Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit

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Abstract

The antioxidant capacity (measured as oxygen radical absorbance capacity, ORAC), total anthocyanins, total phenolics, aroma compounds, and postharvest quality of strawberry fruit (\textit{Fragaria x ananassa} cv. Chandler) kept at 0°C, 5°C, and 10°C were investigated. Strawberry fruit stored at 10°C or 5°C showed higher antioxidant capacity, total phenolics, and anthocyanins than those stored at 0°C. However, the postharvest life based on overall quality was longer at 0°C than at 5°C or 10°C. The production of aroma compounds was markedly influenced by storage time and temperature. Individual aroma compounds were affected differently. For example, ethyl hexanoate, hexyl acetate, methyl acetate, and butyl acetate increased, while 3-hexenyl acetate and methyl hexanoate decreased during storage. In general, strawberries stored at 10°C or 5°C produced higher levels of these volatiles than those stored at 0°C. In conclusion, strawberries stored at 0°C retained an acceptable overall quality for the longest storage duration; however, berries stored at temperatures higher than 0°C showed higher content of aroma compounds and antioxidant capacity during the postharvest period.

Keywords: Storage temperature; Antioxidant; Aroma; Strawberries

1. Introduction

Fruit aroma and taste are the result of a special assortment and mixture of different metabolites. While sugars and acids contribute to sweetness and tartness, aroma is derived from combinations of volatile molecules. The different proportions of the volatile components and the presence or absence of trace components often determine aroma properties. The flavor of cultivated strawberries is mainly determined by a complex mixture of esters, aldehydes, alcohols, and sulfur compounds (Pérez, Sanz, Rios, & Olias, 1993; Zabetakis & Holden, 1997).

The postharvest life of fruit and vegetables has been traditionally defined in terms of visual appearance (freshness, color, and absence of decay or physiological disorders) and texture (firmness, juiciness, and crispness). Although this concept involves aesthetic appeal and mechanical properties associated with quality, it disregards flavor and nutritional quality. Flavor plays an important role in consumer satisfaction and influences further consumption of fruits and foods in general (Pelayo, Ebeler, & Kader, 2003). In addition to their aesthetic qualities, fruits form an important part of our diet mainly as a source of energy, vitamins, minerals, and antioxidants.

Strawberries are good sources of natural antioxidants (Wang, Cao, & Prior, 1996; Heinonen, Meyer, & Frankel, 1998; Wang & Lin, 2000). In addition to the usual nutrients, such as vitamins and minerals,
strawberries are also rich in anthocyanins, flavonoids, and phenolic acids (Heinonen et al., 1998; Rice-Evans & Miller, 1996). Strawberries have shown a remarkably high scavenging activity toward chemically generated radicals, thus making them effective in inhibiting oxidation of human low-density lipoproteins (Rice-Evans & Miller, 1996). Previous studies (Wang & Jiao, 2000; Wang & Lin, 2000) have shown that strawberries have high oxygen radical absorbance activity against peroxyl radicals (ROO·), superoxide radicals (O2·−), hydrogen peroxide (H2O2), hydroxyl radicals (OH·), and singlet oxygen (1O2); and antioxidant activities were different among varieties (Wang & Jiao, 2000). There is a positive correlation between antioxidant activity and total phenolic or anthocyanin content (Wang et al., 1996; Wang & Lin, 2000).

Fruit antioxidants protect tissues against stresses and disease. Postharvest disease resistance can also be induced by specific antifungal molecules, e.g. phenolic compounds such as phytoalexins and proanthocyanidins. Strawberry proanthocyanidins (flavan-3-ol dimers and oligomers) may act both as antifungal chemicals to extend postharvest life, and as antioxidants to enhance quality preservation (Hébert et al., 2002).

