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Total antioxidant capacity and total phenolic and anthocyanin contents in fruit species
grown in Northwest Italy

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Abstract

17 fruit species grown in Piedmont (Northwest Italy) were analysed for SSC, pH, TA, total antioxidant capacity, and total phenolic and anthocyanin contents to evaluate their nutraceutical value and emphasize the value of the local productions. Major fruit species (pear, apple, apricot) were generally higher in SSC and had moderate acidity but were tendentially lower in phenolics (39.89-93.71 mg 100 g⁻¹ GAE) and antioxidant capacity (8.07-11.11 mmol Fe²⁺/kg fw) in comparison with berry fruits. Black mulberry, blackberry, highbush blueberry, currant, raspberry, gooseberry and strawberry confirmed to be excellent antioxidant sources (phenolics 196.98-398.67 mg 100 g⁻¹ GAE; FRAP: 11.51-85.97 mmol Fe²⁺/kg fw) while Damask plums showed intermediate properties (phenolics: 175.30-229.62 mg 100 g⁻¹ GAE; FRAP: 12.12-17.88 mmol Fe²⁺/kg fw). The highest anthocyanin contents were recorded in
black mulberry (341.53 mg 100 g$^{-1}$ fw), black currant (224.79 mg 100 g$^{-1}$ fw) and highbush blueberry (222.74 mg 100 g$^{-1}$ fw). Differences between cultivars of the same species indicated the presence of variability that should be considered in breeding and in the orchards planning.

**Keywords:** berry; major species; FRAP; soluble solid content; titratable acidity

### 1. Introduction

In recent years, the increasing interest for human health, nutrition and the prevention from illness has driven the demand of the consumer to foods and quality raw material with high nutraceutical value.

The attention to a correct diet and to balanced nutrition has encouraged new studies on foods bearing physiological and physical benefits. In particular, clinical tests and chemical analyses have underlined the role of fruits and vegetables in the prevention of pathologies caused by oxidative stress, showing a positive correlation between the daily assumption of fruit and vegetable and the decrease of disease risk (Herrera et al., 2009; Kaur and Kapoor, 2001).

The oxidative stress is implicated in a number of diseases including cardiovascular dysfunction, various typologies of cancer, rheumatism, diabetes, rheumatoid arthritis, pulmonary emphysema, dermatitis, cataract, neurodegenerative diseases, endothelial cell dysfunction (Benzie, 2003) and several autoimmune diseases linked to the degenerative process of ageing (Shukitt-Hale et al., 2008).

The human organism naturally defends itself from the free radicals producing endogenous antioxidants (Prior et al., 1998); yet, this is not sufficient to meet the needs of the body and the intake of antioxidants from external sources is strongly recommended (Jarrett, 2008).
important phytonutrients with strong antioxidant capacities are fibre (Kaur et al., 2001), phenolics (Shukitt-Hale et al., 2008), vitamins A, B, C, E, terpenoids (Espin et al., 2007) and essential minerals such as selenium, copper and zinc. It has been already demonstrated that most of these compounds are found in fruits, in particular berries, and vegetables but their amount widely varies among species and also among cultivars within the same species (Pantelidis et al., 2007; Gil et al., 2002; Bounous et al., 2009).

Recently, research was done to evaluate the antioxidant capacity of fruits and vegetables produced in different countries including Italy (Pellegrini et al., 2003), Norway (Carlsen et al., 2010), United States (Wu et al., 2004), Czech Republic (Stratil et al., 2006), France (Brat et al., 2006) and Belgium (Kevers et al., 2007) highlighting a geographical effect on food composition, but often not considering the different amounts of biochemical compounds present in cultivars of the same species.

The objective of this research was to screen the antioxidant activity, total phenolic content, anthocyanin content, solid soluble content, pH and titratable acidity in 17 fruit species grown in Northwest Italy (Piedmont Region) to provide information useful for planning effective antioxidant dietary consumption using local products. For each species the most important cultivars, selections or landraces, typically grown in the Piedmont area, were considered to evaluate their nutraceutical value and valorising the local productions.

