Influence of Cultivar on Quality Parameters and Chemical Composition of Strawberry Fruits Grown in Brazil

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Six strawberry cultivars grown on the same commercial plantation in Brazil were evaluated for their chemical composition and quality attributes at the ripe stage. The profiles of the main soluble sugars, ascorbic acid, and anthocyanins were also obtained during the developmental stages. Results showed significant differences among cultivars in all of the investigated parameters. Cv. Campineiro showed an average value for texture of 0.63 N, half the value found for cv. Oso Grande. Anthocyanin content ranged from 13 (cv. Campineiro) to 55 (cv. Mazi) mg/100 g. Total ascorbic acid found for cv. Campineiro (85 mg/100 g) was twice the amount found in cv. Dover (40 mg/100 g). Fructose was the predominant soluble sugar in almost all cultivars. The proportion among the main soluble sugars (fructose, sucrose, and glucose) was similar for Oso Grande and Toyonoka cultivars. The flavonol content (quercetin plus kaempferol derivatives) ranged from 2.7 to 7.1 mg/100 g, with a mean value of 6.1 mg/100 g, whereas free ellagic acid ranged from 0.9 to 1.9 and total phenolics varied from 159 to 289 (mean 221) mg/100 g.

KEYWORDS: Strawberries; anthocyanins; soluble sugars; antioxidants; vitamin C; flavonoids

INTRODUCTION

Strawberry is a nonclimacteric fruit that usually takes ~30 days to achieve full size and maturity. This time is highly dependent on light, temperature, soil composition, and other conditions of cultivation (1). Besides its attractive color and taste, strawberry is also a good source of carbohydrates, vitamin C, and other antioxidant compounds, such as phenolics and flavonoids (2).

Sugars are the main soluble components in ripe strawberry fruit, with glucose, fructose, and sucrose accounting for almost 99% of total sugar content. Glucose and fructose are predominant over sucrose, and the total sugar content can change during the growing period; however, the proportion of each sugar remains constant, even for different growing conditions and cultivars (3, 4). Like sugars, organic acids are important for flavor, and the sugar/acid ratio is calculated to determine the optimum time for strawberry harvesting, because it is considered an index of quality (5).

Vitamin C is one of the most important free radical scavengers in plants, animals, and humans (6). It is defined as all compounds exhibiting the biological activity of L-ascorbic acid (AA), such as L-dehydroascorbic acid (DHA), usually present in <10% of the total pool of vitamin C (7, 8). The content of vitamin C in fruits and vegetables depends on various factors such as genotypic differences, preharvest climactic conditions, and postharvest handling procedures (9).

Flavonoids are widely distributed bioactive compounds found in plant foods and can be grouped in several structural classes including anthocyanins, flavones, flavan-3-ols, flavanones, flavonols, and tannins. Besides being closely associated with the sensory attributes of fruits, flavonoids and phenolic acids have received increased attention due to their potential antioxidant activities, which may also exert cardioprotective effects in humans. Strawberries were recently reported as having the highest antioxidant activity among 12 fruits analyzed, and the contribution of vitamin C to the total antioxidant activity was estimated as being <15% (10).

Häkkinen et al. (11) reported a total flavonol content of 12–15 mg/kg of fresh weight (FW) in two strawberry varieties, Senga Sengana and Jonsok, represented by quercetin and kaempferol. For six varieties cultivated in Finland, total flavonol content ranged from 5 to 14 mg/kg of FW and ellagic acid content from 396 to 586 mg/kg of FW. Cultivation technique showed no effect on phenolic levels (12).

The amount of anthocyanins is important for the maturity evaluation of strawberries. The index of maturity used for harvesting is the red color resulting from anthocyanin synthesis corresponding to half or three-fourths of the fruit (13). The main anthocyanin found in strawberries is pelargonidin 3-glucoside, with cyanidin 3-glucoside and pelargonidin 3-rutinoside present as minor components (14).
In this study, six of the most important cultivars produced in Brazil, produced at the same time on the same commercial plantation, were compared in terms of their physicochemical characteristics. The profiles of the main soluble sugars, ascorbic acid, and anthocyanins were also obtained during fruit development of the six strawberry cultivars.

