Ascorbic acid content in different cultivars of strawberries

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Abstract
The strawberry cultivars Arosa, Clery, Miss, Raurica, Queen Elisa, Diamante and Madeleine grown in Slovenia were harvested at the commercially maturity stage and the content of total ascorbic acid and dehydroascorbic acid (DHA) was determined. Ascorbic acid (AA) was measured by using high-performance liquid chromatography and photodiode array detector. The total AA content of cultivars ranged from 32.3 mg/100 g to 62.5 mg/100 g. No significant difference in total AA contents was observed in fresh and freeze-dried samples.

Keywords: strawberry, ascorbic acid, liquid chromatography

Introduction
Epidemiological studies have shown a positive relationship between consumption of vegetables and fruits and lower risk of some common diseases such as heart diseases and other age-related degenerative diseases (Ames et al., 1993., Hollman et al., 1996., Stacewicz-Sapuntzakis et al., 2001.). These effects are mainly associated with biologically active components such as carotenoids, phenolic compounds and vitamins C and E which are naturally present in fruits and vegetables.

The biologically active isomer of ascorbic acid (vitamin C) is L-ascorbic acid while the activity of L-dehydroascorbic (DHA) acid is still a subject of discussion (Deutsch, 2000.). It is known that vitamin C has many biological functions in reduction of plasma cholesterol level, collagen formation and enhancement of immune system. It is also an appropriate marker for monitoring quality change of vegetables and fruit during transportation, storage and processing (Favell, 1998.). The major sources of AA for the human diet are vegetables and fruits because the human body cannot synthesize it.

Strawberries are susceptible to water loss, microbial decay and to mechanical injury. Size, aroma, colour, sweetness, acidity and firmness are a major characteristics of strawberry quality for the market (Azodanlou et al., 2003.). Components which have antioxidant activity in strawberries are phenolic acids, flavonoids, AA and anthocyanins. Anthocyanins are important in colour development and their concentration increases during ripening (Cheng and Breen, 1991.).

The content of AA and other components which have antioxidant activity in strawberries can be dependent on various factors such as cultivar, preharvest climatic conditions, soil quality, sampling time, ripening degree and postharvest handling procedures. Cordenunsi et al. (2002.) reported that strawberries are among fruits one of the richest sources of AA.

The aim of this study was to compare the content of total AA in strawberry fruits among seven cultivars. The sume of the contents of AA and DHA was determined because it is used as an index of the health-related quality of fruits. The AA/DHA ratios obtained by analysing fresh homogenised samples and freeze-dried samples were compared.
Materials and methods

Samples - The fruits of seven strawberry cultivars, namely Arosa, Clery, Miss, Raurica, Queen Elisa, Diamante and Madeleine were obtained from Slovenian farms of professional growers, respectively. Fruits were harvested at commercially mature stage and sorted to eliminate damaged fruit. For each cultivar one kilogram of fruits were collected. After washing, the fruits were cut into pieces and divided in two groups. Samples of one group were immediately frozen in liquid nitrogen and lyophilised at -53 °C and 0.060 mBar (Alpha 1-2 LD, Vacumbrand GMBH, Germany). Then they were ground, vacuum packed using vacuum sealer (SmartVac) and stored at -75 °C until required for analysis. Samples of the second group were blended using an Ultra-turrax high speed blender. Approximately 1 g of the homogenised sample was weighed into a 50 mL centrifuge tube, dipped into liquid nitrogen and stored at -75 °C until analysis.

Reagents - Methanol was of HPLC-grade from Riedel-deHäen. All other applied chemicals were of analytical reagent grade and included: sodium dihydrogen phosphate dihydrate (Fluka), phosphoric acid (85%, Fluka), metaphosphoric acid (MPA) [33.5-36.5% (HPO3)n, Aldrich], dithiothreitol (Sigma-Aldrich), tris(hydroxymethyl)aminomethane (Sigma-Aldrich), L-ascorbic acid (99.5%, Sigma). Deionised water of 18 MΩ/cm resistivity purified with Milli-Q system (Millipore, Bedford, USA) was employed throughout.

Ascorbic acid analysis - AA was extracted with 3% MPA. In brief, to 1,000 ± 0.05 g of the homogenised strawberry sample or to 0,1000 ± 0,05 g of freeze dried powder 20 mL of extraction solution was added and agitated with a vortex for 1 min. Sample extract was centrifuged at 7000 rpm and 4 °C for 15 min. Total AA was measured after reduction of DHA using dithiothreitol (DTT). A 600 μL aliquot of the sample extract was mixed with 190 μL of DTT solution prepared in 0.4 mM Tris buffer to obtain a concentration in the final extract solution of 10 mM DDT and the pH of approximately 6.0. The solution was incubated at room temperature for 15 min in the dark. After a reduction period, 5 μL of concentrated H3PO4 diluted with water (1:1, v/v) was added resulting in a solution pH of 2.3. The solution was transferred to the refrigerated autosampler (4 °C). The concentration of total AA in the final solution prepared as described above was determined by high performance liquid chromatography (HPLC). The chromatographic system consisted of a Waters 600E System controller, column oven (WAT 062079), pumping system Waters 600E Pump, an autosampler Triathlon 900 (Spark) and photodiode array detector (Waters 996) set at 245 nm. Eluent reservoirs were purged with helium. Separation was performed on a reversed-phase column (Synergi 4μ Hydro-RP 80A, 150 x 4.6 mm, Phenomenex, USA) with a precolumn (Securityguard™ C18, 3 mm i.d. x 4 cm) operated under isocratic conditions at 23 °C and a flow rate of 0.5 mL min⁻¹. The mobile phase was a mixture of phosphate buffer adjusted to pH 2.3 by H3PO4 and methanol (95% : 5%). The quantification was made using the calibration graph based on concentration (μg mL⁻¹) vs. peak area (AU), prepared daily by measuring fresh standard solutions.