2. Materials and methods

2.1. Fruit samples

Fruit samples of the following 17 species were collected in the Piedmont Region (Table 1): apple (*Malus x domestica* Borkh.), apricot (*Prunus armeniaca* L.), blackberry (*Rubus*
ulmifolius L.), highbush blueberry (Vaccinium corymbosum L.), black currant (Ribes nigrum L.), white currant (Ribes rubrum L.), gooseberry (Ribes grossularia L.), kiwifruit (Actinidia deliciosa Chev.), black mulberry (Morus nigra L.), white mulberry (Morus alba L.), nectarine [Prunus persica (L.) Batsch], pear (Pirus communis L.), European plum (Prunus domestica L.), Damask plum (Prunus insititia L.), Chino-Japanese plum (Prunus salicina Lindl.), raspberry (Rubus idaeus L.) and strawberry (Fragaria x ananassa Duch.). All fruits but mulberries were sampled from commercial orchards in typical areas of cultivation; mulberries were collected from single grafted plants used in the past for silkworm raising.

For apple, apricot, blueberry, nectarine, pear, plums and raspberry, the three most common cultivars or landraces (Damask plum) were selected for the study; fewer cultivars or selections (mulberry) were sampled for blackberry, mulberry, white currant and gooseberry due to the lower availability of varieties grown in the Region. Only the ‘Hayward’ cultivar, representing almost 100% of production in the Region, was sampled for Actinidia deliciosa Chev..

Sampled cultivars and landraces with their fruit skin colour are listed in Table 2.

10 kg of fruit for each genotype were sampled at ripeness from 3 plants, except for small fruits: in this case 500 g of fruit were collected from each plant (or for linear meter on the row in the case of raspberry and blackberry). Samples were maintained fresh in an icebox during the transport to the laboratory and subsequently stored in a refrigerated room at 4° C; they were extracted and analyzed within 24 hours.

Three replicates of fruits for each genotype were used to determine soluble solids content, pH and titratable acidity; fruit extracts were used for the analysis of the total phenolic content, the total anthocyanin content and the total antioxidant capacity.

2.2. Soluble solids content (SSC), pH and titratable acidity (TA)
50 mL of juice obtained by a juice extractor were centrifuged (centrifuge ALC PK 110) at 6,500 rpm for 10 minutes at 20° C, to get a juice completely devoid of solid residues. The SSC was determined on the juice by a digital refractometer XS DBR 35 and expressed in °Brix. The pH and titratable acidity (10 mL of juice and 90 mL of deionized water) were determined using a pH meter and a semi-automatic titrator CRISON. Titratable acidity has been expressed in meq/L.

2.3. Extraction protocol

Apples, pears and kiwifruits were peeled before extraction of the pulp while apricots, plums and nectarines were extracted with skin, following the common method of eating in Italy. The extracts from berry species were obtained using the whole fruit.

Fruit extract was obtained using 10 g of fruit added to 25 ml of extraction buffer (500 ml methanol, 23.8 ml deionized water and 1.4 ml hydrochloric acid 37%). After 1 hour in the dark at room temperature, the samples were thoroughly homogenized for a few minutes with an ultraturrax (IKA, Staufen, Germany) and centrifuged for 15 minutes at 3,000 rpm.

The supernatant obtained by centrifugation was collected and transferred into glass test tubes and stored at -28° C until analysis.

2.4. Total phenolic content (TPC)

The amount of total phenolics in fruits extracts was measured following the Folin-Ciocalteu procedure, according to the method of Slinkard and Singleton (1977), using gallic acid as standard. The methanolic extract (0.5 g) was mixed with 30 mL of deionized water and 2.5 mL of Folin-Ciocalteu reagent adding 10 mL of sodium carbonate (15% w/v). The solution was brought to volume (50mL) with deionized water. The mixture was left for 2 hours in the
dark at room temperature and shaken every 30 minutes. The absorbance at 765 nm was measured in a Lambda 15, Perkin Elmer spectrophotometer. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (fw).

2.5. Total anthocyanin content (TAnC)

The total anthocyanin content was determined by a pH differential absorbance method (Cheng and Breen, 1991). 1 g of fruit extract was diluted in 100 mL of buffer at pH 1.0 (4.026 g KCl in 1L of distilled water, pH value adjusted to 1.0 with concentrated HCl). One g of fruit extract was diluted in a pH 4.5 solution (32.82 g CH₃CO₂Na.3H₂O in 1 L of distilled water, pH value adjusted to 4.5 with concentrated HCl). The mixtures were left for 20 minutes at room temperature. Absorbance was measured simultaneously at 510 nm (A₅₁₀) and 700 nm (A₇₀₀) using a Lambda 15, Perkin Elmer spectrophotometer. The result was expressed as milligrams of cyanidin-3-glucoside (C₃G) equivalent per 100 g of fw and was calculated using a molar extinction coefficient of cyanidin-3-glucoside of 29,600 L mol⁻¹cm⁻¹ and using the absorbance value obtained with the following equation:

\[ A = \frac{(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}}{} \]