MATERIALS AND METHODS

Material. Strawberry fruit (Fragaria ananassa Duch.) of the cultivars Toyonoka, Pajaro, Mazi, Dover, Campineiro (Brazilian cultivars), and Oso Grande were harvested on the same commercial plantation located in Atibaia (São Paulo State, Brazil). Fruits were classified in five stages: small green (stage 1); medium size green (stage 2); full size green (stage 3); full size white turning red (stage 4); and full size three-fourths red (stage 5). At least 40 fruits in each stage were picked, weighed, and measured in length and diameter. Samples were made into pieces, immediately frozen in liquid nitrogen, and stored at −80 °C. At the time of analysis, samples were thoroughly homogenized by pounding in liquid nitrogen.

Anthocyanin Content. Strawberry samples (2 g of FW) were ground with 20 mL of methanol containing 1% HCl, using a Brinkmann homogenizer (Polytron; Kinematica GmbH, Kriens-Luzern, Sweden), and centrifuged at 2000g for 15 min (4 °C). Anthocyanin content was estimated as pelargonidin 3-glucoside at 510 nm, using a molar absorptivity coefficient of 39000 and expressed as milligrams per 100 g of FW.

Carbohydrate Analysis. Soluble sugars were extracted successively with three portions of boiling 80% (v/v) aqueous ethanol. The supernatants were combined, and the ethanol was evaporated under vacuum. The soluble sugar content was analyzed by HPLC-PAD (Dionex, Sunnyvale, CA), using a PA1 column (Dionex) in an isocratic mixture (100 mL the first time, 50 mL the next two times) comprising methanol/water/acetic acid (final solvent composition 70:30:5, including 1% w/v) and analyzed by reversed-phase HPLC in a Hewlett-Packard 1100 system with a diode array detector. The column used was a Prodigy 5μ ODS3 reversed-phase silica (250 mm × 4.6 mm i.d., Phenomenex Ltd.), and elution solvents were (A) water/tetrahydrodronafril/ trifluoroacetic acid 98:2:0.1 (B) acetonitrile. The solvent gradient was the same one used by Price et al. (17), except for the separation of acidic flavonols, where the initial % B was 25% in order to allow separation of elagic acid from quercetin glucuronide.

RESULTS AND DISCUSSION

Physical Characteristics. The average weight of the fruits ranged from 9.5 to 22 g, with Pajaro and Campineiro cultivars being the smallest ones and cv. Oso Grande the largest (Table 1). Considering that the number of fruits per pack can be affected by regularity of fruit weight, the coefficient of variation (CV) from the average weight was calculated. Two cultivars showed a CV of ~20% (Mazi and Pajaro), whereas cv. Oso Grande and Toyonoka showed CV values of 33%. Cv. Campineiro was the most irregular one (CV = 39%).

Fruits of the Campineiro cultivar were the softest ones, with an average value of 0.63 N, which is half the value observed for cv. Oso Grande. This result is consistent with the empirical observation that cv. Campineiro strawberries are extremely prone to mechanical damage and bruising, which limits their postharvest shelf life.

Water content of the fruits can also make a contribution to the firmness, because cell turgor can affect the mechanical resistance of the tissue. However, as shown in Table 2, the water contents of the six cultivars studied are quite similar, ranging from 89.7 to 93.1%. Even when one considers that the value of water content of cv. Campineiro fruits was one of the highest, and water can contribute to cell turgor, differences in composition of cell wall are more likely to be the reason for the contrasting behaviors in relation to firmness.

