Impact of Shelf Life on Content of Primary and Secondary Metabolites in Apple (*Malus domestica* Borkh.)

Robert Veberic, Valentina Schmitzer, Maja M. Petkovsek, and Franci Stampar

**Abstract:** In this study, we evaluated the changes in apple fruit quality during shelf life. After a month of cold storage, apples of cultivars “Jonagold” and “Golden Delicious” were exposed to ambient temperatures for 21 d, with subsequent sampling every 3 or 4 d. Fruit firmness, changes in amounts of sugars, malic acid, and phenolics were observed during shelf life. Chemical analyses were done with HPLC-PDA system. An interchange between various sugars was noticed, but in general, the sum of sugars remained at the same level. The content of malic acid remained stable or dropped, resulting in sweeter fruit. Levels of phenolics were more constant in the pulp of both cultivars analyzed, while in the peel, the changes were more pronounced. In the pulp, a peak in the content of hydroxycinnamic acids and flavanols was noticed on the 2nd or 3rd sampling and afterwards, the amounts remained constant. In the peel an initial decrease of all analyzed phenolic groups was observed in both cultivars, however it was more pronounced in “Jonagold.” It can be concluded that changes in primary and secondary metabolites are not the main reason for the lower quality of fruit exposed to ambient temperatures. On the other hand, fruit firmness might be the limiting factor for shelf life duration.

**Keywords:** color, firmness, organic acids, phenolics, sugars

**Practical Application:** In our study, we were able to show that various quality parameters react uniquely to exposure to ambient temperatures. The most dramatic change was noticed in fruit firmness, which might be the limiting factor for the practical duration of shelf life. On the other hand phenolics, as the health-beneficial constituents in the flesh and peel of apples, did not change dramatically during shelf life. This information is important for nutritionists as well as for the consumers and specialists in fruit storage and handling.

**Introduction**

On European as well as world fruit markets, apples represent an important produce available year around. This is possible owing to the different ripening periods in the northern and southern hemispheres as well as to excellent apple storability and sophisticated storage conditions. During storage at reduced oxygen concentrations and low-temperature conditions, the general appearance of stored apples can be maintained for a long period of time (Brackmann and others 1994). It is well known that during inappropriate storage, apples can lose some of their quality as a result of metabolic degradation, respiration, and synthesis processes. The storage regimes and storage time have been optimized for minimal losses due to physiological disorders and maximum retention of firmness, juiciness, and sweetness (Varela and others 2008). Particularly at higher temperatures, such as those in supermarkets and ambient temperatures in consumers’ homes, internal and external fruit quality might be affected by the changed conditions (Matthes and Schmitz-Eiberger 2009).

The attractiveness of fruit to consumers is determined by visual attributes that include appearance, size, uniformity, color, and freshness, as well as nonvisual attributes such as taste, aroma, flavor, firmness (texture), nutritional and health value. Among these attributes, firmness and aroma appear to be the most important for consumers (Awad and de Jager 2002).

Apart from vitamins and minerals, apples also contain other phytochemicals, which are the important constituents of the fruit. The group of phenolics has been a particular focus of several studies in recent years. Consumers are becoming more interested in the content of these health-promoting compounds in fruit because of their antioxidant activity (Gil and others 2002). The importance of consuming whole fruit, including apple peel, has also been underlined because in general, the content of phenolics in the peel can be several times higher than that in pulp of the fruit (Mikulic Petkovsek and others 2007; Lata and others 2009). The reason for this involves high quantities of certain groups of flavonoids, like flavonol glycosides, catechins, and chlorogenic acid (Escarpa and Gonzalez 1998). In particular, phenolics in apple skin have shown a much higher contribution to the total antioxidant and antiproliferative activities of the whole apple than those in apple pulp (Wolfe and others 2003).

Several studies have dealt with the evaluation of changes in fruit composition during short- or long-term storage. Patterns of change in the content of organic acids, carbohydrates, and
phenolics during storage are variable. Roth and others (2007) reported that the sucrose content in apples increased during storage, while glucose and sorbitol contents increased. Pinetti and others (1994) found that epicatechin, quercetin glycosides, and procyanidins in “Granny Smith” generally decreased during storage. Similar results were reported for “Boskoop” apples, where a decrease in the content of catechin, epicatechin, and phenolic acids was observed (Moesl and Herrmann 1974). In contrast, some researchers found that the content of phenolics increased during storage (Burd and others 1990; Napolitano and others 2004). The majority of researchers, however, reported that no change in the content of simple phenolics (mainly chlorogenic acid), flavonoids, and anthocyanins occurred during storage (Awad and de Jager 2000; Golding and others 2001).