Interest in the role of antioxidants in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved through crop breeding, cultural practices, and postharvest storage and processing. Preharvest factors, such as genetic background and cultural practices, have the potential to influence antioxidant capacity in crops. Strawberry fruit from a hill plasticulture system consistently had higher flavonoid content and antioxidant capacity than fruit from plants grown using the matted row system (Wang, Zheng, & Galletta, 2002). Postharvest storage can also affect anthocyanin, phenolic compound levels and antioxidant capacity in fruits and vegetables. Controlled atmosphere (CA) storage of strawberry fruit did not affect anthocyanin content in external tissues but decreased anthocyanin content in internal tissues (Holcroft & Kader, 1999). Processing also has marked effects on phenolic content and antioxidant capacity in fruits. Strawberry processing to produce jams decreased the total ellagic acid content by 20% and the flavonoids by 15–20% (Häkkinnen, Kärenlampi, Mykkänen, & Törrönen, 2000). It has also been reported that the freezing process decreased both the total phenolic content and free radical scavenging capacity by 4–20% in four cultivars of raspberries (Ancos, Gonzalez, & Cano, 2000). As antioxidant content is becoming an increasingly important parameter with respect to fruit and vegetable quality, it is of great interest to evaluate changes in antioxidant status during postharvest storage of horticultural crops. However, little information is available regarding the effects of storage conditions, such as temperature, on the changes of anthocyanins, phenolic compounds, and antioxidant capacity in strawberry fruit. This study was undertaken to investigate the effects of different temperatures on total phenolics, total anthocyanins, and antioxidant capacity as well as the main aroma constituents and fruit quality in strawberry fruit during postharvest storage.

2. Materials and methods

2.1. Chemicals

R-Phycocerythrin (R-PE) from Porphydium cruentum was purchased from Sigma (St. Louis, MO, USA). 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA, USA). 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI, USA).

2.2. Plant materials

Strawberry fruit (Fragaria x ananassa cv. Chandler) grown at Butler’s Orchard in Germantown (MD, USA) were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. Berries were placed in trials (200 berries per trial) and stored in cold rooms at 0°C, 5°C, and 10°C. Aroma, antioxidant capacity, total anthocyanins, phenolic compounds, and quality were evaluated on days 0, 5, 7, 11, and 13 after harvest.

2.3. Overall quality

Thirty fruits per treatment were used for each quality evaluation. Samples from each treatment were evaluated subjectively on the initial day and on days 5, 7, 11, and 13 during storage. Overall quality was evaluated on a 1–5 scale according to the percentage of surface area decayed, where 1 = unacceptable (> 50% surface affected), 2 = bad (20–50% surface affected), 3 = acceptable (5 to 20% surface affected), 4 = good (up to 5% surface affected), and 5 = excellent. Results were expressed as an overall quality index.

2.4. Fungal decay index

Fungal decay was visually inspected during the course of the experiment. Strawberry fruits showing surface mycelial development were considered decayed. Fungal decay was evaluated on a 1–5 scale, where 1 = normal, 2 = trace (up to 5% surface affected), 3 = slight (5–20% surface affected), 4 = moderate
(20–50% surface affected), and $5 = $severe ($> 50\%$ surface affected). Results were expressed as overall decay index.

### 2.5. Total soluble solids (TSSs), total titratable acidity (TA), and pH determinations

Twenty fruits from each replicate were wrapped in cheesecloth and squeezed with a hand press, and the juice was analysed for TSSs, pH, and TA. TSSs were determined at 20°C on an Atago DBX-55 refractometer (Atago Co. Ltd, Tokyo, Japan). pH was measured with a pH meter. TA was determined by diluting each 5 mL aliquot of strawberry juice in 95 mL of distilled water and then titrating to pH 8.2 using 0.1 mol/L NaOH.

### 2.6. Surface color measurement

Fruit surface color was measured on 10 fruit from each replicate using a chromameter (CR 200, Minolta, Ramsey, NJ, USA), which provided CIE L*, a*, and b* values. Negative a* values indicate green and higher positive a* values red color. Higher positive b* values indicate a more yellow skin color. These values were then used to calculate hue degree ($h^o = \arctan(b^*/a^*)$), where $0^o = \text{red–purple}; 90^o = \text{yellow}; 180^o = \text{bluish green};$ and $270^o = \text{blue}$, and chroma ($C^* = [a^*^2 + b^*^2]^{1/2}$), which indicates the intensity or color saturation.