2.6. Total antioxidant capacity (FRAP)

Total antioxidant capacity was determined using the FRAP (Ferric Reducing Antioxidant Power) assay, according to Benzie and Strain (1996) method modified by Pellegrini et al. (2003). The method is based on the reduction of the Fe³⁺ TPTZ complex to the ferrous form at low pH. A sample containing 900 µl of freshly prepared FRAP solution (0.3 M acetate buffer pH 3.6 containing 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM...
FeCl$_3$·6H$_2$O), 90 µl of deionized water and 30 µl of extract was incubated at 37°C for 30 min. The reduction is monitored by measuring absorbance (A) change at 595 nm. FRAP values were calculated using a standard curve obtained from the analysis of a standard mix containing increasing concentrations of Fe$^{2+}$ and were expressed as mmol of Fe$^{2+}$ equivalents per kg of edible part of the fruits.

2.7. Statistical Analysis

The data was elaborated by ANOVA for means of comparison and significant differences were calculated using Tukey's test. Correlations were evaluated with Pearson coefficient. Statistical analysis was carried out using the SPSS 17.0 software Inc. (Chicago, USA).

The data obtained by chemical analysis was processed by principal component analysis (PCA) using PAST (PAleontological Statistics) ver. 2.12 software.

3. Results and discussion

3.1 Soluble solids content, pH and titratable acidity

The results obtained for soluble solid content, titratable acidity and pH for each species (means of data of all cultivars, landraces or selections analyzed for each species) grown in Piedmont are given in Table 1. White mulberry showed the highest SSC (21.60 °Brix), followed by plum cultivars and landraces (14.81-16.73 °Brix), pear (14.41 °Brix), black mulberry (14.47 °Brix) and black currant (13.89 °Brix). The lowest SSC were recorded in strawberry (6.71 °Brix), nectarine (9.15 °Brix) and raspberry (9.70 °Brix).

White currant (361.27 meq/L) had the highest TA value followed by gooseberry (280.93 meq/L) and raspberry (249.84 meq/L); TAs of white mulberry (21.90 meq/L), pear (34.48
meq/L), black mulberry (40.67 meq/L) and apple (47.43 meq/L) were the lowest. The highest value of pH was recorded in white mulberry (6.04), followed by black mulberry (5.04), pear (4.44) and strawberry (4.35). The black currant (2.91) and raspberry (2.96) had the lowest pH values. pH values did not grad cultivars following the same order given by titratable acidity as observed also by Konić-Ristić et al. (2011) in some berry juices.

