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Free radical scavenging activity and phenolic content in achenes and thalamus from *Fragaria chiloensis* ssp. *chiloensis*, *F. vesca* and *F.* x *ananassa* cv. Chandler

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Abstract

The total phenolic, flavonoid and anthocyanin content of achenes (true fruit) and thalamus (receptacle) from the native South American *Fragaria chiloensis* ssp. *chiloensis* (f. patagonica and f. chiloensis), *Fragaria vesca* and *Fragaria* x *ananassa* cv. Chandler was determined by spectrophotometric means. Highest phenolic content was found in *F. vesca* while lowest content was measured for white strawberry (*F. chiloensis* ssp. *chiloensis*, f. chiloensis). The total anthocyanin and total flavonoid contents in the samples investigated was lower for the white strawberry and higher in *F.* x *ananassa* cv. Chandler. Total flavonoid content showed a better correlation than total anthocyanins with the free radical scavenging effect of the extracts measured by means of the DPPH discoloration assay. In the superoxide anion assay all the acetone extracts of strawberries showed similar activity. The data presented in this study demonstrate that the amount of phenolic compounds differ significantly between species and subspecies and determine the free radical scavenging activity of fruits. On a w/w basis, higher total phenolics including flavonoids was found in achenes. The highest total anthocyanin content was found in the achenes of *F. chiloensis* and *F. vesca*, while *F. ananassa* presented higher antocyanin content in thalamus. The main anthocyanin in thalamus of *F. ananassa* (95%) were pelargonidin derivatives which were also present in *F. chiloensis* ssp. *chiloensis f. patagonica* (62.6%) but were not detected in *F. vesca* and *F. chiloensis* ssp. *chiloensis* f. chiloensis. In *F. vesca* and *F. chiloensis* ssp. *chiloensis* f. chiloensis only cyanidin derivatives were found both in thalamus and achenes.

Keywords: *Fragaria chiloensis*; Chilean strawberry; Antioxidants; Total phenolic, flavonoid and anthocyanin content; Free radical scavenging effect

1. Introduction

Epidemiological studies have shown that high fruit and vegetable consumption has health benefits in the prevention of chronic diseases (Ness & Powles, 1997; Steinmetz & Potter, 1991). Several studies have suggested that the phytochemical content and antioxidant/free radical scavenging effect of fruits and vegetables contribute to their protective effect against chronic and degenerative diseases (Heinonen, Meyer, & Frankel, 1998; Record, Dreosti, & McInerney, 2001). Fruits and vegetables are a good source of dietary antioxidants, such as vitamin C, vitamin E and β-carotene. The contribution of vitamin C to the total antioxidant activity of 12 different fruits analyzed was estimated as being <15% (Wang, Cao, & Prior, 1996). In the same study, strawberry extracts were found to have higher antioxidant activity, as indicated by the oxygen radical absorbance capacity assay, than extracts from plum, orange, red grape, kiwifruit, pink grapefruit, white grape, banana, apple, tomato, pear, and honeydew melon.

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However, these compounds are not the only ones that contribute to the antioxidant activity of fruits and vegetables.

The antioxidant properties of strawberries have been shown to be due mainly to high content of phenolic compounds more than to vitamin C (Meyers, Watkins, Pritts, & Liu, 2003). Other studies (Heinonen et al., 1998; Vinson, Su, Zubik, & Bose, 2001) have pointed out that strawberry generally possesses a high level of antioxidant activity, which could be linked to the levels of phenolic compounds in the fruit. Wang and Jiao (2000) showed that strawberry juice exhibited a high level of antioxidant capacity against free radical species including superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen.

The polyphenolic composition and antioxidant properties of different strawberries cultivars has been the subject of many investigations. Several strawberry cultivars have been found to display significantly higher levels of antioxidant activity than others (Meyers et al., 2003; Wang, Zheng, & Galletta, 2002), and the individual flavonoid and phenolic acid compounds also differ among cultivars, as determined by high-performance liquid chromatography (HPLC) analysis (Häkkinen & Törrönen, 2000). However, little has been done on the polyphenolic composition and antioxidant properties of pulp and achenes and no comparison has been undertaken of the contribution of achenes and thalamus in the total phenolic and antioxidant activities of the Chilean strawberry, the wild European and cultivated strawberry fruits.

The main objective of this study was to compare the phenolic content in achenes and thalamus of wild Chilean strawberries (red and white fruits), *F. vesca* and *F.* x *ananassa* (cv. Chandler). The free radical scavenging effect was assessed in order to relate the phenolic content with this activity.