Even though many studies have been performed during storage, little information is available about the changes in fruit quality during shelf life. In available literature, the impact of shelf life was mostly followed up to 2 wk and only few quality parameters were considered. After storage, apples are normally kept in stores until they are sold, and then for some additional time they are left at ambient temperatures in consumers’ homes. The changes—not only in firmness and sugars and organic acids content but also in wide range of phenolics—could be of great importance for consumers, particularly if the health benefits of fruit are affected. Such an approach will provide additional knowledge about the changes in fruit quality after cold storage, and the data could be of use for a variety of nutritional studies.

**Materials and Methods**

**Plant material**

This experiment was carried out in 2008. For the experiment, fruits of “Golden Delicious” and “Jonagold” were taken from controlled atmosphere cool storage after 1 mo of storage. Fruit samples were exposed to ambient temperatures to mimic conditions in stores and in consumers’ homes. The fruits were subsequently sampled every 3rd or 4th day after exposure to ambient temperatures of 22 °C (Table 1). At each sampling date, five fruit samples were taken for analysis. Fruit firmness was measured, and the tissue was frozen in liquid nitrogen and stored at −20 °C for subsequent extraction and analysis of sugars, organic acids, and phenolics.

**Firmness and color measurement**

The measurement of fruit firmness (Chatillon penetrometer equipped with 11 mm probe) and skin color (in the case of “Golden Delicious”) was done immediately after sampling. Five fruit samples of each cultivar were used for measurements. Apple color was measured using the Minolta CR-10 Chroma portable colorimeter (Konica Minolta, Osaka, Japan) with C illuminant.

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**Extraction and determination of sugars and organic acids**

Primary metabolites (sucrose, glucose, fructose, sorbitol, and malic acid) were analyzed from the whole edible part of the fruit. For each cultivar, five repetitions per sampling date were carried out ($n = 5$); each repetition included one fruit. Ten grams of fruit for extraction were homogenized in 50 mL of bi-distilled water using Ultra-Turrax T-25 (Ika-Labortechnik, Staufen, Germany) at 12000 rpm for 7 min at 10 °C. The supernatant was filtered through a 0.45-mm cellulose ester filter (Macherey-Nagel, Düren, Germany), transferred into a vial, and 20 μL of the sample was used for analysis. The analysis of sugar (fructose, glucose, sucrose, and sorbitol) and malic acid content was carried out using high-performance liquid chromatography (HPLC) from Thermo Separation Products equipment. The separation of sugars and sorbitol was carried out using a Rezex RCM-mono-saccharide column (300 × 7.8 mm; Phenomenex, Torrance, Calif., U.S.A.) operated at 65 °C from Phenomenex. The mobile phase was bi-distilled water, and the flow rate was 0.6 mL/min; the total run time was 35 min, and a refractive index detector was used to monitor the eluted carbohydrates as described by Dolenc-Sturn and others (1999). Organic acids were analyzed with HPLC using an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad, Hercules, Calif., U.S.A.) associated with a UV detector set at 210 nm as described by Dolenc-Sturn and others (1999). The column temperature was set at 65 °C. The elution solvent was 4 mM sulfuric acid in bi-distilled water at a flow rate of 0.6 mL/min. The duration of the analysis was 30 min.

