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Bioactive compounds and quantification of total ellagic acid in strawberries (*Fragaria x ananassa* Duch.)

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Abstract

Strawberries are one of the most popular edible fruits in Brazil and their consumption has increased with the development of new varieties available at almost all seasons. Fruit of seven full-ripened strawberry cultivars (Dover, Camp Dover, Camarosa, Sweet Charlie, Toyonoka, Oso Grande and Piedade) were characterized in relation to the total phenolics, vitamin C, flavonoids, free and total ellagic acid contents and antioxidant capacity. Camp Dover had the lowest values for anthocyanins and total phenolics but the highest total flavonoid content. Dover presented the highest anthocyanin, total phenolics and ellagic acid contents and also elevated antioxidant capacity. The best conditions for the determination of the total ellagic acid content in strawberries were also optimized and the results showed that the extraction with 80% acetone, and hydrolysis using 2 N TFA at 120 °C for 60 min allowed a 99% recovery.

Keywords: Strawberry cultivars; *Fragaria x ananassa* Duch.; Flavonoids; Ellagic acid; Antioxidant capacity

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.) is a known non-climacteric fruit of frequent human consumption (Avigdori-Avidov, 1986). Besides its attractive colour and taste, strawberry is also a good source of vitamin C, and other antioxidant compounds, such as flavonoids and ellagic acid (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). The main flavonoids present in strawberries are anthocyanins, which are responsible for the attractive colour of the fruit, besides being very important for the evaluation of fruit ripeness.

There is a particular interest in the determination of the ellagic acid content in fruits because of possible chemopreventive effects. Ellagic acid is a polyphenol found in nuts and berries, such as strawberry, raspberry and blackberry (Tomás-Barberán & Clifford, 2000). This compound can exist as free form, glycoside or linked as ellagitannins esterified with glucose (Bate-Smith, 1972; Haddock et al., 1982; Maas & Galletta, 1991), although the free form of this compound is rarely found. Strawberry represents the main source of ellagic acid derivatives in the Brazilian diet, corresponding to more than 50% of all phenolic compounds found in the fruit (Häkkinen, Kärenlampi, Mykkänen, Heinonen, & Törrönen, 2000).

The detection and quantification of ellagitannins are based on the fact that, when these compounds are exposed to acids or bases, the ester bonds are hydrolyzed and the hexahydroxydiphenic acid (HHDP) spontaneously rearranges into water-insoluble ellagic acid (Clifford & Scalbert, 2000). Tomás-Barberán and Clifford (2000) reported that although all the common analytical techniques for measuring ellagic acid have good accuracy and reproducibility, the results differ, depending on the method of extraction used and whether the extract is hydrolyzed before analysis. Another problem is that free ellagic acid is fairly insoluble in water, so it is easy to underestimate

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its content, depending on the solvent used for the extraction.

Many authors have reported the ellagic acid contents of different cultivars of strawberries but huge differences can be observed among them, ranging from 19.9 to 522 μg/g FW (Gil, Holcroft, & Kader, 1997; Häkkinen & Törrönen, 2000). This fact could be attributed, not only to the cultivar studied, but also to the method chosen for this determination.

The objectives of this study were to characterize seven strawberry cultivars grown in Brazil at the ripe stage in relation to vitamin C, flavonoids, total phenolics, free and total ellagic acid contents and antioxidant capacity. The conditions for the determination of total ellagic acid content were first optimized by testing different methods previously reported in the literature and thus choosing the best conditions of extraction and hydrolysis for strawberry fruits.

2. Materials and methods

2.1. Materials

Full-ripened strawberry fruits of the cultivars Dover, Camp Dover, Camarosa, Sweet Charlie, Toyonoka, Oso Grande and Piedade, grown in the Sao Paulo state (winter season of 2005) were harvested on the same commercial plantation, located in Atibaia (Sao Paulo State, Brazil). Two kilograms of each cultivar, grown at the same place and under the same conditions, were cut into pieces, immediately frozen in liquid nitrogen, lyophilized and stored at −18 °C until analyzes. At the time of analysis, samples were thoroughly homogenized. The moisture was measured by drying at 70 °C under vacuum, according to the AOAC (1995). All chemicals and solvents were reagent or HPLC grade. Ellagic acid, quercetin and kaempherol were purchased from Sigma Chemical Co. (St. Louis, USA). The anthocyanidins, cyanidin and pelargonidin and the respective 3-glucosides, were obtained from Extrasynthèse (Genay–France).