Data of cultivars/landraces grouped per species are shown in Table 2. Apple cultivars ‘Red Chief’ and ‘Golden Delicious’ statistically differed for SSC, pH and TA. ‘Golden Delicious’ showed the highest SSC (13.80 °Brix) and TA (56.20 meq/L) values, although not significantly different from those observed with ‘Gala Brookfield’. Between apricot cultivars, ‘Tonda di Costigliole’ showed the highest values of soluble solids content (14.73 °Brix), while the lowest content was recorded in ‘Goldrich’ (12.07 °Brix). ‘Goldrich’ showed the highest titratable acidity value (359.80 meq/L), followed by ‘Laycot’ (238.83 meq/L) and ‘Tonda di Costigliole’ (175.33 meq/L). The pH values followed on reverse the values of TA. Statistical differences were recorded between highbush blueberry cultivars. ‘Elliot’ showed the highest value of soluble solids content and titratable acidity, 13.27 °Brix and 191.73 meq/L, respectively, whereas ‘Duke’ had the lowest soluble solids content (10.93 °Brix) and did not statistically differ from ‘Bluetta’ for pH and titratable acidity. ‘Ben Lomond’ showed the highest SSC (14.27 °Brix), while ‘Tiben’ recorded the lowest pH value (2.64) among the black currant cultivars. Statistical differences between ‘Hynnomyak Rot’ and ‘Hynnomyak Gelb’ were observed for titratable acidity. The black mulberry ‘Selection2’ had higher SSC (16.27 °Brix) and lower TA (33.70 meq/L) than ‘Selection 1’. Nectarine cultivars did not differ significantly for SSC. ‘Big Top’ resulted as the cultivar less acidic with values of pH and titratable acidity of 3.95 and 73.53 meq/L, respectively. No statistical differences were found between pear cultivars for soluble solids content and titratable acidity; however, the pH value of ‘Conference’ fruits resulted the highest. Among Chino-Japanese plum cultivars, ‘Black diamond’ recorded the highest SSC (18.13 °Brix) and pH (3.38), while ‘TC sun’ showed the
highest TA value (194.40 meq/L). Significant differences were observed between the European plum cultivars. ‘D’Ente 707’ showed the highest SSC (21.13 °Brix) and pH (3.66) while ‘Stanley’ recorded the lowest SSC (13.50 °Brix) and TA (111.13 meq/L). No statistical differences was observed between Damask plum landraces for the SSC; instead the ‘landrace 1’ recorded the lowest pH (3.23) and the highest TA value (225.90 meq/L). Among raspberry cultivars, ‘Heritage’ had the highest value of soluble solids content (10.53°Frix) and TA value (350.00 meq/L). The ‘Heritage’ pH value (2.64) was lower than ‘Malahat’ (3.14) and ‘Tulameen’ ones (3.03). The other cultivars resulted slightly less acidic: these results are similar to those obtained by other authors since fruits of primocane cultivars (‘Heritage’) are usually more acidic and present higher SSC than floricanes. For strawberry cultivars, ‘Maya’ showed a lower content in SSC than ‘Alba’ and ‘Arosa’ (5.50, 7.47, 7.17 °Brix, respectively), despite the fact that no significant differences were observed. ‘Alba’ recorded the highest titratable acidity (66.17 meq/L) and the lowest pH value, and showed significant differences with ‘Maya’. No significant differences were found between ‘Alba’ and ‘Arosa’ for the parameters considered. In general, differences between species were larger than within species but there was also variation among genotypes of the same species. This was often related to ripening season (earlier cultivars tend to have less SSC) and the ratio flesh/seeds present in each genotype.

3.2. Total phenolic content (TPC)

The variation of total phenolic content was large among the different species (Table 1). Nectarine, pear, apple and kiwifruit contained small quantities of phenolics (39.89-62.83 mg 100 g⁻¹ GAE), followed by apricot and white mulberry fruits (93.72 mg 100 g⁻¹ and 95.91 mg 100 g⁻¹, respectively). A significantly higher phenolic content was recorded in strawberry (398.67 mg 100 g⁻¹) and in berry fruits (196.98-362.15 mg 100 g⁻¹). Similar results were reported by Prior et al. (1998) and Moyer et al. (2002). The Damask plum landraces showed an
interesting total phenolic content (229.62 mg 100 g$^{-1}$). Macheix et al. (1990) and Ruiz et al. (2005) reported that berry fruits and stone fruits (peach, nectarine and apricot) contain a wide variety of phenolics including hydroxybenzoic and hydroxycinnamic acid derivatives (phenolic acids), anthocyanins, flavonols, condensed tannins (proanthocyanidins) and hydrolysable tannins. Moreover, Aaby et al. (2007) showed that ellagitannins were, together with anthocyanins, the major class of phenolic compounds in strawberry. Generally, total phenolics were abundant in the highly colored fruits, and in particular, in berry fruits; according to Konić-Ristić et al. (2011), phenolic compounds are abundant in berry fruits and in particular in berries with intense black-purple and red color such as blueberry, black currant, blackberry, strawberry and raspberry, representing a rich source of antioxidants (Moyer et al., 2002). Other coloured fruits, such as plums, are rich in antioxidant components (Gil et al., 2002). In this paper white currant was an exception and showed a high TPC (362.15 mg 100 g$^{-1}$, respectively).