2. Materials and methods

2.1. Chemicals

All solvents used were of analytical grade. Methanol was obtained from J.T. Baker (Phillipsburg, NJ). HPLC-grade acetonitrile and formic acid from Merck (Darmstadt, Germany) were used. HCl, KCl, sodium acetate, Folin–Ciocalteu phenol reagent, aluminum chloride hexahydrate and sodium carbonate were from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), quercetin, nitrobluetetrazolium (NBT), xanthine oxidase, and hypoxanthine were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Plant material

Ripe fruits of *F. chiloensis* ssp. *chiloensis* (f. chiloensis) (white fruit) and *F.* x *ananassa* cv. Chandler were harvested in a commercial plantation located in Pichihuillinco, Contulmo, Province of Arauco, VIII Region, Chile at 605 meters above sea level. Voucher herbarium specimens were deposited with the number 2865 in the Herbarium of the Universidad de Talca. The ripe fruits of wild growing *F. vesca* were collected at Lago Ranco, X Región, Chile and a voucher herbarium specimen is kept at the Instituto de Biología Vegetal y Biotecnología de la Universidad de Talca with a serial number 03W111PM. The ripe fruits of *F. chiloensis* ssp. *chiloensis* (f. patagonica) (red fruit) were supplied by Instituto Nacional de Investigaciones Agropecuarias (INIA) with a serial number 3MEL7C. The strawberries were sorted to eliminate damaged, poor quality fruit and to obtain a uniform sample in size and color. After that, the samples were immediately frozen at −80 °C until extraction. The achenes were separated manually from the frozen fruits.

2.3. Total phenolic and flavonoid content

A precisely weighed amount of whole fruit, thalamus and achenes were homogenized for 5 min and extracted with 1% HCl in methanol (MeOH) (2 ml/0.01 g of achenes and 2 ml/g of pulp). The extracts were shaked and allowed to stand for 1.5 h at room temperature. The extracts were filtered through Whatman filter paper and the filtrates were taken to a final volume with distilled water. Extractions were carried out in triplicate. The extracts obtained were used to determine total phenolic and flavonoid content as well as HPLC profiles.

The total phenolic contents of achenes, thalamus and whole fruit were determined using the method described previously (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, the appropriate extract dilution was oxidized with the Folin–Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 700 nm after 30 min using a Heλios α V-3.06 UV/Vis spectrophotometer (Unicam Spectrometry, Cambridge, UK). Quantification was done on the basis of a standard curve of gallic acid. Results were expressed as mg gallic acid equivalents (GAE). The total flavonoid content in the samples was determined by the methodology of Chang, Yang, Wen, and Chern (2002). Quercetin was used as a reference for the calibration curve. The absorbance of the reaction mixture was measured at 415 nm. Results were expressed as mg quercetin equivalents (QE). Data are reported as means ± standard deviation (SD) for at least three replicates.

2.4. Total anthocyanin content

For the total anthocyanin content, acetone extracts of whole fruit, achenes and thalamus were prepared. The extracts were filtered through Whatman filter paper and the filtrates were taken to a final volume (5 ml) with distilled water. The total anthocyanin content of the acetone extract was measured using a modified pH differential method (Meyers et al., 2003). A Heλios α V-3.06 UV/Vis spectrophotometer was used to measure absorbance at 510 and 700 nm in buffers at pH 1.0 and 4.5. Absorbance

readings were converted to total mg of cyanidin 3-glucoside per 100 g fresh weight of strawberry using the molar extinction coefficient of 26,900 and absorbance of $A = [(A_{510} - A_{700})_{pH\,1.0} - (A_{510} - A_{700})_{pH\,4.5}]$. Data are reported as means ± SD for three replications.

2.5. HPLC analysis of anthocyanins

HPLC analysis of samples was performed using HPLC-DAD Merck-Hitachi (LaChrom, Tokyo, Japan) equipment consisting of a L-7100 pump, a L-7455 UV diode array detector and D-7000 chromato-integrator. A 250 × 4.6 mm i.d., 5 μm C18-RP column (Phenomenex, Torrence, CA) was used. The anthocyanins were monitored at 521 nm and the absorbance was measured between 500 and 560 nm. The solvent system was a linear gradient from 100% A (17.6% formic acid) to 50% A in 15 min, followed by 5 min of 100% B (acetonitrile) at a flow rate of 1 ml/min (Einbond, Reynertson, Luo, Basile, & Kennelly, 2004).