Acidity and pH. Strawberry flavor is conditioned in part by the balance between sugars and acids expressed in ripe fruits, and the organic acids are the second contributors (after sugars).
to the soluble solids of strawberries. Besides their importance in flavor, acids are important in processing because they affect the gelling properties of pectin. Citric acid contributes 92% and malic acid 9% of acidity (5). The acidity (calculated as percent w/w) of a typical ripe strawberry is ~1.01, with a minimum of 0.57 and a maximum of 2.26. For the cultivars tested, citric acid contents are all near the minimum, with a variation between 0.59 and 0.71 (Table 2). The sugar/acid ratio in all cultivars, except Mazi, was >5.3, which is the value mentioned in the literature (5), probably because of the low acid content. In the case of cv. Oso Grande, the ratio was 9.2 and for cv. Mazi it was 4.5, because of the low sugar contents. In Mazi and Campineiro cultivars there was no increase limited. In Mazi and Campineiro cultivars there was no increase of accumulation in the tissue during fruit development.

### Table 2. Chemical Composition (Mean ± SD of Triplicate Assays) of Six Strawberry Cultivars Harvested at the Ripe Stage

<table>
<thead>
<tr>
<th>constituent</th>
<th>Mazi (g/100 g)</th>
<th>Oso Grande (g/100 g)</th>
<th>Dover (g/100 g)</th>
<th>Pajaro (g/100 g)</th>
<th>Toyonoka (g/100 g)</th>
<th>Campineiro (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water (%)</td>
<td>91.2 ± 0.2</td>
<td>90.5 ± 0.3</td>
<td>93.1 ± 0.1</td>
<td>90.8 ± 1.3</td>
<td>89.7 ± 0.2</td>
<td>92.8 ± 0.2</td>
</tr>
<tr>
<td>soluble solids (%)</td>
<td>7.5</td>
<td>8.0</td>
<td>5.4</td>
<td>9.0</td>
<td>9.4</td>
<td>6.0</td>
</tr>
<tr>
<td>glucose (mg/100 g)</td>
<td>713 ± 108</td>
<td>1713 ± 23</td>
<td>1500 ± 82</td>
<td>951 ± 52</td>
<td>1495 ± 271</td>
<td>1371 ± 368</td>
</tr>
<tr>
<td>fructose (mg/100 g)</td>
<td>1327 ± 353</td>
<td>1928 ± 37</td>
<td>1852 ± 99</td>
<td>1232 ± 66</td>
<td>1591 ± 182</td>
<td>1547 ± 372</td>
</tr>
<tr>
<td>sucrose (mg/100 g)</td>
<td>661 ± 120</td>
<td>1803 ± 67</td>
<td>908 ± 30</td>
<td>807 ± 48</td>
<td>1316 ± 212</td>
<td>847 ± 298</td>
</tr>
<tr>
<td>citric acid (mg/100 g)</td>
<td>600</td>
<td>590</td>
<td>680</td>
<td>ND</td>
<td>590</td>
<td>710</td>
</tr>
<tr>
<td>total ascorbic acid (mg/100 g)</td>
<td>50.9 ± 0.4</td>
<td>63.3 ± 2.3</td>
<td>40.1 ± 5.5</td>
<td>69.3 ± 2.1</td>
<td>55.6 ± 3.8</td>
<td>85.3 ± 0.8</td>
</tr>
<tr>
<td>anthocyanin (mg/100 g)</td>
<td>54.9 ± 5.6</td>
<td>42.2 ± 8.3</td>
<td>52.2 ± 6.6</td>
<td>21.2 ± 2.0</td>
<td>19.1 ± 1.5</td>
<td>13.4 ± 2.3</td>
</tr>
<tr>
<td>total phenolics (mg/100 g)</td>
<td>174.3 ± 2.3</td>
<td>249.8 ± 0.7</td>
<td>219.7 ± 0.6</td>
<td>233.1 ± 2.1</td>
<td>198.6 ± 3.0</td>
<td>289.2 ± 8.7</td>
</tr>
</tbody>
</table>

*Values of SD for citric acid were under 2%.