The results and the statistical analysis of data of the different cultivars within the species are shown in Table 2. Among apricot cultivars ‘Goldrich’ contained the highest quantities of phenolics (119.03 mg 100 g$^{-1}$), followed by ‘Tonda di Costigliole’ (97.77 mg 100 g$^{-1}$) and ‘Laycot’ (64.33 mg 100 g$^{-1}$). The black currant cultivar ‘Ben Lomond’ showed the highest TPC (626.84 mg 100 g$^{-1}$), statistically different from ‘Tiben’ and ‘Titania’ that recorded the lowest total phenolic content (448.33 and 405.03 mg GAE 100 g$^{-1}$, respectively). The gooseberry cultivar ‘Hynnomyak Gelb’ showed a TPC value (184.03 mg 100 g$^{-1}$) lower than ‘Hynnomyak Rot’ (209.98 mg 100 g$^{-1}$), probably due to the different colour of the skin, red in ‘Hynnomyak Rot’ and dark-yellow in the other cultivar. In the black mulberry selections significantly higher phenolic content was observed in ‘Selection 1’ (405.08 mg 100 g$^{-1}$) respect to ‘Selection 2’ (280.76 mg 100 g$^{-1}$). Among nectarine cultivars, significantly higher phenolic content was recorded in ‘Caldesi 2000’ (72.73 mg 100 g$^{-1}$), followed by ‘Venus’ (30.53 mg 100 g$^{-1}$) and ‘Big Top’ (16.40 mg 100 g$^{-1}$). Chino-Japanese plum ‘Angeleno’, a black-purple skinned cultivar, showed a higher phenolic content (237.37 100 g$^{-1}$) than the two other P. salicina species.
Among Damask plums the ‘Ramassin landrace 2’ (264.83 mg 100 g⁻¹) and the ‘Ramassin landrace 3’ (237.03 mg 100) recorded the highest phenolic quantities. Significant differences in TPC among European plums were observed. ‘D’Ente 707’ with 212.87 mg 100 g⁻¹ showed the highest phenolic content, followed by ‘Stanley’ (172.40 mg 100 g⁻¹) and ‘President’ (140.63 mg 100 g⁻¹). ‘Malahat’ showed the highest TPC (372.60 100 g⁻¹) between raspberry cultivars, followed by ‘Tulameen’ (330.00 100 g⁻¹) and ‘Heritage’ (287.70 100 g⁻¹) with the lowest phenolic content. Among strawberry cultivars, ‘Arosa’ showed the highest phenolic content (461.46 mg 100 g⁻¹), however no statistical differences with ‘Maya’ (424.33 mg 100 g⁻¹) were observed. ‘Alba’ recorded the lowest value (310.17 mg 100 g⁻¹) with no significant differences with ‘Maya’. No statistical differences were observed between cultivars within apple, blueberry, and pear. Compared to major species, Damask plum landraces recorded the highest TPC and can be considered as a good source of phenolic compounds.

Differences among fruits for the quantity and the composition of phenolic compounds can be due to factors as genotype, environmental conditions, agrotechniques, and storage conditions as observed by Aaby et al. (2007) in strawberry fruits.

### 3.3. Total anthocyanin content (TAnC)

Significant differences in anthocyanin content were recorder in the different species and genotypes (Table 1). Black mulberry contained the highest anthocyanin content (341.53 mg 100 g⁻¹ cyanidin-3-glucoside fw), followed by black currant (224.79 mg 100 g⁻¹ fw), blueberry (222.74 mg 100 g⁻¹ fw) and blackberry cultivars (106.40 mg 100 g⁻¹ fw). Berry species with light skin colour, such as white mulberry, white currant, gooseberry, raspberry, and the fruits belonging to the major species contained a lower amount of anthocyanins (0.03-44.43 mg 100 g⁻¹ fw), as expected. Määttäälä et al. (2003) observed that delphinidin 3-O-glucoside, delphinidin 3-O-rutinoside, cyaniding 3-O-glucoside and cyaniding 3-O-rutinoside represent the 4 major anthocyanins present in black currant with 97% of the total content. Although the white currant
showed a low anthocyanin value, the high value of the phenolic content was probably due to hydroxycinnamic acid derivatives (caffeic acid hexose derivative, p-coumaric acid 4-O-glucoside, p-coumaroylglucose and hexoses, feruloylhexose and ferulic acid hexose derivatives) and flavonol glycosides (rutin, quercetin 3-O-glucoside and quercetin hexoside-malonate) as reported by Määttää et al. (2003). The values of anthocyanin content in the analyzed berry fruits (blueberry, raspberry, blackberry and gooseberry cultivars in particular) are consistent with anthocyanin amounts presented by other authors (Wang and Lin, 2000; Prior et al., 1998; Moyer et al., 2002; Pantelidis et al., 2007). Among major fruits, strawberry and plum cultivars recorded the highest total anthocyanin content (44.43 mg 100 g⁻¹ fw and 13.70-33.38 mg 100 g⁻¹ fw, respectively). Similar results for anthocyanin content were reported by others researchers, both in strawberry (Rababah et al., 2011; Wang et al., 2000) and plum (Usenik et al., 2009). A direct correlation existed between the levels of anthocyanins and the fruit skin and flesh colour. Anthocyanins are responsible for the red, purple and blue colour of the fruits, and they have been shown to have potent antioxidant activities (Baliga et al., 2011).