2.6. Superoxide anion

The enzyme xanthine oxidase (XO) is able to generate superoxide anion by oxidation of reduced products from intracellular ATP metabolism. In this reaction, the XO oxidizes the substrate hypoxanthine generating a superoxide anion which reduces the nitro blue tetrazolium dye (NBT), leading to a chromophore with absorption maxima at 560 nm. Superoxide anion scavengers reduce the generation speed of the chromophore. The activity of dried acetone extracts was measured spectrophotometrically as reported previously (Schmeda-Hirschman et al., 2003) using a Genesys-10 UV scanning spectrophotometer (Thermo Spectronic, Rochester, USA). Dried acetone extracts were evaluated at 50 μg/ml and values are presented as means ± SD of three determinations. The percentage of superoxide anion scavenging effect was calculated as follows:

$$\%\ \text{of Scavenging activity} = \frac{E - S}{E} \times 100$$

where $E = A - B$ and $S = C - (B + D)$; A: optical density of the control; B: optical density of the control blank; C: optical density of the sample; D: optical density of the sample blank.

2.7. DPPH discoloration assay

Some 20 μl of juice diluted with water (1:1) of whole fruits and thalamus and 2 μl of MeOH achenes extracts (0.04 g of achenes/ml MeOH:H_2O, 1:1) were assayed by the discoloration of a methanolic solution of DPPH as previously reported (Cheel, Theoduloz, Rodríguez, & Schmeda-Hirschmann, 2005; Galati et al., 2003) with some modifications. The scavenging of free radicals by extracts and juices was evaluated spectrophotometrically at 517 nm against the absorbance of the DPPH radical. The percentage of discoloration was calculated as follows:

$$\%\ \text{of Discoloration} = 1 - \frac{\text{Absorbance of compound/extract}}{\text{Absorbance of blank}} \times 100$$

The degree of discoloration indicates the free-radical scavenging efficiency of the substances. Quercetin was used as a free radical scavenger reference compound. Values are reported as means ± SD of three determinations.

2.8. Statistical analysis

To determine whether there was any difference between activity or phenolic, flavonoid and anthocyanin content of samples, a one-way analysis of variance (ANOVA) was applied. Values of $p < 0.05$ were considered as significantly different. The differences between means were determined using the Tukey's multiple comparison test. To assess the relationship between the activities and the phenolic content, Pearson's correlation coefficients were calculated with 95% confidence. The Statistical Package S-Plus 2000 for Windows was used to analyze the data.

3. Results and discussion

The total phenolic, flavonoid and antocyanin content of whole fruits, achenes and thalamus from two forms of the Chilean strawberry, the European *F. vesca* and the Chandler cultivar from *F.* x *ananassa* as well as the free radical scavenging effect of the corresponding extracts or juice were determined by spectrophotometric means.

3.1. Phenolic contents in whole fruits

The total phenolic contents in the whole fruits are shown in Fig. 1. *F. vesca* had the highest phenolic content, with 268.1 mg gallic acid equivalents/100 g fresh fruit, while lowest content was measured for white strawberry (*F. chiloensis* ssp. *chiloensis*, f. chiloensis) with 106.3 mg

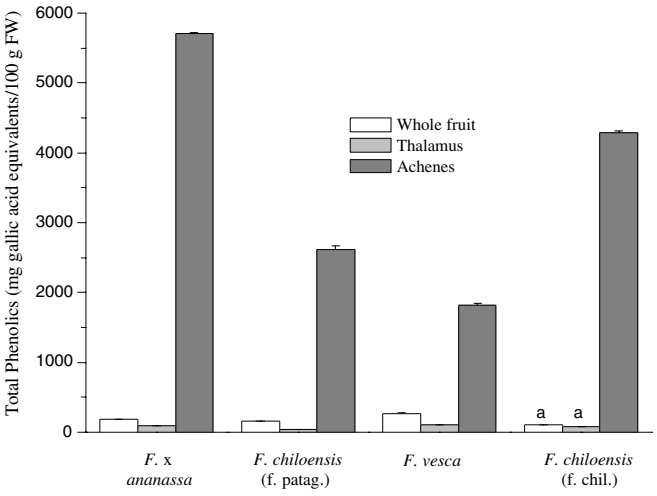

Fig. 1. Total phenolic content of strawberry whole fruit, thalamus and aquenes (means ± SD, $n = 3$). Bars showing the same letter (a) are significantly different among them ($p < 0.05$).

gallic acid equivalents/100 g fruit. In the study of Cordenunsi, Oliveira do Nascimento, Genovese, and Lajolo (2002) the total phenolic content ranged from 159 (*F.* x *ananassa* cv. Toyonoka) to 289 (*F.* x *ananassa* cv. Campineiro) mg equivalent of catechin/100 g of fresh fruit. Other investigations reported values of 330 mg gallic acid equivalent/100 g of fresh fruit for extracts of *F.* x *ananassa* American Class I Driscoll, British (Proteggente et al., 2002) and 161 to 295 mg gallic acid equivalent/100 g of fresh fruit from *F.* x *ananassa* cv. Chandler (Heinonen et al., 1998). A recent study (Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005) with six cultivars of *F.* x *ananassa* (Don, Idea, Camarosa, Wave, Sveva and Patty) reported phenolic contents between 181.4 and 212.8 mg gallic acid equivalent/100 g fresh fruit.