### 2.7. Total phenolic compound analysis

Total soluble phenolics in the fruit juice extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1997) using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight.

### 2.8. Analysis of total anthocyanin content

Total anthocyanin contents in fruit juice were determined using the pH differential method (Cheng & Breen, 1991). Absorbance was measured in a Shimadzu spectrophotometer (Shimadzu UV-160) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{\text{pH} 1.0} - (A_{510} - A_{700})_{\text{pH} 4.5}]$ with molar extinction coefficients of pelargonidin 3-glucoside (22400) for strawberry fruit juice. Results were expressed as milligrams of pelargonidin 3-glucoside equivalents per 100 g of fresh weight.

### 2.9. Oxygen radical absorbance capacity (ORAC) assay

The procedures for the ORAC assay on strawberries were modified from a previously described method by Cao, Sofic, and Prior (1996). This assay measures the effect of antioxidant components in fruit juices of strawberries on the decline in R-PE fluorescence induced by a peroxyl radical generator, AAPH. The reaction mixture contained 1.7 mL of 75 mM phosphate buffer (pH 7.0), 100 μL of R-PE (3.4 mg/L), 100 μL of 320 mM AAPH, and 100 μL of sample. Phosphate buffer was used as a blank, and 1 μM of Trolox (a water-soluble α-tocopherol analogue) was used as a standard during each run. The final volume of 2 mL was used in a 10-mm-wide fluorometer cuvette. R-PE, phosphate buffer, and samples were preincubated at 37°C for 15 min. The reaction was started by the addition of AAPH. Fluorescence was measured and recorded every 5 min at the emission of 570 nm and excitation of 540 nm using a Shimadzu RF-Mini 150 recording fluorometer (Columbia, MD, USA) until the fluorescence of the last reading declined to less than 5% of the first reading (approximately 70 min). One blank, one standard, and a maximum of 10 samples were analysed at the same time. Each sample was repeated three times. The ORAC value refers to the net protection area under the quenching curve of R-PE in the presence of an antioxidant. The final results (ORAC value) were calculated and expressed using Trolox equivalents (TE) per gram on a fresh weight basis (Cao et al., 1996):

\[
\text{ORAC value (μmol TE/g F.W.)} = 20K(S_{\text{sample}} - S_{\text{blank}})/(S_{\text{trolox}} - S_{\text{blank}}),
\]

where $K$ is the sample dilution factor and $S$ the area under the fluorescence decay curve of the sample, Trolox, or blank. $S$ is calculated as follows:

\[
S = (0.5 + f_5/f_0 + f_{10}/f_0 + f_{15}/f_0 + f_{20}/f_0 + f_{25}/f_0 + f_{30}/f_0 + \cdots + f_{60}/f_0 + f_{65}/f_0 + f_{70}/f_0) \times 5,
\]

where $f_i$ is the initial fluorescence at 0 min and $f_i$ the fluorescence measurement at time $i$.

### 2.10. Analysis of aroma compounds

Strawberry fruit (100 g) were placed in a hermetically closed container (500 mL) housed within a thermostated water-bath (25°C). After 10 min equilibrium time period, volatile compounds were adsorbed on an SPME fiber (65 μm, poly(dimethylsiloxane)/DBD) (Supelco, Bellefonte, PA, USA). Sampling time was 20 min. Two samples per day per treatment were obtained with this procedure. Desorption of volatile compounds trapped in the SPME fiber was carried out directly into the GC injector. Volatiles were analysed using a GC HP-6890 (Hewlett-Packard, Rockville, MD, USA) equipped with a fused silica capillary column 5-HP (5 m × 0.25 mm). Oven temperature was initially held at 40°C for 1.5 min and then a temperature ramp of 5°C/min was programmed up to 250°C. Authentic standards were used for identification of volatile compounds. Quantification
was achieved by integrating the area under the curve of each identified compound (Pérez et al., 1993).