### Table 3. Flavonol and Free Ellagic Acid Content (Milligrams per 100 g of FW) (Mean ± SD of Duplicate Assays) of Six Strawberry Cultivars at the Ripe Stage

<table>
<thead>
<tr>
<th>constituent</th>
<th>Mazi (mg/100 g)</th>
<th>Oso Grande (mg/100 g)</th>
<th>Dover (mg/100 g)</th>
<th>Pajaro (mg/100 g)</th>
<th>Toyonoka (mg/100 g)</th>
<th>Campineiro (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>free ellagic acid</td>
<td>1.36 ± 0.01</td>
<td>1.89 ± 0.08</td>
<td>1.01 ± 0.01</td>
<td>0.90 ± 0.04</td>
<td>0.38 ± 0.03</td>
<td>1.62 ± 0.03</td>
</tr>
<tr>
<td>neutral flavonols</td>
<td>0.39 ± 0.02</td>
<td>0.45 ± 0.02</td>
<td>0.32 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.30 ± 0.01</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>quercetin derivative</td>
<td>0.25 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.75 ± 0.02</td>
<td>0.67 ± 0.02</td>
<td>0.14 ± 0.01</td>
<td>0.56 ± 0.02</td>
</tr>
<tr>
<td>kaempferol derivative</td>
<td>5.32 ± 0.06</td>
<td>4.82 ± 0.11</td>
<td>4.14 ± 0.10</td>
<td>4.14 ± 0.14</td>
<td>1.90 ± 0.09</td>
<td>4.08 ± 0.12</td>
</tr>
<tr>
<td>acid flavonols</td>
<td>1.01 ± 0.04</td>
<td>1.56 ± 0.05</td>
<td>1.31 ± 0.04</td>
<td>1.51 ± 0.05</td>
<td>0.33 ± 0.02</td>
<td>1.57 ± 0.03</td>
</tr>
<tr>
<td>quercetin derivative</td>
<td>5.71 ± 0.27</td>
<td>5.27</td>
<td>4.66</td>
<td>4.6</td>
<td>2.20</td>
<td>4.37</td>
</tr>
<tr>
<td>kaempferol derivative</td>
<td>1.26</td>
<td>1.84</td>
<td>2.06</td>
<td>2.18</td>
<td>0.47</td>
<td>2.13</td>
</tr>
<tr>
<td>total quercetin</td>
<td>6.97</td>
<td>7.11</td>
<td>6.52</td>
<td>6.78</td>
<td>2.67</td>
<td>6.50</td>
</tr>
<tr>
<td>total flavonols</td>
<td>108 ± 1.08</td>
<td>1713 ± 23</td>
<td>231 ± 2.1</td>
<td>298 ± 3.1</td>
<td>208 ± 4.0</td>
<td>289.2 ± 8.7</td>
</tr>
</tbody>
</table>

### Figure 2

During development and ripening, the levels of AA in cv. Dover and Pajaro fruits, any interpretation of changes in AA content of these cultivars is limited. In Mazi and Campineiro cultivars there was no increase in total AA associated with ripening (change in color from white-green to red). Because cv. Campineiro and Mazi are the AA-rich and AA-poor cultivars, respectively, it appears that there is no relationship between final AA content and pattern of accumulation in the tissue during fruit development.

### Soluble Sugars

As shown in Figure 1, the total sugar content of strawberries during development changed in a similar way to that of AA, reaching maximum values at full maturity. Sucrose, fructose, and glucose were the main soluble sugars detected in all cultivars studied, and they showed similar profiles during development and ripening (Figure 2). There was a close relationship between fructose and glucose content and also between the proportion of these two sugars during development. At the full maturity stage, the proportion among sugars was very similar in all strawberry cultivars except for Dover and Pajaro fruits, which presented a significantly higher amount of fructose. In general, the proportion of sucrose in the cultivars was higher than that described by Wrostade and Shallenberger (19), who found a sucrose maximum of 17% of total sugars. The sucrose profile shown in Figure 2 for all cultivars is different from that found in cv. Brighton (4), because a decrease of sucrose content concomitantly with the color appearance was found at ripening time. The results presented here point to values of ~33% for each sugar in cv. Oso Grande and Toyonoka. The other cultivars showed at least 22% of sucrose.