2.2. Flavonoid and free ellagic acid contents

Extraction was performed according to the method of Cordenunsi, Nascimento, Genovese, and Lajolo (2002), with some modifications. Samples of lyophilized strawberry powder (1 g) were extracted three times in a solvent mixture (100 ml the first time, 50 ml the next two times) comprising methanol/water/acetic acid (70:30:5) at moderate speed for 1 min (Brinkmann homogenizer, Polytron; Kinematica GmbH), while cooled in ice. The homogenate was filtered under reduced pressure through filter paper (Whatman No. 1), and the combined fractions were evaporated under vacuum at 40 °C to ~20 ml in a rotatory evaporator and made up to 50 ml with water. An aliquot of 20 ml of the extract was added to a 1 g polyamide SC6 column (Macherey-Nagel GmbH and Co., Düren, Germany) preconditioned with methanol (20 ml) and water (60 ml). The column was washed with water (20 ml) and further eluted with methanol (40 ml), to elute the neutral flavonols, and with methanol/ammonia (99.5:0.5), to elute the acidic flavonols and ellagic acid. These fractions were evaporated to dryness under pressure at 40 °C, redissolved in HPLC grade methanol (1 ml), filtered through 0.22 μm PTFE (polytetrafluoroethylene) filters (Millipore Ltd., Bedford, MA), and analyzed by HPLC.

2.3. HPLC quantitation

Identification and quantification of flavonoids and ellagic acid were achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector. The column used was 250 mm × 4.6 mm, i.d., 5 μm, Prodigy ODS3 reversed-phase silica (Phenomenex Ltd., Torrance, CA) and elution solvents were A, water:tetrahydrofuran:trifluoroacetic acid (98:2:0.1) and B, acetonitrile. Solvent gradient was the same as used by Arabbi, Genovese, and Lajolo (2004), in the proportion of 17% B for 2 min increasing to 25% B after 5 min, to 35% B after a further 8 min and to 50% B after 5 min, except for the separation of acidic flavonols, where the initial% B was 25% in order to allow separation of ellagic acid from quercetin glucuronide. Calibration was performed by injecting the standards three times at five different concentrations ($R^2 > 0.999$). Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards and the library spectra. Cochromatography was used when necessary. Samples were injected in duplicate. Results were expressed as mg aglycone/100 g sample fresh weight (FW).

2.4. Total ascorbic acid (AA)

AA was determined according to the method of Pasternak, Potters, and Caubergs (2005), with some modifications. AA was extracted with *meta*-phosphoric acid (6% w/v) and analyzed by reversed-phase HPLC in a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector. The column used was 150 mm × 3.6 mm i.d., HP®, NucleoSil 100C18 and elution (flow rate of 0.8 ml/min) was performed under isocratic conditions with 2 mM potassium chloride buffer (pH 2.5), monitored at 245 nm. Total AA was estimated after reduction of dehydroascorbic acid (DHA) with 10 mM dithiothreitol. Results were expressed as mg/100 g sample FW.

2.5. Sample extraction for total phenolics and antioxidant capacity assays

Samples of lyophilized strawberry powder (1 g) were extracted three times in a solvent mixture (100 ml the first time, 50 ml the next two times) comprising methanol/water/acetic acid (70:30:5). The homogenate was filtered under reduced pressure through filter paper (Whatman

No. 1) and it was stored at −18 °C prior to analysis. All extractions were done in duplicate, and the subsequent assays were run in triplicate.

2.6. Total phenolics

The analysis was performed according to Zieliński and Kozlowska (2000), with some modifications. A 0.25 ml aliquot was mixed with 0.25 ml of the Folin–Ciocalteu reagent and 2 ml of distilled water. After 3 min at room temperature, 0.25 ml of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture placed at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm with a spectrophotometer (Ultrospec 2000 UV/Visible, Amersham Biosciences, Cambridge, UK). Results were expressed as mg of gallic acid/100 g sample FW.