In particular, the anthocyanins are abundant in brightly coloured berry fruits (Shukitt-Hale et al., 2008) and represent one of the most dominant classes of bioactive compounds in berries (Clifford, 2000).

Usenik et al. (2009) reported that in plum cultivars the total anthocyanin content varied in relation to cultivars and ripening stage. Present results indicate that a great variability exists in anthocyanin content among cultivars of the same species (Table 2). The concentration of anthocyanins is clearly related to the colour of the fruit. Significantly higher anthocyanin content was recorded in Ribes grossularia L., between cultivar ‘Hynnomyk Rot’ (5.99 mg 100 g⁻¹ fw), with a red fruit skin, and the cultivar ‘Hynnomyk Gelb’ (0.44 mg 100 g⁻¹ fw), with a dark-yellow skin. The Chino-Japanese plums ‘Black diamond’ and ‘Angeleno’, with a black-purple skin, showed a higher TAnC than ‘TC sun’ characterized by a yellow-orange skin, 47.93 mg 100 g⁻¹ fw, 43.53 mg 100 g⁻¹ fw and 8.67 mg 100 g⁻¹ fw, respectively. The violet Damask
plum ‘Ramassin landrace 3’ recorded the highest anthocyanin content 50.63 mg 100 g\(^{-1}\) fw, followed by ‘Ramassin landrace 2’ (29.27 mg 100 g\(^{-1}\) fw) and ‘Ramassin landrace 1’ (19.77 mg 100 g\(^{-1}\) fw) with a blu skin colour. Among European plums the cultivar ‘Stanley’, characterised by a dark blue-violet skin colour, showed a higher TAnC (19.77 mg 100 g\(^{-1}\) fw) than ‘D’Ente 707’ (14.70 mg 100 g\(^{-1}\) fw) and ‘President’ (6.63 mg 100 g\(^{-1}\) fw), that are red-violet skinned cultivars.

The data indicated statistical differences also in genotypes of the same species with similar colour of the skin, such as for \(\text{Ribes nigrum}\) L., cv ‘Tiben’ (271.33 mg 100 g\(^{-1}\) fw) and ‘Titania’ (184.10 mg 100 g\(^{-1}\) fw), and for \(\text{Morus nigra}\) L. ‘Selection 1’ and ‘Selection 2’.

Anthocyanins are probably the largest group of phenolic compounds in the human diet and the antioxidant capacity of anthocyanins may be one of their most significant biological properties in humans (Wang et al., 2000). However, in humans the bioavailability of anthocyanins is low relative to the amount consumed (Wu et al., 2002).

### 3.4 Total antioxidant capacity (FRAP)

The values of total antioxidant capacity, expressed as FRAP, are shown in Table 1. The results showed large variations between the different species. In general berries, and in particular the white currant cultivar. ‘Werdavia’ (85.97 mmol Fe\(^{2+}\)/kg fw) and the blackberry cv. ‘Navaho’ (79.17 mmol Fe\(^{2+}\)/kg fw) presented the highest antioxidant capacity. The high antioxidant capacity of berry fruits, according to the literature (Carlson et al., 2010; Kalt et al., 1999), is likely due to the high content of phenolic acids and flavonoids, such as anthocyanins, in fruits with dark skin (Macheix et al., 1990; Pantelidis et al., 2007) which have demonstrated a high antioxidant activity (Pellegrini et al., 2003; Shukitt-Hale et al., 2008; Beccaro et al., 2006).

