> cartridge (Waters). The eluate was used for sugars analysis by an HPLC system consisting of a refractive index detector, Shim-pack SCR-101P column kept at 80°C. Twenty microliters of the eluate was injected into the HPLC in which water flowing at 1.0 ml/min was used as eluent. Quantitation of individual sugars was done using a standard curve derived using the peak areas of individual sugar standards.

To determine the acid contents of the fruit, 10–50 ml of the extraction was freeze-dried. The residue was dissolved in 2 ml of water and passed through a column of cation exchange resin (Dowex 50) with 50 ml of water. The eluate containing the acids was analyzed by an HPLC Organic Acid Analysis System (Japan Spectroscopic, Ltd., Tokyo) using a Shodex Ionpak C-811 column. A 20 µl eluate was auto-injected. The flow rate was 1.0 ml/min using 3 mM HClO<sub>4</sub> as eluent, and the flow rate was 1.5 ml/min using 0.2 mM bromothymol blue (BTB), with 15 mM NaH<sub>2</sub>PO<sub>4</sub> as reagent. The column temperature was 60°C. Organic acid components were detected at a wavelength of 445 nm and quantified by an external standard method.

**Data analysis** All analyses were carried out in triplicate unless others stated. Means and standard errors were calculated. The data were analyzed by the Kaleida Graph I of the data analysis and graphic presentation for business, science and engineering (Abelbeck software, 1990).

### Table 1. Quality and physical parameters of loquat (cv. Mogi) fruits at three stages of ripeness.

<table>
<thead>
<tr>
<th>Ripeness stage</th>
<th>Description</th>
<th>Weight (g)</th>
<th>Skin color (a value)</th>
<th>Respiration rate (CO&lt;sub&gt;2&lt;/sub&gt; ml/kg h)</th>
<th>Total soluble solids (TSS) (%)</th>
<th>Titratable acidity (TA) (g/100 g FW)</th>
<th>Organic acid (mg/100 g FW)</th>
<th>Malic</th>
<th>Citric</th>
<th>Succinic</th>
<th>Fumaric</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Less-ripe; full yellow</td>
<td>40.3</td>
<td>9.5</td>
<td>69.5</td>
<td>9.7</td>
<td>0.74</td>
<td>612</td>
<td>34</td>
<td>4.8</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ripe; light orange; suitable for fresh fruit market</td>
<td>42.6</td>
<td>14.1</td>
<td>58.6</td>
<td>10.7</td>
<td>0.45</td>
<td>390</td>
<td>35</td>
<td>5.2</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Full-ripe; fully ripe; dark orange</td>
<td>41.3</td>
<td>18.0</td>
<td>41.8</td>
<td>11.8</td>
<td>0.30</td>
<td>248</td>
<td>37</td>
<td>5.6</td>
<td>3.3</td>
<td></td>
</tr>
</tbody>
</table>

Data from 20 fruits per stage of ripeness.

### Table 2. Changes in sugar contents (g/100 g FW) of loquats (cv. Mogi) harvested at three stages of ripeness during storage at 5°C.