2.11. Statistical analysis

Experiments were performed according to a completely randomized design. Analysis of variance (ANOVA) of data was performed for this experiment using NCSS (2000). The effect of temperature storage and time storage on fruit quality (decay, TSS, TA, pH, fruit color, and aroma compounds) and the values of phenolics, anthocyanins, and their antioxidant capacity were evaluated by the Fischer test. Differences between means of data were compared by least significant difference (LSD). Differences at \( p \leq 0.05 \) were considered to be significant.

3. Results and discussion

3.1. Overall quality index

Strawberry fruit has a very short postharvest life, mostly due to their relatively high water content and high metabolic activity, and the incidence of microbial molds and rots. Fig. 1 shows the overall quality index of strawberries stored at 0°C, 5°C, and 10°C. Overall quality loss increased continuously at a higher rate in strawberries stored at 10°C than in those stored at 5°C and 0°C. Storage temperature of 0°C was the most effective in maintaining the highest overall quality of strawberry fruit during the storage period. Strawberries at 5°C maintained an acceptable quality up to 7 days. Temperature is the most important factor in the postharvest life of fresh produce because of its dramatic effects on rates of biological reactions and microbial growth (Li & Kader, 1989). Water loss during storage is a major cause of fruit deterioration. Reduction in turgidity as a result of water loss causes shriveling and faster depletion of nutrients.

3.2. Fungal decay

Fungal decay increased rapidly in berries stored at 10°C especially after 7 days of storage (Fig. 2). Berries stored at 5°C showed slight fungal decay during 13 days of storage. Storage temperature of 0°C was very effective in suppressing fungal decay of strawberries. Therefore, temperature is an important factor that significantly affects the fungal decay of strawberries. Disease caused by *Botrytis cinerea* is the most serious disease of strawberry (Harvey & Pentzer, 1960). Botrytis fruit rot, also known as gray mold, is widespread in the environment. It can infect strawberry flowers and cause flowers to rot or it can become dormant. Dormant infections resume activity on the berries later in the season anytime before or after harvest when sugars increase and conditions become favorable to disease development.

3.3. TSSs, total TA, and pH

Fig. 3 shows the changes of TSSs in strawberry stored at 0°C, 5°C, and 10°C. Soluble solids content decreased in all treatments during storage. Strawberries stored at 10°C had the lowest soluble solids content after 11 days of storage. Soluble solids content in fruit stored at 0°C and 5°C also declined but to a lesser extent. Storage time and temperature treatments showed significant effects \( (p < 0.05) \) on soluble solids content of strawberries.
High depletion of TSSs in strawberries stored at 10°C could be explained by high respiratory activities of these fruit. Comparatively, lower rates of respiration in strawberries stored at 0°C and 5°C might have helped to conserve carbohydrates in tissue.

High sugar and relatively high acid content are required for good strawberry flavor (Kader, 1990). Although not all strawberries with high TSSs are high quality, the absence of high TSSs makes good quality unlikely. Galletta, Maas, Enns, Draper, and Swartz (1995) reported that TSSs in strawberries generally are in the range of 7–12% depending on genotype. Fructose and glucose were found to be the two major sugars in strawberry fruit comprising more than 65% of the TSSs (Wang & Camp, 2000).

No differences in pH among temperature treatments were found (Table 1). Different storage temperatures also did not affect the total TA.

### 3.4. Color

Nonsignificant differences were found in skin color parameters among different storage temperatures ($p > 0.05$) (Table 2). However, there were differences in skin color among the various storage times ($p < 0.05$). Wang and Camp (2000) reported that higher growth temperatures caused more rapid development of fruit color than lower growth temperatures.