2.7. Antioxidant capacity

2.7.1. β-Carotene/linoleic acid bleaching method

The antioxidant capacity was determined by the bleaching β-carotene method according to Miller (1971), with some modifications (Hassimotto, Genovese, & Lajolo, 2005). For the preparation of the reactive solution, aliquots (30 μl) of β-carotene in chloroform (2 mg/ml) were mixed with linoleic acid (50 μl), chloroform (1 ml) and Tween 40 (510 μl). After this, chloroform was completely evaporated under nitrogen flow and 100 ml of distilled water saturated with oxygen were added to the mixture. The absorbance was adjusted with water to 0.7. For the oxidation reaction, an aliquot of the sample (100 μl) was mixed with 2400 μl of the β-carotene solution in a cuvette. The samples were submitted to auto-oxidation at 50 °C for 2 h. The absorbance at 470 nm was measured with a spectrophotometer (Ultrospec 2000 UV/Visible, Amersham Biosciences, Cambridge, UK), using a methanolic solution of BHT (butylhydroxytoluene) (50 μM) as control. Results were expressed as μmol BHT equivalents/g sample FW.

2.7.2. DPPH radical-scavenging activity

The antioxidant capacity was determined also by the DPPH· (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to Brand-Williams, Cuvelier, and Berset (1995), with some modifications (Duarte-Almeida, Santos, Genovese, & Lajolo, 2006). A 50 μl aliquot of the extract previously diluted and 250 μl of DPPH· (0.5 mM) were shaken and, after 25 min, the absorbance was measured at 517 nm using a Microplate Spectrophotometer (Benchmark Plus, Biorad, Hercules, CA), using a methanolic solution of BHT, at different concentrations, as control. The antioxidant capacity was expressed as μmol BHT equivalents/g sample FW.

2.8. Optimization of the total ellagic acid determination

The lyophilized powdered sample (1 g) was extracted three times (100 ml the first time, 50 ml the next two times) in a solvent mixture comprising methanol/water (70:30). The homogenate was filtered under reduced pressure through filter paper (Whatman No. 1), and the three fractions were combined. An aliquot of this extract was taken and hydrolyzed under the following hydrolysis conditions:

1. 2 N TFA/120 °C/90 min (Carpita, 1983).
2. 2 N HCl/95 °C/1 h (Lee & Talcott, 2004).
3. methanolic 2 N TFA/100 °C/2 h (Bushman et al., 2004).
4. 2 N HCl/100 °C/10 h (Wilson & Hagerman, 1990).
5. TFA (0.1 ml/10 ml sample)/100 °C/1 h/under reflux (Maas, Wang, & Galletta, 1991).
6. 2 N TFA/100 °C/2 h/under reflux (Daniel et al., 1989).
7. 1.2 M HCl (final concentration)/85 °C/20 h/under reflux (Häkkinen et al., 2000).

Different solvents were tested to determine the best one for the extraction of ellagic acid derivatives. Recovery was measured by adding pure ellagic acid standard to the samples and following the optimized hydrolysis conditions. All the results obtained were expressed as mg/100 g sample FW.

2.9. Total ellagic acid content

All the strawberry cultivars were hydrolyzed under the optimized conditions. An aliquot of 2 ml of the extract in 80% acetone was dried under nitrogen and, after this, 2 ml of 2 N TFA were added and the hydrolysis was performed at 120 °C for 60 min. Results were expressed as mg/100 g sample FW.

2.10. Statistical analysis

All analyses were run in triplicate and were expressed as means ± standard deviation (SD). Statistical analysis was done by using the Statistic software package version 5.0 (StatSoft Inc., Tulsa, OK). Differences between means were first analyzed by ANOVA test and then least significant difference (LSD) test ($p < 0.05$).

3. Results and discussion

3.1. Contents of bioactive compounds

Strawberries are good sources of vitamin C and polyphenolics such as flavonoids, especially anthocyanins and flavonols, and ellagic acid derivatives, to which many beneficial effects have been attributed (Hannum, 2004). Seven of the most common strawberry cultivars grown in Brazil, grown at the same place and under the same conditions, were compared in relation to the contents of bioactive compounds (Table 1).