**Extraction and determination of phenolic compounds**

The apples were peeled with a mechanical peeler, and the peel was separated from the pulp. The extraction of samples (peel and pulp separately) was made as described by Escarpa and Gonzalez (2000). The samples of 10 g of pulp and 5 g of peel were extracted with methanol containing 1% of 2,6-di-tert-butyl-4-methylphenol (BHT) in a cooled water bath using sonification. BHT was added to the samples to prevent oxidation during the extraction. The samples were extracted with 10 mL of solvents for 1 h, 10 mL for 30 min, and then 5 mL for 30 min. The extracts were then combined to a final volume of 25 mL. After centrifuging at 10000 rpm for 10 min at 4 °C, the supernatants were filtered through a 0.45 mm membrane filter (Macherey-Nagel), prior to injection into the HPLC. The phenolic compounds were analyzed on a Thermo Finnigan Surveyor HPLC system, using a diode array detector at 280, 350, and 530 nm. The hydroxycinnamic acids (chlorogenic and caffeic acids) and the monomeric flavonoids (catechin, epicatechin) detected at 280 nm, while the flavonols (quercetin rutinoside, quercetin rhamnoside, quercetin glucoside, and quercetin galactoside) were estimated at 350 nm and cyanidin galactoside at 530 nm. The spectra of the compounds were also recorded between 200 and 600 nm. The column used was a Phenomenex Gemini C18 (150 × 4.6 mm 3 μm; Phenomenex).

**Table 1—Sampling dates and corresponding days of shelf life.**

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Dates</th>
<th>Shelf life (days after exposure to ambient temperatures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>26 Nov</td>
<td>0</td>
</tr>
<tr>
<td>2nd</td>
<td>30 Nov</td>
<td>4</td>
</tr>
<tr>
<td>3rd</td>
<td>3 Dec</td>
<td>7</td>
</tr>
<tr>
<td>4th</td>
<td>6 Dec</td>
<td>10</td>
</tr>
<tr>
<td>5th</td>
<td>10 Dec</td>
<td>14</td>
</tr>
<tr>
<td>6th</td>
<td>13 Dec</td>
<td>17</td>
</tr>
<tr>
<td>7th</td>
<td>17 Dec</td>
<td>21</td>
</tr>
</tbody>
</table>
operated at 25 °C. The elution solvents were 1% formic acid in bi-distilled water (A) and 100% acetonitrile (B). The samples were eluted according to the linear gradient described by Marks and others (2007), with an injection amount of 20 μL and a flow rate 1 mL/min. The phenolics were identified by comparing their UV–Vis spectra and retention times. Quantification was achieved according to concentrations of the corresponding standard. The concentrations were expressed in milligram per kilogram of fresh weight.

Chemicals
Malic acid was purchased from Merck (Darmstadt, Germany). Fructose, glucose, sucrose, sorbitol, and acid were obtained from Fluka (Buchs, Switzerland). For determining the phenolic peaks, standards acquired from Sigma-Aldrich (Steinheim, Germany) (rutin, chlorogenic acid), Roth (Karlsruhe, Germany) ((+)-catechin), and Fluka ((-)-epicatechin, quercetin-3-rhamnoside, quercetin-3-galactoside, quercetin-3-glucoside, caffeic acid) were used; and methanol was acquired from Sigma-Aldrich. Water was bi-distilled and purified with the Milli-Q system (Millipore, Bedford, Mass., U.S.A.).

Statistic analysis
The data were analyzed with the Statgraphics Plus 4.0 program (Statgraphics, Herndon, Va., U.S.A.) using one-way analysis of variance. The differences between sampling dates for an individual cultivar were tested using the Duncan test at the 0.05 significance level. The means and the standard errors of the means are also presented. Different letters in columns denote statistically significant differences between sampling dates at P < 0.05.

Results and Discussion
Fruit firmness and color measurement
Instrumental measurements of fruit firmness and texture have become important tools of fruit quality assessment. In these tests, a penetrometer is the most widely used device to assess fruit firmness. In our research, “Golden Delicious” showed higher values in fruit firmness than “Jonagold” (Table 2). In both cultivars, firmness decreased during shelf life, resulting in softer and mealler fruit at the end of the trial.

For a high-sensory estimation by the consumer, the firmness of “Golden Delicious” fruit should not be lower than 5.5 kg cm⁻². In our case, firmness above this value was maintained only at the 1st two sampling dates. As we assume that beyond this date, the fruit would be less acceptable to consumers.

Consumers worry that “old,” stored apples get “mealy” if kept at home, which in many cases is true, since apple texture can deteriorate during cold storage, resulting in softness and mealiness. Varela and others (2008) studied consumer acceptability, and descriptive sensory analyses for storage periods of up to 28 d at 20 °C indicated that the greatest quality loss in stored “Fuji” apples was associated with increased mealiness, a perceived overripe taste, and an alcoholic taste and odor.