Total flavonoid content in our samples ranged between 30.0 mg quercetin equivalents/100 g fresh fruit for the white strawberry to 123.2 mg quercetin equivalents/100 g fresh fruit for *F.* x *ananassa* cv. Chandler (Fig. 2). Meyers et al. (2003) reported values in the range from 46.2 to 78.0 mg catechin equivalents/100 g fresh fruit for *F.* x *ananassa* cultivars. The anthocyanin content in the samples investigated (Fig. 3) was lower for the white strawberry (2.3 mg cyanidin 3-glucoside/100 g fresh fruit) and higher in *F.* x *ananassa* cv. Chandler (30.6 mg cyanidin 3-glucoside/100 g fresh fruit). According to the anthocyanin content of our samples, the strawberries investigated can be placed into high-anthocyanin and low-anthocyanin-containing groups. The first one comprises *F.* x *ananassa*, *F. chiloensis* ssp. *chiloensis* f. patagonica and *F. vesca* with 15.7–30.6 mg cyanidin 3-glucoside equivalents/100 g fresh fruit which is 7–15 times higher than the content found in the native white strawberry. For eight cultivars of *F.* x *ananassa* analyzed by Meyers et al. (2003), the anthocyanin content ranged from 22.0 to 48.0 mg cyanidin 3-glucoside equivalents/100 g fresh fruit. Similar variation was found

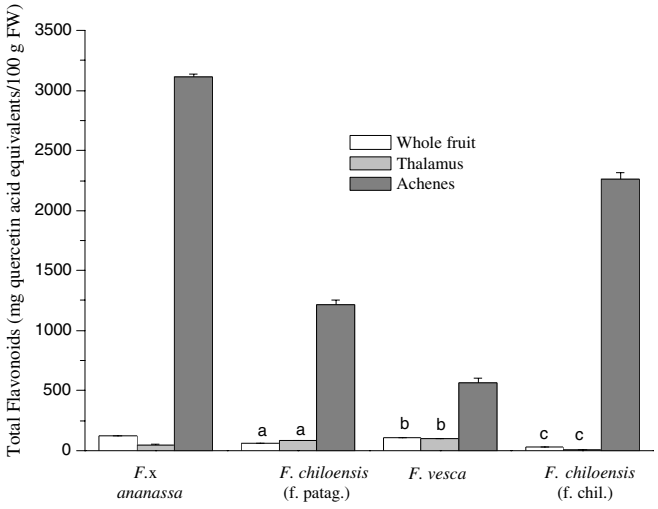

Fig. 2. Total flavonoid content of strawberry whole fruit, thalamus and aquenes (means ± SD, $n = 3$). Bars showing the same letter (a, b, c) are not significantly different among them ($p < 0.05$).

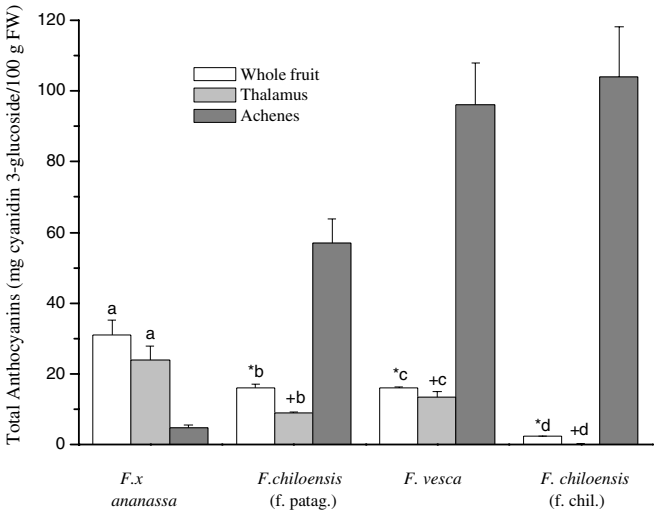

Fig. 3. Total anthocyanin content of strawberry whole fruit, thalamus and aquenes (means ± SD, $n = 3$). Bars showing the same letter (a, b, c, d) and symbol (∗, +) are not significantly different among them ($p < 0.05$).

by Cordenunsi et al. (2002) for six strawberry cultivars with anthocyanin contents from 13.0 to 55.0 mg pelargonidin 3-glucoside equivalents/100 g fresh fruit. Woodward (1972) reported 30.0 mg cyanidin 3-glucoside/100 g fresh fruit for *F.* x *ananassa* cv. Red Gauntlet while a higher value (80.0 mg cyanidin 3-glucoside/100 g fresh fruit) was reported by Montero, Mollá, Esteban, and López-Andréu (1996). In our study with the whole fruit, a positive correlation was observed ($r = 0.797$, $p < 0.05$) between total phenolic content and total flavonoid content. However, there was no significant correlation between total phenolic and total anthocyanin content. This last finding was similar to those reported by Cordenunsi et al. (2002).