Moisture content of full-ripened strawberry cultivars was similar for all the cultivars, around 90%. In relation to the vitamin C content, the cultivar Piedade presented the highest content (112 mg/100 g FW), almost twice those

Table 1
Moisture (%), total phenolics, and vitamin C contents (mg/100 g FW) of strawberry cultivars

Cultivar	Constituent		
	Moisture (%)	Vitamin C	Total phenolics
Piedade	88 ± 2^{a}	112 ± 5^{a}	252 ± 8^{a}
Oso Grande	90.6 ± 0.7^{a}	65 ± 6^{b}	230 ± 5^{b}
Sweet Charlie	90.2 ± 0.6^{a}	73 ± 5^{b}	209 ± 3^{c}
Camp Dover	92 ± 1^{a}	71 ± 1^{b}	205 ± 6^{c}
Dover	89 ± 1^{a}	93 ± 9^{c}	318 ± 3^{d}
Camarosa	91 ± 1^{a}	65 ± 3^{b}	262 ± 8^{a}
Toyonoka	87.9 ± 0.2^{a}	97 ± 7^{c}	212 ± 4^{c}

Values are expressed as means ± SD for triplicates. Means in the same column with common letters are not significantly different ($p < 0.05$).

found for the cultivars Oso Grande and Camarosa (65 mg/100 g FW). The average content (82 mg/100 g FW) was high enough to consider strawberry to be one of the richest sources of ascorbic acid among fruits although this content is lower than those of such known sources as orange (*Citrus sinensis*), cashew (*Anacardium occidentale*) and acerola (*Malpighia glabra*). Total phenolic content presented a lower variation among cultivars, ranging from 205 (Camp Dover) to 318 (Dover) mg/100 g FW, with a mean value of 241 mg/100 g FW.

The amount of anthocyanins is important for the maturity evaluation of strawberries. The main anthocyanin found in strawberries is pelargonidin-3-glucoside, with cyanidin-3-glucoside and pelargonidin-3-rutinoside present as minor components (Cordenunsi et al., 2005; Gil et al., 1997).

The anthocyanin content ranged from 12.4 (Camp Dover) to 44.2 (Dover) mg/100 g FW, and this difference can be considered very important, taking into account the impact of anthocyanin content on antioxidant capacity (Hassimotto et al., 2005). These compounds are the main flavonoids present in the strawberry fruit, ranging from 52% to 92% of the total flavonoid contents (Table 2).

Contents of quercetin derivatives, the main flavonols, ranged from 1.2 to 4.4 mg/100 g FW. Flavanols such as catechin and epicatechin were not present in detectable amounts in some cultivars (Sweet Charlie and Camarosa) and in others the mean value was around 5.7 mg/100 g FW. Arts, Van De Putte, and Hollman (2000) reported (in strawberries) moderate levels of catechin (4.47 and 4.91 mg/100 g FW). Among all strawberry cultivars studied in this work, three of them had already been characterized in relation to the content of bioactive compounds and significant differences could be observed (Cordenunsi et al., 2002).

Häkkinen and Törrönen (2000) reported that the total flavonol content ranged from 0.5 to 1.4 mg/100 g FW for six varieties cultivated in Finland and that the cultivation technique did not affect the phenolic levels. However, it is known that the contents of bioactive compounds in fruits and vegetables depend on various factors, such as genotypic differences, preharvest climactic conditions and post harvest handling procedures.

3.2. Antioxidant capacity

The β-carotene/linoleic acid bleaching method assesses the ability of a compound to inhibit the bleaching caused by free radicals formed during linoleic acid peroxidation (Yanishilieva & Marinova, 1995). The DPPH method is based on the reduction of DPPH radical in the presence of a hydrogen-donating antioxidant. Fig. 1 shows the results for the antioxidant capacity determined by two different methods, β-carotene bleaching and DPPH.