Color measurements were done only on “Golden Delicious” apples (Table 2). The main purpose of these measurements was to monitor the changes from green ground color to yellow as a result of chlorophyll degradation. This was not done on “Jonagold” apples, since the major part of the fruit was covered with red blush, and any changes in the green ground color would be influenced by the red blush. In the case of “Jonagold,” we measured anthocyanins, which are causing the red blush of apples.

As expected, the color parameters (L⁺, a⁺, b⁺) increased during shelf life, indicating degradation of chlorophyll and exposure of yellow pigments. L⁺ increased from 66.4 at the exposure to 69.1 indicating lighter hues in the fruit. a⁺ increased from −1.8 to 8.5, where negative values represent green and positive red. b⁺ increased from 44.3 to 54.8, where higher values represent yellow coloration. Owing to high b⁺ values, apples had a bright yellow coloration. Similar data for “Golden Delicious” were also reported by Rutzkowski and others (2008). Intensification of the yellow coloration can also be seen from the hue value, which represents the best predictor of sensory color perception. A hue angle with a value of 90 indicates a yellow coloration, while 0 means red coloration. In our case, the values decreased from 92.2 to 81.4, indicating that yellow coloration intensified with shelf life.

Table 2—Changes in colorimetric parameters of “Golden Delicious” apple peel and changes in fruit firmness for both cultivars during shelf life (numbers 1 to 7 represent sampling dates). Average values ± standard errors are presented. Different letters in columns denote statistically significant differences between sampling dates at P < 0.05.

<table>
<thead>
<tr>
<th>Date</th>
<th>L⁺</th>
<th>a⁺</th>
<th>b⁺</th>
<th>b⁺</th>
<th>Fruit firmness“Jonagold”</th>
<th>Fruit firmness“Golden Delicious”</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66.42 ± 0.52</td>
<td>a</td>
<td>−1.79 ± 0.65</td>
<td>a</td>
<td>44.30 ± 0.67</td>
<td>a</td>
</tr>
<tr>
<td>2</td>
<td>68.53 ± 0.57</td>
<td>a</td>
<td>−1.42 ± 0.56</td>
<td>a</td>
<td>45.24 ± 0.56</td>
<td>b</td>
</tr>
<tr>
<td>3</td>
<td>70.75 ± 0.56</td>
<td>bc</td>
<td>1.60 ± 0.81</td>
<td>b</td>
<td>48.09 ± 0.74</td>
<td>b</td>
</tr>
<tr>
<td>4</td>
<td>70.57 ± 0.51</td>
<td>bc</td>
<td>4.26 ± 0.88</td>
<td>c</td>
<td>51.76 ± 0.90</td>
<td>c</td>
</tr>
<tr>
<td>5</td>
<td>71.35 ± 0.47</td>
<td>c</td>
<td>7.35 ± 0.56</td>
<td>d</td>
<td>52.93 ± 0.64</td>
<td>cd</td>
</tr>
<tr>
<td>6</td>
<td>70.62 ± 0.62</td>
<td>bc</td>
<td>9.22 ± 0.95</td>
<td>d</td>
<td>55.29 ± 0.66</td>
<td>e</td>
</tr>
<tr>
<td>7</td>
<td>69.10 ± 1.04</td>
<td>ab</td>
<td>8.45 ± 1.21</td>
<td>d</td>
<td>54.80 ± 1.23</td>
<td>de</td>
</tr>
</tbody>
</table>

Firmness is expressed in kg/cm².

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Figure 1—Changes in the sum of total sugars during shelf life (numbers 1 to 7 represent sampling dates) expressed in g kg\(^{-1}\) FW. Average and standard error are presented. JG = “Jonagold,” GD = “Golden Delicious.” Different letters denote statistically significant differences between sampling dates for individual cultivars at \(P \leq 0.05\).

Figure 2—Changes of sucrose content during shelf life (numbers 1 to 7 represent sampling dates) expressed in g kg\(^{-1}\) FW. Average and standard errors are presented. JG = “Jonagold,” GD = “Golden Delicious.” Different letters denote statistically significant differences between sampling dates for individual cultivars at \(P \leq 0.05\).