3.2. Phenolic contents in achenes and thalamus

A comparison of quantitative distribution of phenolics in achenes and thalamus of native strawberries has not been previously reported. The phenolic content and antioxidant activity of thalamus and achenes of ripe *F.* x *ananassa* has been recently published (Aaby, Skrede, & Wrolstad, 2005). In the present study, *F. vesca* thalamus showed the highest value of total phenolic content with 99.8 mg gallic acid equivalent/100 g of fresh weight (FW) and the native red strawberry the lowest value with 37.5 mg gallic acid equivalent/100 g FW (Fig. 1). Strawberry achenes contained high amounts of total phenolics, averaged 3.6 g GAE/100 g FW which is in agreement with those reported by Aaby et al. (2005), who showed an average value of 3.6 g GAE/100 g FW for *F.* x *ananassa* achenes manually separated from freeze-dried berries.

With respect to the total flavonoid content in thalamus, *F. vesca* showed the highest value (99.7 mg quercetin equivalent/100 g FW) with lowest value (7.8 mg quercetin equivalent/100 g FW) for the native white strawberry (Fig. 2). The total flavonoid content in achenes were in the range

of 564.2–3113.9 mg quercetin equivalent/100 g FW. The total anthocyanin content of thalamus ranged from 0.2 to 23.6 mg cyanidin 3-glucoside equivalents/100 g FW (Fig. 3). As expected, the native white strawberry thalamus showed the lowest value. The total anthocyanin content in achenes were in the range of 4.8–104.3 mg cyanidin 3-glucoside equivalents/100 g FW. The native white strawberry achenes showed the highest value. The total anthocyanin contents in thalamus of two cultivars of *F.* x *ananassa* (32.0–60.0 mg pelargonidin 3-glucoside equivalents/100 g FW) was previously reported (Aaby et al., 2005). In the same report the total anthocyanin contents of achenes separated from freeze-dried strawberries was about 59.0 mg pelargonidin 3-glucoside equivalents/100 g FW, more than four times the content of achenes separated from mashed strawberry. According to the author it was possible that achenes separated from freeze-dried berries contained thalamus remnants.

Our results on phenolic contents (based on 100 g FW basis) show that phenolics are higher in achenes compared to thalamus. The achene fraction constitutes a low proportion of the whole fruit. In order to determine the real contribution of the achenes to the total phenolic content of strawberries, the phenolic content of each fruit part was calculated on the basis of their actual weights in whole fruit. Percent contributions of each fraction are given in Table 1. The strawberry achenes on average contributed 50% to total phenolics, 43% to total flavonoids, and 37% to the total anthocyanin content from the whole fruit. The lowest contribution (24%) was observed for *F.* x *ananassa*. These results show that the achenes should be taken into account as important phenolic contributors for strawberries. Aaby et al. (2005) reported that *F.* x *ananassa* fruits contain 1% achenes on a fresh weight basis. However, they contributed to about 11% of the total phenolic mainly as ellagic acid and its derivatives. The catechin and flavonols content of achenes, except the flavonol content in achenes from freeze-dried berries, were about 4-fold higher compared to thalamus. According to Maas, Wang, and Galletta (1991) the highest level of total ellagic acid was found in strawberry leaves followed by achenes and finally flesh. Williner, Pirovani, and Güemes (2003) reported that in strawberry pulp with achenes the total ellagic acid content for red fruit (cv. Camarosa) was about 6-fold higher than that of pulp without achenes.

According to Aaby et al. (2005), when strawberry fruits are processed to give juice and puree, substantial waste material that contains high levels of achenes is generated. This processed waste could be a potential source of nutraceuticals instead of being fed to livestock or sent to sanitary landfill. A similar study was carried out by Toor and Savage (2005) who showed that the skin and seeds of the three tomato cultivars on average contributed 53% to the total phenolics, 52% to the total flavonoids, 48% to the total lycopene, 43% to the total ascorbic acid and 52% to the total antioxidant activity present in tomatoes. These results show that removal of achenes of strawberries during home cooking and processing would result in a significant loss of important phenolics.