The results showed that there was no correlation among the antioxidant capacities obtained by the two different methods ($r = 0.29$). By using the β-carotene method, most of the strawberry cultivars showed similar antioxidant capacities, in the range of 3.6–5.0 μmol BHT equivalents/g FW. However, the antioxidant capacity determined by the DPPH method varied significantly. The cultivars Piedade, Dover, Oso Grande and Camarosa had the highest antioxidant activities, coincident with their highest total phenolic content, while Toyonoka and Sweet Charlie presented values 38% and 55% lower, respectively. Phenolic

Table 2
Flavonoid compositions and contents (mg/100 g sample FW) of strawberry cultivars

Constituent	Cultivar						
	Piedade	Oso Grande	Sweet Charlie	Camp Dover	Dover	Camarosa	Toyonoka
Anthocyanins							
Pelargonidin derivatives	34.9 ± 0.8^{a}	17.0 ± 0.2^{b}	29 ± 2^{c}	11.9 ± 0.2^{d}	22.8 ± 0.6^{e}	43 ± 2^{f}	18 ± 1^{b}
Cyanidin derivatives	0.85 ± 0.04^{a}	2.1 ± 0.2^{b}	0.31 ± 0.02^{c}	0.54 ± 0.03^{d}	1.1 ± 0.1^{e}	1.2 ± 0.1^{e}	1.3 ± 0.1^{e}
Flavonols							
Quercetin derivatives	1.21 ± 0.04^{a}	2.8 ± 0.1^{b}	2.27 ± 0.04^{c}	3.1 ± 0.1^{d}	3.3 ± 0.1^{d}	2.7 ± 0.2^{b}	4.4 ± 0.2^{e}
Kaempferol derivatives	0.60 ± 0.04^{a}	1.02 ± 0.02^{b}	0.58 ± 0.02^{a}	0.77 ± 0.02^{c}	2.3 ± 0.1^{d}	0.79 ± 0.03^{c}	1.11 ± 0.05^{b}
Flavanols							
Catechin	3.0 ± 0.4^{a}	2.8 ± 0.6^{a}	n.d.	5.7 ± 0.1^{b}	2.7 ± 0.3^{a}	n.d.	3.3 ± 0.5^{a}
Epicatechin	2.1 ± 0.2^{a}	1.4 ± 0.2^{b}	n.d.	2.0 ± 0.2^{a}	2.2 ± 0.2^{a}	n.d.	2.2 ± 0.1^{a}
Total Flavonoids	42.7 ± 0.6^{a}	27.1 ± 0.5^{b}	32 ± 1^{c}	24.0 ± 0.4^{d}	34.4 ± 0.6^{e}	48 ± 2^{f}	30 ± 2^{c}

Values are expressed as means ± SD for triplicates. Means in the same column with common letters are not significantly different ($p < 0.05$).

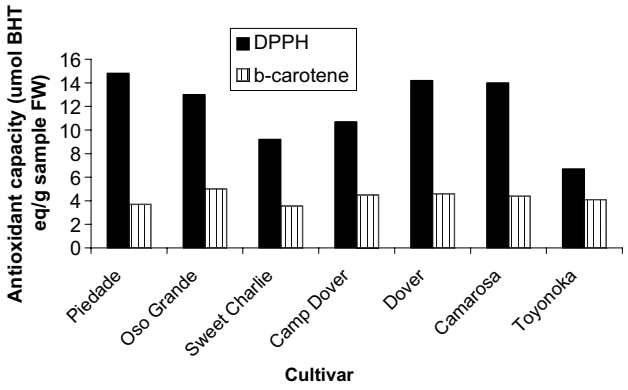

Fig. 1. Antioxidant capacity of strawberry cultivars determined by the β-carotene bleaching and DPPH methods.

content presented a higher correlation with the DPPH method ($r = 0.71$) than with the β-carotene method ($r = 0.27$) probably because of the similarity between the mechanisms of action of the DPPH and Folin–Ciocalteu methods.

When comparing absolute values obtained by the DPPH method to those obtained by the β-carotene/linoleic acid bleaching method, it can be observed that the DPPH results were higher than the β-carotene ones. The explanation is that, in the β-carotene method, vitamin C presents prooxidant capacity, leading to lower values of antioxidant capacity (Hassimotto et al., 2005). So, for samples that are known to be rich in vitamin C, this method would not give accurate results and consequently a negative correlation is observed between the results ($r = -0.47$). The DPPH method, on the other hand, measures the reduction capacity of all the compounds present in the sample, including vitamin C.

Wang, Cao, and Prior (1996) reported that, among 12 fruits analyzed, strawberries showed the highest antioxidant capacity, but the contribution of vitamin C was estimated as being only 15% on average. Beekwilder et al. (2005) developed a method to determine, on-line, the antioxidant capacity of all compounds present in raspberry samples. The authors observed that the main antioxidant compounds were anthocyanins, ellagitannins and proathocyanidins. Among these compounds, ellagitannins were the principal antioxidants found in these fruits, contributing to 30–60% of the total antioxidant capacity measured.