Figure 3—Changes of glucose content during shelf life (numbers 1 to 7 represent sampling dates) expressed in g kg\(^{-1}\) FW. Average and standard errors are presented. JG = “Jonagold,” GD = “Golden Delicious.” Different letters denote statistically significant differences between sampling dates for individual cultivars at \(P \leq 0.05\).
Shelf life and apple quality can be observed, while a steady decrease during the remaining shelf life can be seen. This was noticed for both cultivars. Twenty-one days after exposure, "Jonagold" had about 40% less sucrose and "Golden Delicious" about 25% less sucrose compared to the 1st sampling date. The rise in the amount of sucrose on the 4th day after exposure to ambient conditions was probably the result of starch degradation, although starch content was not measured. As fruit ripen, starch is degraded and sugar content increases, providing the sweetness associated with changing fruit taste (Blanco and others 1992). Thammawong and Arakawa (2007) studied the degradation of starch in two apple cultivars and noticed that starch degradation after harvest seems highly related to climacteric and physiological changes in fruit, such as increased ethylene production and respiration rate. Even more starch degradation related is glucose, the content of which generally rises in both cultivars during the shelf life of fruit (Figure 3). When the changes between 1st and last sampling dates are considered, glucose content increased by 18% and 47% in "Golden Delicious" and "Jonagold," respectively. An increase in glucose content in the fruits of "Elstar" and "Jonagold" cultivars during storage was also reported by Ackermann and others (1992) and Roth and others (2007). The sugar with the highest content in apple fruit was fructose (Figure 4). If only the 1st and the last sampling dates are compared, there were no significant changes in its content in the case of "Jonagold." On the other hand, "Golden Delicious" showed some increase in the fructose content after 21 d at room temperature. The increase in the content was about 18%. However, oscillations were present in the content in both cultivars during shelf life, probably owing to metabolic activity in the fruit. Similarly, Mikulic Pekovsek and others (2009) noticed an increase in the fructose content in the "Golden Delicious" cultivar, while in many other cultivars in their study this was not the case. The content of sorbitol, a sugar alcohol, decreased during shelf life; and again an increase at the end was measured. However, the difference between 1st and last sampling dates was insignificant in both cultivars (data not shown). Since sorbitol represents only a small part of the total sugar content, the oscillations in its quantity are of lesser importance.

Malic acid is the main acid in apple fruit and has an important influence on the sour taste of apples. In cultivars with low
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amounts of malic acid, the sweet taste becomes predominant. In the case of the “Jonagold” cultivar, the content of malic acid decreased between the 1st and last sampling dates; meanwhile, in “Golden Delicious” the 1st and last sampling dates exhibited similar amounts of malic acid (Figure 5). In both cases, a slight increase with a maximum on the 3rd or 4th sampling dates was noticed. In studies by Ackermann and others (1992), Roth and others (2007), and Mikulic Perkovsek and others (2009), the content of malic acids decreased during storage. Malic acid is the main substrate for respiration; therefore, its content decreases during storage, particularly when high-oxygen content is present (Roth and others 2007).

Phenolic compounds

Hydroxycinnamic acids. In this group, the content of the chlorogenic and caffeic acids was analyzed. Their content is shown in Figure 6. Approximately 85% is represented by the content of chlorogenic acid, which is a caffeic acid derivative. It is interesting that, contrary to other phenolics, the content of chlorogenic acid is high in peel as well as in pulp. This was already demonstrated in previous research (Veberic and others 2005). Regarding changes in content during shelf life, the analyzed hydroxycinnamic acids in pulp showed a peak at the 2nd sampling date, and after that a more or less constant amount till the end of shelf life was recorded. Awad and de Jager (2000) reported a constant level of chlorogenic acid in apple peel during 2 wk of shelf life although their experiment conditions differed in several points. They have examined the peel of cultivars “Elstar” and “Jonagold,” the latter also being the case of our study. On the contrary, in our experiment “Jonagold” apple, an increase in the levels of hydroxycinnamic acids in peel was measured on the 2nd sampling date similar to that of the pulp. In the skin of both cultivars, an increase in the concentration of hydroxycinnamic acids can be observed in the 2nd part of the shelf life. The increase in total phenols during storage could be a result of ethylene activity. This hormone stimulates the activity of phenylalanine ammonia lyase, the key enzyme in biosynthesis of phenolic compounds, with the consequent accumulation of phenolic constituents (Ritenour and others 1995). At ambient temperatures, ethylene production is higher, thus stimulating the biosynthetic pathway of phenolic compounds (Napolitano and others 2004).