3.3. Free radical scavenging activity

F. x *ananassa* cv. Chandler whole fruits showed the highest free radical scavenging activity in the DPPH assay (86%) (Fig. 4). The lowest activity was found in native red strawberry (42%), but without statistically significant difference with the native white strawberry ($p < 0.05$). In the superoxide anion assay all the acetone extracts of strawberries showed similar activity (averaging 55%) at 50 μg/ml (results not shown). Using the same assay Wang and Jiao (2000) reported values in the range from 57% to 69% for juices of six *F.* x *ananassa* cultivars at 50 μl of juice/ml.

Several investigations have reported significant differences in antioxidant activity among strawberry cultivars (Meyers et al., 2003; Wang et al., 2002). However, little has been done comparing the free radical scavenging activity between native and cultivated strawberries. Scalzo et al. (2005) indicated that *F. vesca* fruits were 2.5 times more active than cultivated strawberries in the TEAC assay (Trolox equivalent antioxidant capacity). A similar finding was reported by Halvorsen et al. (2002) on FRAP assay (ferric ion reducing antioxidant power). Even though

Table 1
Contribution of thalamus and achenes to the phenolic content in strawberries

Species	Fruit part	Part % in fresh fruit	% Contribution[a]		
			Total phenolics	Total flavonoids	Total anthocyanins
F. x *ananassa* cv. Chandler	Thalamus	99.5	75.9	74.7	9.9
	Achenes	0.5	24.1	25.3	0.1
F. chiloensis ssp. *chiloensis* f. patagonica	Thalamus	96.7	29.4	66.5	81.6
	Achenes	3.3	70.6	33.5	18.4
F. vesca	Thalamus	90.3	33.8	62.2	56.2
	Achenes	9.7	66.2	37.8	43.8
F. chiloensis ssp. *chiloensis* f. chiloensis	Thalamus	98.9	59.4	22.8	15.0
	Achenes	1.1	40.6	77.2	85.0

[a] Each value corresponds to the percentual contribution of each fraction from the mean of phenolic content ($n = 3$).

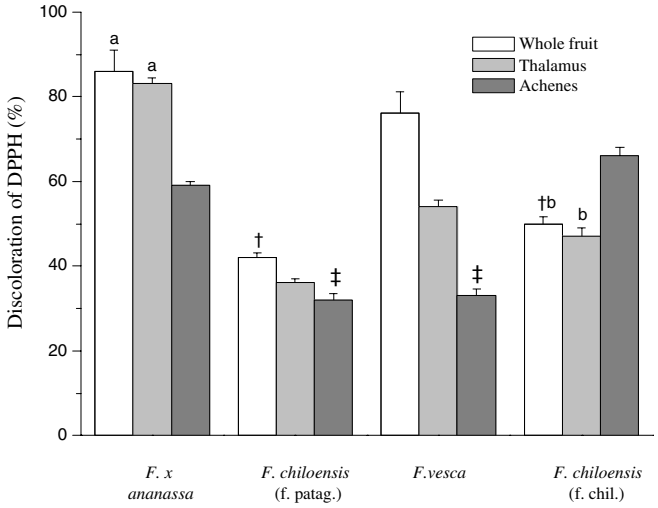

Fig. 4. Free radical scavenging activity (DPPH) of strawberry whole fruit, thalamus and achenes (means ± SD, $n = 3$). Bars showing the same letter (a, b) and symbol (†, ‡) are not significantly different among them ($p < 0.05$).

TEAC, FRAP and DPPH assays are methods based on electron transfer reaction (Huang, Ou, & Prior, 2005) it is difficult to compare the results from different assays as Frankel and Meyer (2000) have already concluded. These assays differ from each other in terms of substrates, probes, reaction conditions and quantitation methods.

In our study with whole fruit a high and positive correlation between flavonoid content and DPPH activity ($r = 0.879$, $p < 0.05$) was observed (Fig. 5). However, weaker correlations were observed between total anthocyanin content and DPPH activity ($r = 0.727$, $p < 0.05$) followed by the correlation between total phenolic content and DPPH activity ($r = 0.657$, $p < 0.05$). The two last associations were similar to those reported by Meyers et al. (2003) using another antioxidant assay. According to Häkkinen et al. (1999) the flavonoids in strawberries represent 11% of all phenolic compounds with quercetin as major flavonoid, kaempferol and myricetin as minor flavonoids. The ellagic- and *p*-coumaric acid comprises 51% and 34%, respectively, of total phenolics except for anthocyanins.

In our study, the achenes of the native white strawberry showed the highest free radical scavenging activity with lowest value for the native red strawberry, the latter being statistically similar to *F. vesca* ($p < 0.05$) (Fig. 4). In thalamus the activity was similar to those observed in whole fruits which obviously suggests a direct correlation. *F.* x *ananassa* thalamus showed the highest DPPH activity with lowest activity in native red strawberry. Taking into account that the highest anthocyanin content was found in the native white strawberry achenes, the high DPPH activity can be related to the anthocyanins content. According to Aaby et al. (2005) the phenolic content based on fresh weight is higher in achenes than in thalamus as well as the antioxidant activity. In the same study, the achenes contributed about 14% of antioxidant activities in strawberries in spite of its about 1% content on a fresh fruit basis. The authors showed that the main contribution was from ellagic acid and its derivatives.