Together with color and vitamin C content, sweetness is another important attribute of strawberries, and because the relative sweetness in relation to sucrose can be equal or higher for fructose and lower for glucose, it is obvious that it can be affected by changes or differences in sugar composition. However, because taste is a complex phenomenon, which is also related to acids and volatiles and is dependent on food matrix, it is not possible to establish a simple relationship between sugar content and sweetness of the six cultivars of strawberries studied based only on chemical analysis. Soluble solids (Table 2) were also different among cultivars; whereas cv. Dover has 5.4%, cv. Pajaro has almost twice the amount, 9.4%.

### Anthocyanins

Besides size and shape, color is another important component of strawberry fruit appearance, and it is defined by the anthocyanin content. Although all cultivars have started accumulating anthocyanin at the first stage, they showed...
a great increase in anthocyanin content between stages 4 and 5, associated with the ripening process (Figure 1). Cv. Mazi was an exception because it showed a dramatic increase in...
anthocyanins from stage 3 to 4 and had a higher content of anthocyanin. It was observed that the inner part of cv. Mazi fruits is strongly colored, giving them a more homogeneous appearance. In contrast, in cv. Campineiro fruits anthocyanins are predominantly distributed on the surface, resulting in fruits slightly colored red in the surface and pink to white in the inner parts.

The amount of anthocyanin determined for the six cultivars ranged from 13 to 55 mg/100 g of fresh fruit (Table 2). These values are compatible with other cultivars described in the literature such as Chandler [80 mg/100 g of FW (20)] and Red Gauntlet with [30 mg/100 g of fresh fruit (3)]. On the basis of the data presented here, groups of strawberries can be clearly defined in relation to the anthocyanin content. One group would be composed of the Mazi, Oso Grande, and Dover cultivars, which showed 2 times more anthocyanin than the group composed of cv. Pajaro, Toyonoka, and Campineiro fruits at the full ripening stage. This difference in anthocyanin composition can be reflected not only in the color of the fruits or their attractiveness but also in the benefits to the consumer’s health, as a consequence of its antioxidant activity. However, besides anthocyanins, other flavonoids, phenolic acids, and vitamins can contribute to the protective effect against oxidative damage to cells. Considering the AA and anthocyanin contents together, there might be an inverse relationship between the levels of these compounds in strawberry (Table 2). Cv. Campineiro fruits were the richest in AA but showed a poor red color, as indicated by the lower anthocyanin content. On the other hand, Mazi and Dover cultivars showed high levels of anthocyanin and the lowest levels of AA, whereas the other cultivars had intermediate contents. It would be possible that AA and anthocyanin have a complementary or superimposing action in strawberry, probably as antioxidant agents, acting to protect cells against oxidative damage. This would explain why fruits that are rich in one of these two compounds are relatively poor in the other one.

**Total Phenols.** Total phenolics content varied from 159 (cv. Toyonoka) to 289 (cv. Campineiro) mg/100 g of FW and was not correlated to anthocyanin or flavonol content (Tables 2 and 3). This finding was expected because, as previously reported, strawberries are characterized by a very high content of ellagic and p-coumaric acids, which together represent ~85% of total phenols, excluding anthocyanins (21). The variation found here in total phenolics content among cultivars, of 1.8 at maximum, was not as extensive as that found for flavonoids (4.1 for anthocyanins). According to their biological activities and influence on consumer acceptance, the content of anthocyanins would be a more significant parameter of quality than the content of total phenols.