<table>
<thead>
<tr>
<th>Ripeness</th>
<th>Stage</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Sorbitol</th>
<th>Galactose</th>
<th>Other&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day (at harvest)</td>
<td>Stage 1</td>
<td>2.94±0.26</td>
<td>1.72±0.18</td>
<td>2.92±0.28</td>
<td>0.54±0.08</td>
<td>0.06</td>
<td>0.16</td>
<td>8.34</td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>2.41±0.21</td>
<td>2.53±0.14</td>
<td>3.89±0.16</td>
<td>0.82±0.13</td>
<td>0.10</td>
<td>0.16</td>
<td>9.91</td>
</tr>
<tr>
<td></td>
<td>Stage 3</td>
<td>1.80±0.21</td>
<td>3.08±0.23</td>
<td>4.52±0.23</td>
<td>0.81±0.12</td>
<td>0.13</td>
<td>0.21</td>
<td>10.56</td>
</tr>
<tr>
<td>15 days</td>
<td>Stage 1</td>
<td>2.58±0.17</td>
<td>2.25±0.21</td>
<td>3.45±0.25</td>
<td>0.62±0.12</td>
<td>0.05</td>
<td>0.15</td>
<td>9.10</td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>2.02±0.35</td>
<td>2.81±0.24</td>
<td>4.02±0.27</td>
<td>0.72±0.13</td>
<td>0.10</td>
<td>0.15</td>
<td>9.92</td>
</tr>
<tr>
<td></td>
<td>Stage 3</td>
<td>1.32±0.14</td>
<td>3.38±0.27</td>
<td>4.73±0.32</td>
<td>0.92±0.12</td>
<td>0.12</td>
<td>0.20</td>
<td>10.67</td>
</tr>
<tr>
<td>30 days</td>
<td>Stage 1</td>
<td>1.62±0.18</td>
<td>2.38±0.21</td>
<td>3.67±0.19</td>
<td>0.72±0.07</td>
<td>0.04</td>
<td>0.18</td>
<td>8.61</td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>1.05±0.16</td>
<td>2.92±0.23</td>
<td>4.35±0.28</td>
<td>0.91±0.10</td>
<td>0.08</td>
<td>0.17</td>
<td>9.48</td>
</tr>
<tr>
<td></td>
<td>Stage 3</td>
<td>0.89±0.07</td>
<td>3.18±0.26</td>
<td>4.68±0.36</td>
<td>1.03±0.09</td>
<td>0.10</td>
<td>0.19</td>
<td>10.07</td>
</tr>
<tr>
<td>45 days</td>
<td>Stage 1</td>
<td>0.87±0.13</td>
<td>2.36±0.16</td>
<td>3.64±0.28</td>
<td>0.88±0.17</td>
<td>trace</td>
<td>0.19</td>
<td>7.94</td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>0.62±0.07</td>
<td>2.53±0.23</td>
<td>3.98±0.37</td>
<td>1.12±0.03</td>
<td>0.05</td>
<td>0.18</td>
<td>8.68</td>
</tr>
<tr>
<td></td>
<td>Stage 3</td>
<td>0.78±0.11</td>
<td>3.16±0.14</td>
<td>4.57±0.26</td>
<td>1.18±0.14</td>
<td>0.06</td>
<td>0.18</td>
<td>9.95</td>
</tr>
<tr>
<td>60 days</td>
<td>Stage 1</td>
<td>0.63±0.06</td>
<td>2.20±0.15</td>
<td>3.41±0.26</td>
<td>0.93±0.12</td>
<td>trace</td>
<td>0.17</td>
<td>7.34</td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>0.42±0.08</td>
<td>2.36±0.20</td>
<td>3.87±0.27</td>
<td>1.28±0.18</td>
<td>trace</td>
<td>0.22</td>
<td>8.35</td>
</tr>
<tr>
<td></td>
<td>Stage 3</td>
<td>0.73±0.08</td>
<td>2.85±0.28</td>
<td>4.34±0.23</td>
<td>1.22±0.12</td>
<td>trace</td>
<td>0.24</td>
<td>9.38</td>
</tr>
</tbody>
</table>

<sup>a</sup> Other: showed one peak on the HPLC chromatograms, but unidentified.
Results and Discussion

The physical and chemical characteristics of loquats (cv. Mogi) harvested at different ripeness are shown in Table 1 and the data for harvest day in Table 2. In general, the contents of total soluble solids (TSS), fructose and glucose, TSS/TA ratio and skin color (a value) increased with advanced harvest ripeness. The weight and the contents of citric acid, succinic acid and fumaric acid did not vary significantly with ripeness. Respiration rate, titratable acidity, malic acid and sucrose contents decreased significantly with advanced ripeness. Data showed that the fruit had higher sugar and lower but optimal acid contents associated with high quality at harvest if they were harvested in the later stage of ripeness.

The physical and chemical characteristics of all three ripeness stages of loquats showed variable changes during storage at 5°C. The weight of the loquats of all three ripeness stages decreased steadily during storage with an average decrease of about 0.15%/day and 9% total decrease after 60 days of storage (Fig. 1). The weight losses showed no significant difference by ripeness in spite of the fruit shriveling observed at the full-ripe stage. Uchino et al. (1994) showed that the water content of loquat fruits decreased with advanced ripeness. We observed severer weight loss and overripe symptoms (shriveling) in the full-ripe fruit (stage 3) after 60 days of storage. That may be due to the lower water content in the full-ripe fruits at harvest.

During storage at 5°C, full-ripe fruit (stage 3) showed less decay than less-ripe (stage 1) and ripe (stage 2) fruits. After 60 days of storage, the incidence of rotten fruits harvested at less-ripe, ripe and full-ripe stages were 13%, 8% and 5%, respectively (Fig. 1). The spoilage of loquat fruits was caused predominantly by an internal physiological disorder. The common symptoms of the disorder started with internal flesh browning of fruits and then became rotten during storage at 5°C which is typical of CO₂ injury for fruits and vegetables developed upon massive accumulation of CO₂ in the core or the pulp tissue (Herner, 1987). Therefore, the high respiration rate of the less ripe fruits (Table 1) may offer an explanation for the higher incidence of decay occurring in the less-ripe fruits. It should also be noted out that the flesh firmness of loquats was increased progressively during fruit ripening (Chachin et al., 1990; Uchino et al., 1994) and also Chachin et al. indicated that loquat fruits contained much more pectate than water soluble pectin during ripening. The higher moisture content and the softer texture in less-ripe fruits may also contribute to the higher decay rate in those fruits.

The effects of low-temperature storage and harvest ripeness on the skin color (a value) and carotenoid contents are shown in Figs. 2 and 3. The Hunter a values and carotenoid contents of all three ripeness categories increased significantly during
Harvest Ripeness and Storage of Loquat

Fig. 4. Changes in the total phenolic and chlorogenic acid contents of loquats (cv. Mogi) harvested at three stages of ripeness during storage at 5°C. Symbols and vertical lines are the same as those in Fig. 1.