Although no differences in skin color were found among different storage temperatures ($p > 0.05$), we observed differences in total anthocyanin content (Fig. 4). This could reflect an increase in internal

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**Table 1**

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Temperature</th>
<th>0°C</th>
<th>5°C</th>
<th>10°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>TA</td>
<td>pH</td>
<td>TA</td>
</tr>
<tr>
<td>0</td>
<td>3.24 ± 0.01</td>
<td>0.59 ± 0.01</td>
<td>3.24 ± 0.01</td>
<td>0.59 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>3.19 ± 0.01</td>
<td>0.68 ± 0.01</td>
<td>3.17 ± 0.02</td>
<td>0.73 ± 0.01</td>
</tr>
<tr>
<td>7</td>
<td>3.13 ± 0.02</td>
<td>0.71 ± 0.01</td>
<td>3.20 ± 0.02</td>
<td>0.74 ± 0.01</td>
</tr>
<tr>
<td>11</td>
<td>3.20 ± 0.01</td>
<td>0.69 ± 0.01</td>
<td>3.19 ± 0.01</td>
<td>0.68 ± 0.01</td>
</tr>
<tr>
<td>13</td>
<td>3.24 ± 0.01</td>
<td>0.65 ± 0.00</td>
<td>3.28 ± 0.01</td>
<td>0.69 ± 0.04</td>
</tr>
</tbody>
</table>

*Data expressed as mean ± SEM, ES, end of shelf-life, LSDpHTreat = 0.0209, LSDpHTime = 0.0220, LSDTATreat = 0.0272, LSDTATime = 0.0220, at $p < 0.05$.

**Table 2**

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Temperature</th>
<th>0°C</th>
<th>5°C</th>
<th>10°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L *</td>
<td>C *</td>
<td>H°</td>
<td>L *</td>
</tr>
<tr>
<td>0</td>
<td>33.9 ± 0.5</td>
<td>35.1 ± 0.6</td>
<td>28.5 ± 0.9</td>
<td>33.9 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>33.7 ± 0.8</td>
<td>37.6 ± 0.8</td>
<td>28.1 ± 1.3</td>
<td>33.4 ± 0.9</td>
</tr>
<tr>
<td>7</td>
<td>31.8 ± 0.6</td>
<td>34.5 ± 0.9</td>
<td>29.4 ± 1.8</td>
<td>32.6 ± 0.6</td>
</tr>
<tr>
<td>11</td>
<td>30.9 ± 0.8</td>
<td>33.6 ± 1.5</td>
<td>27.9 ± 1.2</td>
<td>32.2 ± 0.7</td>
</tr>
<tr>
<td>13</td>
<td>29.9 ± 0.9</td>
<td>32.1 ± 1.4</td>
<td>28.5 ± 1.7</td>
<td>31.6 ± 0.4</td>
</tr>
</tbody>
</table>

*Data expressed as mean ± SEM, ES, end of shelf-life, LSDL *Treat = 0.8957, LSDL *Time = 1.1839, LSDC *Treat = 1.2855, LSDC *Time = 1.6991, LSDH *Treat = 1.8406, LSDH *Time = 2.4328, at $p < 0.05$. 

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Fig. 3. Effect of different storage temperatures on soluble solids content in strawberries (cv. Chandler) stored at 0°C (●), 5°C (○), and 10°C (▲). Data points are means of three replicates and LSDs at 0.05 level for treatment and time are: LSDTreat: 0.2019; LSDTime: 0.2626.
anthocyanin content in strawberry flesh. Changes in anthocyanins in external and internal tissues might not be necessarily the same in response to different treatments. For example, high carbon dioxide decreased anthocyanins of internal tissues in strawberries while it did not affect the anthocyanins of external tissues (Holcroft & Kader, 1999). Pelayo et al. (2003) reported that distribution of these pigments in the fruit tissues of different strawberry cultivars is not uniform; the internal color of ‘Aromas’ and ‘Diamante’ strawberries is mostly white, whereas it is light red in ‘Selva’.