Among the fruits belonging to major species, strawberry had the highest total antioxidant capacity (40.84 mmol Fe\(^{2+}\)/kg fw), in agreement with Szeto et al. (2002). Strawberries were also higher in antioxidant capacity than other berry fruit such as gooseberry and raspberry, in
agreement with Kalt et al. (1999). No statistical differences were found between apple, apricot, nectarine, pear and plum. On the contrary, plums and Damask plums had the highest FRAP values; in particular the ‘Ramassin’ landraces had 17.88 mmol Fe$^{2+}$/kg fw against the 13.02 mmol Fe$^{2+}$/kg fw of Chino-Japanese plums and 12.12 mmol Fe$^{2+}$/kg fw of European plums. This data is in agreement with those reported by Pellegrini et al. (2003), Szeto et al. (2002) and Gil et al. (2002) that described a higher antioxidant activity of plums in comparison to other stone fruits, in particular nectarines. Among major fruit species kiwifruit recorded an antioxidant capacity similar to those observed for the others peeled fruits with values in agreement with Szeto et al. (2002).

Significant differences in total antioxidant capacity among genotypes of the same species were recorded (Table 2). The most interesting results were observed in blueberry, black currant, gooseberry, black mulberry, nectarine, European plum and strawberry.

‘Elliot’ had the highest total antioxidant capacity between blueberry cultivars (71.23 mmol Fe$^{2+}$/kg fw), followed by ‘Duke’ (47.83 mmol Fe$^{2+}$/kg fw) and ‘Bluetta’ with the lowest value (29.30 mmol Fe$^{2+}$/kg fw). Significant differences in FRAP were observed in black currants. ‘Tiben’ and ‘Ben Lomond’ had the highest antioxidant capacity (79.60 and 68.53 mmol Fe$^{2+}$/kg fw, respectively). The gooseberry cultivar ‘Hynnomaky Rot’ showed twice as much antioxidant capacity than ‘Hynnomaky Gelb’ (24.07 mmol Fe$^{2+}$/kg fw and 12.76 mmol Fe$^{2+}$/kg fw, respectively). Among black mulberry cultivars, ‘Selection 1’ recorded the highest FRAP (77.15 mmol Fe$^{2+}$/kg fw). Between nectarine cultivars, ‘Caldesi 2000’ showed the highest FRAP value (12.73 mmol Fe$^{2+}$/kg fw) despite the fact that no statistical differences were observed with ‘Big Top’ (9.83 mmol Fe$^{2+}$/kg fw). ‘Venus’ had the lowest total antioxidant capacity (5.57 mmol Fe$^{2+}$/kg fw) without statistical differences with ‘Big Top’. ‘Ramassin landrace 2’ showed the highest FRAP (27.73 mmol Fe$^{2+}$/kg fw), twice as much as the other plum cultivars (11.63-13.80 mmol Fe$^{2+}$/kg fw). Among strawberry cultivars, ‘Arosa’ showed the highest FRAP (74.97 mmol Fe$^{2+}$/kg fw), statistically different from ‘Alba’ (24.03 mmol
Fe$^{2+}$/kg fw) and ‘Maya’ (23.53 mmol Fe$^{2+}$/kg fw). These differences are related principally to the colour of the flesh and in particular of the skin, where the highest content of anthocyanins and carotenoids is concentrated, and with genotype and the size of the fruit (skin/flesh ratio) (Howard et al., 2003).

FRAP values were highly correlated with phenolic content ($r=0.697^{**}$) and anthocyanin content ($r=0.636^{**}$). Similar results have been reported by Pantelidis et al. (2007) and Deighton et al. (2000) which found a similar correlation between total antioxidant capacity and anthocyanins ($r=0.635$ and $r=0.588$, respectively). However, they recorded a higher linear correlation between total antioxidant capacity and phenolic content ($r=0.947$ and $r=0.965$, respectively). This difference is probably due to the greater number of species considered in this study resulting in a larger variability of fruit composition and structure and in differences of the type of antioxidant compounds present in the pulp and skin. Moreover, this research did not take into account components of variance such as sun exposure, water availability and agricultural practices that can influence the fruits composition.

In general, major fruit species showed different chemical characteristics compared to berry fruits. In order to simplify the multivariate model based on the analysis of 6 parameters, and classify the species according to their the nutraceutical characteristics, a PCA was carried out. The first two principal components (PC1 and PC2) accounted for 74.05% of the total variance and were used to obtain a scatter plot (Figure 1). PC1 represented 42.81% of the total variation and was associated mainly to total phenolic content, SSC and antioxidant activity on the discrimination of the species. PC2 accounted for 31.24% of the total variation and was mostly associated to pH, titratable acidity and anthocyanin content. The PCA scatter plot split the 16 species into two groups: major fruit crops and white mulberry were higher in SSC and lower in phenolics and FRAP and were placed in the lower half of the plot; berry fruits, being more rich in phenolics, were placed in the upper half of the plot. In the upper left part of the plot the most acidic berry fruits: raspberry, goosberry and white currant were grouped. In the
upper right part of the plot the berry fruits high in phenolics, anthocyanins and FRAP were placed.