In our study with strawberry achenes, total phenolic content and total flavonoid content are correlated to the DPPH activity ($r = 0.858$, $p < 0.05$ and $r = 0.856$, $p < 0.05$, respectively) but not with the DPPH activity of whole fruits. There was no relationship between total anthocyanin content and DPPH activity. In thalamus high positive correlations were observed between total phenolic and anthocyanin content and DPPH activity ($r = 0.737$, $p < 0.05$ and $r = 0.792$, $p < 0.05$, respectively). The total phenolic and the total anthocyanin content in thalamus correlated to DPPH activities of whole fruits ($r = 0.880$ and $r = 0.826$, $p < 0.05$, respectively).

The high and positive correlation observed between DPPH activity in thalamus and the DPPH activity in whole fruits ($r = 0.889$, $p < 0.05$) (Fig. 6) suggests that 79% of the DPPH activity of the studied strawberries results from the

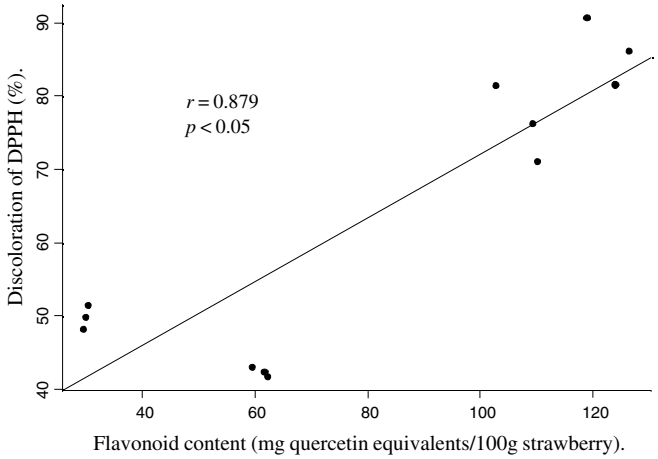

Fig. 5. Correlation between total flavonoid content and DPPH activity in strawberries.

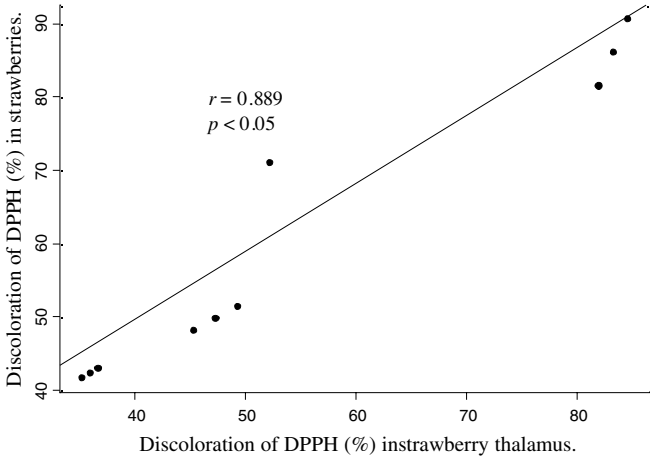

Fig. 6. Correlation between DPPH activity of strawberries and DPPH activity of strawberries thalamus.

contribution of thalamus. According to Aaby et al. (2005) thalamus contributed to about 86% of the antioxidant activity in strawberries.