**Flavonols.** Total flavonol content of cv. Toyonoka was much lower than those of the other five cultivars (Table 3), corresponding to only 38–41% of their content. Myricetin was not found, and queretic derivatives were present in higher amounts than kaempferol derivatives, with a practically constant ratio between queretic and kaempferol for four of the cultivars (~2–3) and a higher ratio for cv. Mazi (4.5) and for Toyonoka (4.7). Also, the main derivatives present seem to be glucoronides because a much higher content of flavonols was present in the fraction eluted from the polyamide column with MeOH/NH₃. The presence of queretic and kaempferol glucosides and glucuronides in strawberries was previously reported, although the amounts of each derivative were not determined (14). The contents of queretic (4 mg/100 g of FW) and kaempferol (1.4 mg/100 g of FW) derivatives found by these authors for the cv. Selva were similar to those reported here, except for the Toyonoka cultivar. However, these results are different from those reported by Hakkinen et al. (11), who found a similar and much lower content of quereticin and kaempferol for the varieties Senga Sengana and Jonosk. Hakkinen and Toirinen (12) found a kaempferol content ranging from 0.2 to 0.9 mg/100 g of FW and a quereticin content from 0.3 to 0.5 mg/100 g of FW for nine strawberry cultivars grown in Finland. According to these results, the cultivars analyzed here would be more promising in relation to beneficial effects on health, due to their much higher content of flavonols. However, besides varieties and environmental conditions, some discrepancies in flavonol contents could be related to maturity stage and/or to the methodology used (sample preparation, determination of aglycons after acid hydrolysis vs glycosyl derivatives, etc.). In this sense, Hakkinen and Toirinen (12) observed that the conditions used by them for sample storage (−20 °C/−9 months) could lead to some losses, kaempferol being more susceptible than quereticin. In any case, the differences found could not be explained solely on the basis of differences of methodology and are most probably inherent to the cultivars used.

**Ellagic Content.** There is an increasing interest in the amounts of ellagic acid in fruits due to the evidence of its supposed anticarcinogenicity. The main sources of ellagic acid in our diet are by far strawberries, raspberries, and blackberries (22). For the six cultivars analyzed, free ellagic acid ranged from 0.9 to 1.9 (mean = 1.3) mg/100 g of FW, similar to the values reported by Amakura et al. (23) and Gil et al. (14). However, most of the ellagic acid in berries is present as an ellagitannin esterified with glucose, demanding an acid hydrolysis step to liberate it (24). The total ellagic acid content of red pulp was previously reported as ranging from 43 to 464 mg/100 g of DW (25), which, considering a mean water content of 90%, would correspond to 4–46 mg/100 g of FW. A lower variation was found by Hakkinen et al. (12) for six strawberry cultivars analyzed, which had ellagic acid contents ranging from 39.6 to 52.2 mg/100 g of FW.

The overall results indicated that, depending on the intended use of the fruits, different cultivars could be more or less suitable. Cv. Toyonoka had the lowest values for vitamin C, anthocyanins, soluble solids, total phenolics, and total flavonols. However, it had a good texture, a high amount of total sugars, and a low amount of citric acid, counting for a good taste. Cv. Oso Grande had the best values concerning weight, texture, total flavonols, free ellagic acid, total phenolics, and total soluble sugars and had good amounts of anthocyanins and vitamin C, being the most suitable cultivar for fresh consumption in terms of nutritional value and quality parameters. Cv. Mazi had the highest amounts of anthocyanins and flavonols but had some of the lowest values of vitamin C, citric acid, and total soluble sugars.

**ACKNOWLEDGMENT**

We appreciate the technical assistance of Marcia Morais, Alberto Bernal, Lucia Justino da Silva, and Ricardo José dos Santos.

**LITERATURE CITED**

2586 J. Agric. Food Chem., Vol. 50, No. 9, 2002


Received for review October 29, 2001. Revised manuscript received January 28, 2002. Accepted January 30, 2002. This work was supported by FAPESP.

JF011421I