Conclusion

This current study highlights the difference in total phenolic and anthocyanin contents, and total antioxidants capacity found in different fruits species and cultivars grown in Piedmont.

According to considerate parameters, the species could be categorized into two groups: berry fruits and major fruits. Major fruits were generally higher in SSC and had moderate acidity but were tendentially lower in phenolics and antioxidant capacity. The results indicated that most fruits present in Piedmont area, in particular the berry fruits blackberry, blueberry, currant, black mulberry and strawberry and the Damask plum are excellent sources of antioxidants and bioactive compounds, especially phenolics. Henning et al. (2010) demonstrated that in particular the consumption of 250 g of strawberry by healthy people was associated with a modest but significant increase in antioxidant activity in serum, which has the potential to improve the body’s defense against chronic disease. Unfortunately the consumption of most berry fruits is low in Italy; only strawberries have a significant demand but their availability is limited to a relatively short period during spring.

Including cultivation and post harvest considerations, the berry species with higher chances of a larger direct use is highbush bluberry. Damask plum showed intermediate properties between berry and major species and their consumption should be encouraged. In Piedmont, Damask plums are sold in local markets being frequently found in small orchards and family gardens; they are consumed during summer mostly as fresh fruit but are also processed for the production of ice-creams. The intake of phenolics and antioxidants from seasonal fruit during autumn and winter would be potentially lower than in summer, due to the
type of local fruit available (apple, pears, kiwifruit) but in the global market the offer of fruit
and vegetables is large enough to provide products from other areas, such as citrus fruits or
berry fruits from the Southern hemisphere, that can help the needs.

Finally we can mention that the differences found among cultivars highlight a great
variability within species that should be considered for the choice of the cultivar and in
breeding programs aimed at selecting varieties with improved antioxidant capacity and
nutraceutical properties.

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challenging reactions to occur. A microbial enzyme does so by simple electron transfer,
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electrospray ionization mass spectrometric (MS) detection: *Ribes* Species. *J. Agric. Food
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### Tables

**Table 1.**

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<thead>
<tr>
<th>Species</th>
<th>SSC (°Brix)</th>
<th>pH</th>
<th>TA (meq L⁻¹)</th>
<th>TPC (mg GAE 100 g⁻¹ fw)</th>
<th>TAnC (mg cyanidin-3-glucoside equivalents 100 g⁻¹ fw)</th>
<th>FRAP (mmol Fe²⁺ kg⁻¹ fw)</th>
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<table>
<thead>
<tr>
<th>Fruit</th>
<th>Mean</th>
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<th>Median</th>
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<th>Q3</th>
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Means followed by the same letter in a column are not significantly different at $p = 0.05$ (Tukey’s test).
Table 2.

Skin colour, soluble solids content (SSC; °Brix), titratable acidity (TA; meq L⁻¹) pH, total phenolic content (TPC; mg GAE 100 g⁻¹ fw), total anthocyanins content (TAnC; mg cyanidin-3-glucoside equivalents 100 g⁻¹ fw) and total antioxidant capacity (FRAP; mmol Fe²⁺ kg⁻¹ fw) of the analyzed cultivars and landraces.

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<tr>
<th>Species</th>
<th>Cultivar/landrace</th>
<th>Skin colour</th>
<th>SSC</th>
<th>pH</th>
<th>TA</th>
<th>TPC</th>
<th>TAnC</th>
<th>FRAP</th>
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<td>3.03b</td>
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<td>330.00ab</td>
<td>25.63b</td>
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<td>4.10b</td>
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<td>424.33ab</td>
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<td>23.53b</td>
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</table>

Data of cultivars/landraces within a species were compared by Tukey’s test: means with the same letter are not significantly different at \( p = 0.05 \) (Tukey’s test).

Figures

**Figure 1**

PCA two-dimensional scatter plot based on the first two principal components (PC1 and PC2) generated for the species studied and based on data of soluble solids content (SSC), titratable acidity (TA), pH, total antioxidant capacity (FRAP), total phenolic content (TPC) and total anthocyanin content (TAnC). C.J_plum (Chino-Japanese plum); E._plum (European plum).
578  Major species
579  Berry species
580