Results and discussion

Ascorbic acid was measured by UV detection at 245 nm. Figure 1 represents typical chromatograms of AA standard solution and AA extracted from strawberries. The retention time was 6.0 min using the separation conditions described in materials and methods. The six point calibration curve obtained by measurement of peak areas was linear in the range from 10 μg mL⁻¹ to 100 μg mL⁻¹ with the correlation coefficient (R²) > of 0.999. The effect of sample matrix on the accuracy of the analysis was evaluated by a recovery test. Known amounts of AA standard substance were added to strawberry samples prior to...
extraction at two different concentration levels. The mean recoveries ranged from 95% to 105%.
Dehydroascorbic acid has weak UV response and even though DHA can be resolved from AA, the sensitivity is insufficient to measure DHA naturally present in fruits (Gökmen et al., 2000.). Prior to HPLC measurements DHA may be reduced to AA using homocysteine, dithiothreitol (Silva, 2005.) and L-cysteine or it can be derivatised with O-phenyldiamine to form the fluorophore.
The reduction of DHA to AA in strawberry samples was catalyzed using DTT and it was optimised with regard to the reaction time and pH at the DTT concentration in final solution of 10 mM. The pH of the sample extract was adjusted to 2, 3, 4, 5, 6, 7, 8 and 9 by adding 0.4 mM Tris. The reduction efficiency was optimal in the pH range between 4 and 6. The strawberry extracts in 3% MPA were made to react with DDT solution in dark at room temperature and the reaction was completed at 10 min. The DHA content of sample was calculated by subtracting the measured AA content from that of total AA analysed after reduction of the DHA.

Figure 1. Chromatograms obtained at 245 nm for AA standard solution (full line) and for AA in strawberry extract (dashed line).

Ascorbic acid concentrations were measured in extracts of fresh homogenised strawberries and also in extracts prepared from freeze-dried strawberry samples. Results are reported of fresh fruit after adjustment for water loss caused by freeze-drying (Table 1).
The total AA content in strawberry samples ranged from 32.3 mg/100 to 62.5 mg/100 g. These values are in the range of those previously reported by other authors (Hakala et al., 2003., Skupień and Oszmiański, 2004.). The cultivars with the highest total AA content were Arosa and Madeleine with more than 61 mg/100 g and the lowest content was observed in Raurica.
Strawberry fruits of the cultivars Arosa and Raurica were harvested in the two different commercial plantations located near Maribor. They were grown under the same pre-harvest climatic conditions and also the post-harvest handling procedures were the same. The similar levels of total AA were found in two different samples of Arosa cultivar (61.8 mg/100 g and 61.3 mg/100 g). The cv. Raurica was also presented twice, and the total AA content varied between 32.3 mg/100 g and 37.1 mg/100 g, respectively. The results show that cultivar type can be an important factor affecting AA content.
Table 1. AA and DHA contents in strawberry cultivars (mg/100 g FW, mean value of two replicate analyses of each sample ± standard deviation).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fresh samples</th>
<th>Freeze-dried samples</th>
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<tbody>
<tr>
<td></td>
<td>Total AA (AA + DHA)</td>
<td>DHA</td>
</tr>
<tr>
<td>Arosa</td>
<td>61.8 ± 0.6</td>
<td>9.6 ± 0.2</td>
</tr>
<tr>
<td>Arosa</td>
<td>61.3 ± 0.3</td>
<td>17.5 ± 0.4</td>
</tr>
<tr>
<td>Miss</td>
<td>43.8 ± 0.1</td>
<td>12.4 ± 0.3</td>
</tr>
<tr>
<td>Raurica</td>
<td>37.1 ± 0.4</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>Raurica</td>
<td>32.3 ± 0.4</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>Queen Elisa</td>
<td>51.1 ± 0.5</td>
<td>7.4 ± 0.1</td>
</tr>
<tr>
<td>Diamante</td>
<td>52.1 ± 0.7</td>
<td>25.6 ± 0.4</td>
</tr>
<tr>
<td>Madeleine</td>
<td>61.4 ± 0.5</td>
<td>25.3 ± 0.4</td>
</tr>
</tbody>
</table>

No significant difference in total AA contents was observed in fresh and freeze-dried samples (Table 1), while the amounts of DHA were consistently higher in fresh samples. In freeze-dried samples DHA did not account for more than 13% of total ascorbic acid content in any of the analysed cultivars. Among the cultivars studied, Diamante and Madeleine have shown the highest content of oxidized form of ascorbic acid (DHA) measured in fresh samples. The amounts of DHA in fresh samples were between 13% of total AA content for cv. Raurica and 49% for cv. Diamante. The results indicated that AA in fresh samples was more susceptible to oxidation.

**Conclusion**

The total AA content among the cultivars varied much. The lowest AA content was determined in cv. Raurica (35 mg/100 g), while cv. Arosa contained almost twice as much (61 mg/100 g). European epidemiological studies have revealed that the recommended daily intake of vitamin C is 60 – 100 mg (National Research Council, 1989.). Strawberries were found to be a good source of vitamin C. Serving of 100 g of strawberries can contribute 35 – 62% the recommended daily intake of vitamin C.

**References**