Fig. 5. Changes in contents of malic acid and titratable acidity of loquats (cv. Mogi) harvested at three stages of ripeness during storage at 5°C. Symbols and vertical lines are the same as those in Fig. 1.

The first 30 days of storage at 5°C and then showed a slight increase. After 60 days storage at 5°C, less-ripe fruits still had lower β-carotene and cryptoxanthin contents in comparison with riper fruits, although they were increased during storage. The increase in Hunter a value showed that loquats at all three ripeness stages were becoming more orange in color. This result indicated that loquats after harvest would develop more yellow and orange color and increased carotenoids even in low temperature storage (5°C).

Total phenolic and chlorogenic acid contents increased with advanced ripeness. The changes in phenolics during storage are presented in Fig. 4. Chlorogenic acid is predominant and accounts for about 50% of the total phenolic content. During storage at 5°C, no significant variation occurred in the total phenolic and chlorogenic acid contents of loquats, although they appeared to have a slight decreasing trend after 30 days of storage. The result indicated that no significant browning of all three ripeness stages fruits occurred during low-temperature storage.

The changes in sugars of loquats are shown in Table 2. Total sugars (TS) increased greatly with advanced ripeness but did not change significantly during storage. The changes in individual sugars, on the contrary, were greater during storage at 5°C. Sucrose is the prominent sugar in less-ripe fruit (stage 1). However, fructose became the predominant sugar in ripe (stage 2) and full-ripe fruits (stage 3), followed by glucose, sucrose, sorbitol, an unidentified component and galactose. Fructose and glucose increased steadily during the first 30 days of storage and then decreased slightly. On the other hand, the sucrose content declined rapidly to a low constant level during the storage. Because the sucrose content decreased and glucose and fructose increased with storage, it is probable that sucrose was hydrolyzed during storage, yielding glucose and fructose. Sorbitol content is relatively low and showed a steadily increasing trend during storage. The sorbitol produced on the tree but was not accumulated in fruit but was continuously converted into fructose, sucrose and glucose (Hirai, 1980). The steady increase observed during storage can be attributed to the anaerobic conversion of fructose (Ackermann et al., 1992). Galactose does not normally occur in its free state; however, soluble, monomeric galactose has been detected in the pulp of loquats (cv. Mogi). The galactose content increased 2-fold from stage 1 to stage 3 (from 60 to 130 mg/100 g FW). The same increase in galactose was observed in tomatoes (cv. Rutgers) during fruit ripening; however, the galactose content was one-fifth of those in loquats (Kim et al., 1991). The source(s) of the free galactose is unknown. Gross (1983) suggested that the accumulation of soluble galactose during ripening could be due to a loss of the ability of the fruit to metabolize the sugar into the cell wall. During storage at 5°C, the concentration of galactose decreased and reached a trace level. Those results showed that loquats picked at a less-ripe stage did not significantly improve in total sugar content and quality during storage.

The changes in titratable acidity (TA) and organic acid contents during storage are shown in Fig. 5. Malic acid, between 0.61 and 0.25%, was the principal nonvolatile organic acid and represented about 90% at harvest. During storage at 5°C, the malic acid concentration rapidly declined associated with the decrease in TA; while citric acid, succinic acid and fumaric acids, on the contrary, remained relatively constant (data not shown). After 60 days of storage, malic acid in fruits had decreased by 60–65%. The decrease can be attributed to respiration because malic acid is the principal metabolic
substrate together with the sugars.

Fruit taste may be influenced mainly by sugars and acids. While studies have emphasized the importance of sugars, the contribution of the acids has not been of much concern. Ulrich (1970) indicated high acidity in fruit has been suggested to contribute in part to the flavor retention of ripened fruit. An optimal acid concentration (about 0.2-0.4%) in loquat fruits for preferred flavor quality may be necessary. If the organic acid content declines below 0.2% during storage, the taste of the loquat will be worse or it will lose the "loquat-like" flavor. The results of this study indicated that the decrease of organic acid contents can not be inhibited during low-temperature storage. Therefore, the determination of at what stage of ripeness loquats should be harvested must consider both characteristics, at what ripeness they have high quality and yet possess suitable sourness after storage. Based on the data from this study, loquats harvested at a less-ripe stage have higher organic acids, lower sugars and less orange color which resulted in low quality at harvest. During storage at low temperature, titratable acidity and malic acid, a predominant acid, in the fruits decreased to an optimal level, but total sugar content did not significantly increase. Therefore, the quality of less-ripe fruits cannot be improved during storage. The fruits harvested at the full-ripe stage had the best flavor at harvest, but they had too low content of organic acids and became overripe during storage and resulted in loss of loquat flavor and shriveling in the fruits stored at 5°C. The fruits harvested at ripe stage (stage 2) had acceptive quality characteristics such as a lower and optimal acid level, higher sugar content and increased yellow color after storage. So the optimum harvesting ripeness of loquats (cv. Mogi) for storage is the ripeness of stage 2. The quality of loquats available to the consumer would be improved if storage methods can protect against the decrease in organic acid so that the fruit could be harvested at a later stage of ripeness.

References