3.4. Anthocyanin composition

The main anthocyanin found in strawberries is pelargonidin 3-glucoside, with cyanidin 3-glucoside and pelargonidin 3-rutinoside present as minor components (Gil, Holcroft, & Kader, 1997). The relative composition of anthocyanins in strawberries has been reported by several researchers (Bakker, Bridle, & Bellworthy, 1994; Hong & Wrolstad, 1990; Wang et al., 2002). However, the differential anthocyanin composition of achenes and thalamus was recently reported for a cultivated strawberry (Aaby et al., 2005). In our study anthocyanins in strawberries achenes and thalamus were tentatively identified by HPLC and comparison of the corresponding UV/Vis spectra. Peaks showing visible λmax at 521 were initially identified as cyanidin derivatives while peaks showing visible λmax at 503 were preliminarily assigned to pelargonidin derivatives (Hong & Wrolstad, 1990). The relative composition (% area) of anthocyanins in thalamus and achenes was also determined (Table 2). The qualitative anthocyanin profile of achenes from *F. chiloensis* ssp. *chiloensis* (f. chiloensis), *F. chiloensis* ssp. *chiloensis* (f. patagonica) and *F.* x *ananassa* were different from that of thalamus. No difference was observed in the anthocyanin profile of thalamus and achenes from *F. vesca*. A preponderance of cyanidin derivatives was observed in all strawberry achenes. According to Aaby et al. (2005) about 56% of the relative anthocyanin composition of achenes corresponded to cyanidin derivatives while the remaining to pelargonidin derivatives. The same authors reported that in thalamus about 99% of the anthocyanins are pelargonidin derivatives and the remaining 1% cyanidin derivatives. In our study of the four strawberries, only *F. vesca* contained cyanidin derivatives in both thalamus and achenes. On the other hand, thalamus and achenes of *F. chiloensis* ssp. *chiloensis* (f. chiloensis) and *F. chiloensis* ssp. *chiloensis* (f. patagonica) showed small peaks with visible λmax at 531 nm which were tentatively identified as petunidin or malvidin derivatives (Hong & Wrolstad, 1990). The HPLC analysis of the hydrolysis products showed two common peaks with λmax at 525 and 532 nm which are compatible with the anthocyanidins pelargonidin and cyanidin. The visible wavelength maxima at 542 nm in thalamus and achenes of *F. chiloensis* ssp. *chiloensis* (f. chiloensis) and *F. chiloensis* ssp. *chiloensis* (f. patagonica), respectively, confirmed the occurrence of petunidin or malvidin (Harborne, 1973).

A positive correlation between the total phenolic content and the antioxidant activity in strawberries has been found using the Folin–Ciocalteau method for the determination of total phenolic compounds, which, in spite of being widely accepted, is not very specific (Santos-Buelga & Scalbert, 2000). Furthermore, in the fruits, variable quantities of diverse antioxidant vitamins can be found. The fact that the antioxidant activity did not show a correlation with the content of phenolics in the samples assayed suggest the synergic or antagonistic effect of still unidentified compounds (Garcia-Alonso, de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004). Kähkönen et al. (1999) found no significant correlations between the total phenolic content and antioxidant activity of the plant extracts. Different phenolic compounds have diverse responses in the Folin–Ciocalteu method.

Different phenolic contents were observed in the strawberry samples investigated. To our knowledge, the phenolic profiles of wild strawberries have not been previously reported. Several strawberry cultivars have been shown to contain different phenolic content profiles or relative proportions of compounds within the profile (Kosar, Kafkas, Paydas, & Can Baser, 2004), differences in these profiles may subsequently result in complex changes in antioxidant activity or other bioactivities (Meyers et al., 2003). Bakker et al. (1994) found 13 different anthocyanins in a total of 39 strawberry cultivars. Only 14 cultivars contained 10 or more of the anthocyanins identified, and there was variation in the relative ratio measured. As noted in our study the DPPH activity of strawberries could be associated with the anthocyanin content. However, anthocyanins were not relevant as superoxide anion scavengers. In the superoxide anion assay all the acetone extracts of the studied strawberries showed similar activity.

Table 2
Anthocyanin composition (area % HPLC) in thalamus and achenes of strawberries

Species	Fruit part	Anthocyanin composition[a]		
		Pelargonidin derivatives	Cyanidin derivatives	Petunidin or malvidin derivatives
F. x *ananassa* cv. Chandler	Thalamus	95.0 ± 0.3	5.0 ± 0.1	ND
	Achenes	ND	100.0 ± 0.3	ND
F. chiloensis ssp. *chiloensis* f. patagonica	Thalamus	62.6 ± 0.4	37.4 ± 0.1	ND
	Achenes	ND	82.2 ± 0.4	17.8 ± 0.1
F. vesca	Thalamus	ND	100.0 ± 0.5	ND
	Achenes	ND	100.0 ± 0.2	ND
F. chiloensis ssp. *chiloensis* f. chiloensis	Thalamus	ND	53.5 ± 0.2	46.5 ± 0.3
	Achenes	ND	100.0 ± 0.3	ND

[a] Each value represents mean ± SD of three determinations. ND, not determined.

Fruit phenolics may exhibit antagonistic as well as additive/synergistic activities and interact with other phenolics and phytochemicals in a particular fruit (Heinonen et al., 1998), this balance of synergism and antagonism may be altered dependent upon the relative proportions and existence of particular phenolic compounds.

Antioxidant activities of fruits are well-known and reported in the literature. The data presented in this study demonstrates that the amount of phenolic compounds differ significantly between species and subspecies and determine the free radical scavenging activity of fruits. Because many consumers are now concerned with the health aspects of their food products, this knowledge could be of interest for consumers to estimate the value of each cultivar or species.

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