Flavanols. In the case of flavanols, the sum of catechin and epicatechin is presented (Figure 7). Higher values were noticed in the sum of both constituents in the fruit peel compared to pulp. Values in the skin followed a similar pattern in both cultivars. After the decrease of flavanol concentration in the peel at the beginning of the shelf life, their increase was again noticed on later sampling dates. In the apple pulp, a pattern similar to the case of the hydroxycinnamic acids could be observed. The flavanols showed an increase at the 2nd or 3rd sampling, followed...
by a constant rate till the end of shelf life. In the study of MacLean and others (2006), in the apple skin of “Red Delicious” after 1 wk of shelf life no changes in the levels of flavonols were noticed. They however claim that an increase in certain group of phenolics could be due to ethylene-stimulated up-regulation of transcription factors of enzymes involved in the phenylpropanoid pathway.

Flavonols. Flavonols are presented as a sum calculated from the concentrations of quercetin rutinosid, quercetin glucoside, quercetin galactoside, and quercetin rhamnoside. Quercetins are mainly located in apple skin and found only in traces in apple pulp (Figure 8). Therefore, only the results for apple peel are presented. Similar to other phenolic groups, there is a considerable decrease in the group of flavonols at the beginning of shelf life, with a peak in its 2nd part. In the study of Awad and de Jager (2000), constant rates were reported with no evident peak after 2 wk of shelf life.

Idaein. Idaein (cyanidin galactoside) is a typical anthocyanin in apple skin. All other anthocyanins are present only in minor amounts. Idaein content was measured only in “Jonagold,” where it is responsible for the formation of the red blush covering the major part of the first quality apples. “Golden Delicious,” on the other hand, lacks the ability to accumulate anthocyanins in high amounts. During shelf life, the concentration of idaein decreased rapidly in the beginning of shelf life and stabilized on the 17th day after exposure of fruit to ambient temperatures (Figure 9). In the study by Ju and others (1996), a decrease in anthocyanin amount was also noted during 7 d of shelf life especially in the case of less-ripe fruits. The authors discuss that high content of anthocyanins is important for preventing the onset of scald during shelf life. After 1 wk of shelf life, a slight decline in the content of cyanidin galactoside was also reported by MacLean and others (2006). However, both-mentioned research studies followed anthocyanin response during 1 wk of shelf life and therefore no stabilization in the amount of idaein in apple peel has been reported contrary to data obtained in our study that was carried out for longer period.

Conclusions
In the present study with a broad tracking of the changes in several quality parameters—ranging from appearance and firmness to the content of primary and secondary metabolites—we were able to demonstrate that various parameters react uniquely to a prolonged exposure to ambient temperatures. Regarding the

Figure 8–Changes in the sum of the analyzed flavonols (quercetin rutinoside, quercetin rhamnoside, quercetin glucoside, and quercetin galactoside) in the apple peel during shelf life (numbers 1 to 7 represent sampling dates) expressed in mg kg⁻¹ FW. Average and standard errors are presented. JG = “Jonagold,” GD = “Golden Delicious.” Different letters denote statistically significant differences between sampling dates for individual cultivars at P ≤ 0.05.

Figure 9–Changes in the amount of cyanidin galactoside—idaein in the apple peel of “Jonagold” cv. during shelf life (numbers 1 to 7 represent sampling dates) expressed in mg kg⁻¹ FW. Average and standard errors are presented. Different letters denote statistically significant differences between sampling dates at P ≤ 0.05.
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primary metabolites, an interchange between individual sugars was noticed, but total levels remained similar, while the content of malic acid remained at the same level or dropped, thus making the fruit sweeter. Phenolics showed different patterns in fruit peel and in pulp. They were more constant in the peel and after regular and ultralow oxygen storage. Postharvest Biol Technol 27:53–8.


References

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References