3.3. Determination of total ellagic acid

There is a particular interest in the amount of ellagic acid in fruits because of increasing evidence of its chemopreventive and antioxidant effects. This compound exists in plants in many derivative forms that differ in solubility, mobility and reactivity, in plant as well as in animal systems (Maas & Galletta, 1991). However, most of the ellagic acid in berries is present as an ellagitannin esterified with glucose, demanding an acid hydrolysis step to liberate it (Daniel et al., 1989), which is the basis for determination of the total ellagic acid content in fruits.

Different hydrolysis methods previously reported were tested to choose the best conditions for the determination of the total ellagic acid content in strawberries. The tests were performed with a 70% methanolic extract of the cultivar Dover as it was the one with the highest free ellagic acid content.

The results showed that there is a significant difference among all methods tested (Table 3). The hydrolysis condition proposed by Häkkinen et al. (2000), 1.2 M HCl for 20 h, resulted in a high ellagic acid content, as did the method proposed by Wilson and Hagerman (1990), using stronger acid concentration and a higher temperature for a shorter period. However, the highest content was obtained by the hydrolysis method proposed by Carpita (1983), using 2 N TFA at 120 °C for 90 min. The other methods tested resulted in lower recoveries, indicating incomplete hydrolysis of ellagitannins.

The best solvent for the extraction of ellagic acid derivatives from strawberries was also determined, among those normally cited in the literature (Table 4).

The results showed a huge difference in the total ellagic acid content according to the solvent used for the extraction. Pure methanol was the least efficient (19.3 mg/100 g FW) while the extraction with 80% acetone (48.3 mg/100 g FW) was the best.

Besides this, other parameters, such as type of acid and hydrolysis temperature, were tested and the results are presented in Table 5.

Table 3
Total ellagic acid content (mg/100 g sample FW) obtained by different hydrolysis conditions of a 70% methanolic extract of the cultivar Dover

Hydrolysis conditions	mg/100 g FW
2 N TFA/120 °C/90 min	34 ± 1^{a}
2 N HCl/95 °C/1 h	23.5 ± 0.4^{b}
Methanolic 2 N TFA/100 °C/2 h	5.5 ± 0.5^{c}
2 N HCl/100 °C/10 h	27 ± 2^{d}
TFA (0.1 ml/10 ml sample)/100 °C/1 h/under reflux	6.1 ± 0.5^{c}
2 N TFA/100 °C/2 h/under reflux	8.0 ± 0.2^{e}
1.2 M HCl/85 °C/20 h/under reflux	28 ± 2^{d}

Values are expressed as means ± SD for triplicates. Means in the same column with common letters are not significantly different ($p < 0.05$).

Table 4
Total ellagic acid content (mg/100 g sample FW) obtained for Dover cultivar after extraction using different solvents and subsequent hydrolysis with 2 N TFA (120 °C for 90 min)

Solvent	mg/100 g FW
100% methanol	19.3 ± 0.04^{a}
70% methanol	35 ± 1^{b}
80% methanol	37.0 ± 0.02^{b}
80% acetone	48.3 ± 0.1^{c}

Values are expressed as means ± SD for triplicates. Means in the same column with common letters are not significantly different ($p < 0.05$).

Table 5
Total ellagic acid content (mg/100 g sample FW) obtained by different hydrolysis conditions of an 80% acetone extract from the Dover cultivar

Hydrolysis conditions	mg/100 g FW
2 N HCl/120 °C/90 min	34.0 ± 0.1^{a}
2 N TFA/100 °C/1 h	29.4 ± 0.4^{b}
2 N TFA/100 °C/2 h	24.9 ± 0.3^{c}
2 N TFA/120 °C/90 min	49.0 ± 0.5^{d}
2 N TFA/120 °C/2 h	50 ± 2^{d}

Values are expressed as means ± SD for triplicates. Means in the same column with common letters are not significantly different ($p < 0.05$).