3.5. Total anthocyanin content and total phenolic compounds

Total anthocyanin content was significantly affected by the temperature and storage period ($p<0.05$). As observed in Fig. 4, anthocyanin content decreased in strawberry fruit stored at 0°C and 5°C during the first 5 days. Meanwhile, anthocyanin content in fruit stored at 10°C increased gradually during the storage period and reached its highest values near the end of the storage period. Anthocyanins occur almost universally, and they are largely responsible for the red color of ripe strawberries. Two anthocyanidin glycosides, pelargonidin 3-glucoside and cyanidin 3-glucoside, contribute primarily to the red color of strawberries (Timberlake & Bridle, 1982). The antioxidant capacity of anthocyanidin may be one of their most significant biological properties (Wang et al., 1996).

Fig. 5 shows the effect of storage temperatures on total phenolic compounds on strawberry fruit. Total phenolic compounds increased continuously in berries stored at 10°C and 5°C. However, strawberry fruit stored at 0°C maintained a constant value of total phenolic compounds during the storage period. Both temperature and storage time had a significant effect ($p<0.05$) on total phenolic compounds of strawberry fruits.

3.6. ORAC assay

In this study, we found that storage temperatures significantly affected the ORAC of strawberry fruit (Fig. 6). The ORAC values in strawberries changed very little during storage at 0°C. However, significant increases of ORAC values were found in strawberries stored at 5°C and 10°C. The higher the storage temperature, the greater the increase. One explanation
for this difference could be related to different total phenolic and anthocyanin contents.

Strawberry stored at 10°C resulted in significantly increased total phenolic and anthocyanin content (Figs. 4 and 5). However, even though antioxidant activity was the highest at 10°C, this elevated temperature may not be optimal for obtaining the best quality of strawberry fruit (Galletta & Bringhurst, 1990).

3.7. Aroma compounds

Strawberry aroma compounds were markedly affected by storage time and temperature (Fig. 7). Even though individual aroma compounds were affected differently by the storage temperatures, strawberry fruit stored at 10°C and 5°C generally produced higher levels of these volatiles. Ethyl hexanoate and hexyl acetate were the compounds most affected by high storage temperature. Their levels increased rapidly in the first 7 days at 10°C, then declined. Methyl acetate and butyl acetate showed a steady increase in all three temperatures during storage and the higher the storage temperature, the higher the increase. Methyl metanoate increased only in berries stored at 10°C. Ethyl butanoate showed increases at 5°C and 10°C only during the later part of storage. Methyl hexanoate decreased after 5 days.
of storage in all three temperatures. Steady decreases of 3-hexenyl acetate were also detected in all three temperatures during the entire storage period.

The typical aroma of strawberries comes from not just one or a few impact aroma compounds, but from numerous volatiles present at certain concentrations and in a particular balance among them. Thus, strawberry aroma is the result of the combined perception of many aromatic constituents (Pérez et al., 1993). Although over 360 compounds have been identified in the aroma of strawberries (Latrasse, 1991; Zabetakis & Holden, 1997), only a few volatiles (primarily methyl and ethyl esters) appear to be the most important contributors to strawberry aroma (Sanz, Olías, & Pérez, 1997; Zabetakis & Holden, 1997). Volatile esters contributing to aroma increased during storage (Forney, Kalt, McDonald, Jane, & Jordan, 1998). Our study revealed that storage temperature has a profound effect on the production of these aroma compounds. Strawberries seem to produce higher levels of most of these methyl or ethyl esters at higher storage temperatures. In addition to storage temperature, other factors such as maturity, storage atmosphere, and light have also been shown to affect the production of aroma compounds in strawberries (Forney, Kalt, & Jordan, 2000).

In summary, the data presented in this paper indicate that storage temperature significantly affect strawberry antioxidant capacity, anthocyanin, phenolic compounds, aroma compounds, and overall quality. New detailed information is presented on the effect of storage temperature on strawberry aroma and antioxidant capacity which suggest that even though overall quality was better maintained at 0°C, storage temperature at 10°C positively enhanced antioxidant capacity and the production of aroma compounds.

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References