Table 6
Free and total ellagic acid (EA) contents (mg/100 g sample FW) of the strawberry cultivars

Cultivar	Free EA	Total EA
Piedade	0.61 ± 0.05^{a}	19 ± 1^{a}
Oso Grande	2.22 ± 0.05^{b}	28 ± 2^{b}
Sweet Charlie	0.75 ± 0.02^{c}	24.7 ± 0.5^{c}
Camp Dover	1.3 ± 0.1^{d}	32 ± 1^{b}
Dover	2.60 ± 0.05^{e}	47 ± 1^{d}
Camarosa	2.2 ± 0.1^{b}	42 ± 1^{e}
Toyonoka	1.05 ± 0.04^{f}	17 ± 2^{a}

Values are expressed as means ± SD for triplicates. Means in the same column with common letters are not significantly different ($p < 0.05$).

The results showed that hydrolysis using 2 N TFA at 120 °C for 60, 90 or 120 min did not significantly change the total ellagic acid contents obtained, which were the highest among the hydrolysis conditions tested. On the other hand, the hydrolysis conditions using 2 N HCl (120 °C for 90 min) or those at 100 °C (2 N TFA for 1 or 2 h) presented the lowest values for the ellagic acid content, indicating that these conditions lead to a partial breakdown of the ellagitannins present. According to these results, the hydrolysis condition using 2 N TFA at 120 °C for only 60 min is enough to determine the total ellagic acid content in strawberries and there is no need for longer times (up to 20 h) as previously reported in the literature (Häkkinen et al., 2000).

A recent study by Vrhovsek et al. (2006) tested the best hydrolysis conditions for the determination of the ellagic acid content in raspberry and blackberry fruits. The authors also concluded that aqueous acetone was the best solvent although the optimized hydrolysis conditions (4 M HCl for 6 h) implied a much longer time than that reported here (2 N TFA for 1 h) for strawberries.

Finally, known amounts of a standard solution containing 1 mg/ml of ellagic acid, at levels of 0.05, 0.5 and 1 mg/ml of strawberry extract, were hydrolyzed under the optimized conditions. After this, the percentage recovery was determined to verify the stability of ellagic acid under the conditions used. The results showed excellent recovery values (>99%) at the levels tested.

Most of the proposed methods found in the literature do not present the recovery percentage of a proposed condition. Häkkinen et al. (2000) calculated the recovery percentage for strawberries (80%) and strawberry jams (85%) and they considered these good recoveries since, according to Mangels, Holden, Beecher, Forman, and Lanza (1993), a recovery over 80% would be acceptable.

3.4. Ellagic acid content

Free ellagic acid levels are generally low, although substantial quantities of this compound are detected after acid hydrolysis of extracts, as a result of ellagitannin breakdown (Beattie, Crozier, & Duthie, 2005). Table 6 shows the results obtained for free and total ellagic acid contents for all strawberry cultivars.

For the seven cultivars analyzed, free ellagic acid content ranged from 0.6 to 2.6 (mean 1.6) mg/100 g FW, and these values were similar to those reported by Amakura, Okada, Tsuji, and Tonogai (2000) of 1.8 mg/100 g FW for strawberries and 0.58 mg/100 g FW for raspberries.

The total ellagic content varied significantly among cultivars, from 17 to 47 mg/100 g FW. These results are in agreement with those previously reported by Maas et al. (1991), from 43 to 464 mg/100 g DW, which, considering a mean water content of 90%, would correspond to 4–46 mg/100 g FW. Häkkinen et al. (2000), however, found a lower variation for six strawberry cultivars from Finland analyzed, whose total ellagic acid content ranged from 40 to 52 mg/100 g FW.

4. Conclusions

The results indicated that there are considerable differences in the contents of bioactive compounds among strawberry cultivars grown in Brazil. Camp Dover had the lowest values for anthocyanins and total phenolics but the highest flavanols content. Dover presented the highest anthocyanin, total phenolics and ellagic acid contents and also elevated antioxidant capacity and, as a result, this cultivar seems to be the most suitable for the selection of promising varieties in relation to beneficial effects for human health. Besides this, it was shown that, in contrast to flavonoids, whose determination is well established, the correct ellagic acid content estimation in foods relies on an adequate extraction and hydrolysis, as this compound is normally present as derivatives, and these conditions must be determined for the different food matrices.

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