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

29
30
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39
40
41 **Abstract**

42 17 fruit species grown in Piedmont (Northwest Italy) were analysed for SSC, pH, TA,
43 total antioxidant capacity, and total phenolic and anthocyanin contents to evaluate their
44 nutraceutical value and emphasize the value of the local productions. Major fruit species (pear,
45 apple, apricot) were generally higher in SSC and had moderate acidity but were tendentially
46 lower in phenolics (39.89-93.71 mg 100 g⁻¹ GAE) and antioxidant capacity (8.07-11.11 mmol
47 Fe²⁺/kg fw) in comparison with berry fruits. Black mulberry, blackberry, highbush blueberry,
48 currant, raspberry, gooseberry and strawberry confirmed to be excellent antioxidant sources
49 (phenolics 196.98-398.67 mg 100 g⁻¹ GAE; FRAP: 11.51-85.97 mmol Fe²⁺/kg fw) while
50 Damask plums showed intermediate properties (phenolics: 175.30-229.62 mg 100 g⁻¹ GAE;
51 FRAP: 12.12-17.88 mmol Fe²⁺/kg fw). The highest anthocyanin contents were recorded in

52 black mulberry (341.53 mg 100 g⁻¹ fw), black currant (224.79 mg 100 g⁻¹ fw) and highbush
53 blueberry (222.74 mg 100 g⁻¹ fw). Differences between cultivars of the same species indicated
54 the presence of variability that should be considered in breeding and in the orchards planning.

55
56
57 **Keywords:** berry; major species; FRAP; soluble solid content; titratable acidity
58
59

60 **1. Introduction**

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

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

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

74 The human organism naturally defends itself from the free radicals producing endogenous
75 antioxidants (Prior et al., 1998); yet, this is not sufficient to meet the needs of the body and the
76 intake of antioxidants from external sources is strongly recommended (Jarrett, 2008). The most

77 important phytonutrients with strong antioxidant capacities are fibre (Kaur et al., 2001),
78 phenolics (Shukitt-Hale et al., 2008), vitamins A, B, C, E, terpenoids (Espin et al., 2007) and
79 essential minerals such as selenium, copper and zinc. It has been already demonstrated that
80 most of these compounds are found in fruits, in particular berries, and vegetables but their
81 amount widely varies among species and also among cultivars within the same species
82 (Pantelidis et al., 2007; Gil et al., 2002; Bounous et al., 2009).

83 Recently, research was done to evaluate the antioxidant capacity of fruits and vegetables
84 produced in different countries including Italy (Pellegrini et al., 2003), Norway (Carlsen et al.,
85 2010), United States (Wu et al., 2004), Czech Republic (Stratil et al., 2006), France (Brat et al.,
86 2006) and Belgium (Kevers et al., 2007) highlighting a geographical effect on food
87 composition, but often not considering the different amounts of biochemical compounds
88 present in cultivars of the same species.

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

96 **2. Materials and methods**

98 **2.1. Fruit samples**

99
100 Fruit samples of the following 17 species were collected in the Piedmont Region (Table
101 1): apple (*Malus x domestica* Borkh.), apricot (*Prunus armeniaca* L.), blackberry (*Rubus*

102 *ulmifolius* L.), highbush blueberry (*Vaccinium corymbosum* L.), black currant (*Ribes nigrum*
103 L.), white currant (*Ribes rubrum* L.), gooseberry (*Ribes grossularia* L.), kiwifruit (*Actinidia*
104 *deliciosa* Chev.), black mulberry (*Morus nigra* L.), white mulberry (*Morus alba* L.), nectarine
105 [*Prunus persica* (L.) Batsch], pear (*Pirus communis* L.), European plum (*Prunus domestica* L.),
106 Damask plum (*Prunus insititia* L.), Chino-Japanese plum (*Prunus salicina* Lindl.), raspberry
107 (*Rubus idaeus* L.) and strawberry (*Fragaria x ananassa* Duch.). All fruits but mulberries were
108 sampled from commercial orchards in typical areas of cultivation; mulberries were collected
109 from single grafted plants used in the past for silkworm raising.

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

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

117 10 kg of fruit for each genotype were sampled at ripeness from 3 plants, except for small
118 fruits: in this case 500 g of fruit were collected from each plant (or for linear meter on the row
119 in the case of raspberry and blackberry). Samples were maintained fresh in an icebox during the
120 transport to the laboratory and subsequently stored in a refrigerated room at 4° C; they were
121 extracted and analyzed within 24 hours.

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

125
126 **2.2. Soluble solids content (SSC), pH and titratable acidity (TA)**

127

128 50 mL of juice obtained by a juice extractor were centrifuged (centrifuge ALC PK 110) at
129 6,500 rpm for 10 minutes at 20° C, to get a juice completely devoid of solid residues. The SSC
130 was determined on the juice by a digital refractometer XS DBR 35 and expressed in °Brix. The
131 pH and titratable acidity (10 mL of juice and 90 mL of deionized water) were determined using
132 a pH meter and a semi-automatic titrator CRISON. Titratable acidity has been expressed in
133 meq/L.

134

135 ***2.3. Extraction protocol***

136

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

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

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

146

147 ***2.4. Total phenolic content (TPC)***

148

149 The amount of total phenolics in fruits extracts was measured following the Folin-
150 Ciocalteu procedure, according to the method of Slinkard and Singleton (1977), using gallic
151 acid as standard. The methanolic extract (0.5 g) was mixed with 30 mL of deionized water and
152 2.5 mL of Folin-Ciocalteu reagent adding 10 mL of sodium carbonate (15% w/v). The solution
153 was brought to volume (50mL) with deionized water. The mixture was left for 2 hours in the

154 dark at room temperature and shaken every 30 minutes. The absorbance at 765 nm was
155 measured in a Lambda 15, Perkin Elmer spectrophotometer. Results were expressed as
156 milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (fw).

157

158 **2.5. Total anthocyanin content (TAnC)**

159

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

170

$$171 \quad A = [(A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}].$$

172

173 **2.6. Total antioxidant capacity (FRAP)**

174

175 Total antioxidant capacity was determined using the FRAP (Ferric Reducing Antioxidant
176 Power) assay, according to Benzie and Strain (1996) method modified by Pellegrini et al.
177 (2003). The method is based on the reduction of the Fe³⁺ TPTZ complex to the ferrous form at
178 low pH. A sample containing 900 µl of freshly prepared FRAP solution (0.3 M acetate buffer
179 pH 3.6 containing 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM

180 FeCl₃·6H₂O), 90 µl of deionized water and 30 µl of extract was incubated at 37° C for 30 min.
181 The reduction is monitored by measuring absorbance (A) change at 595 nm. FRAP values were
182 calculated using a standard curve obtained from the analysis of a standard mix containing
183 increasing concentrations of Fe²⁺ and were expressed as mmol of Fe²⁺ equivalents per kg of
184 edible part of the fruits.

185

186 **2.7. Statistical Analysis**

187

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

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

193

194 **3. Results and discussion**

195

196 **3.1 Soluble solids content, pH and titratable acidity**

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

203 White currant (361.27 meq/L) had the highest TA value followed by gooseberry (280.93
204 meq/L) and raspberry (249.84 meq/L); TAs of white mulberry (21.90 meq/L), pear (34.48

205 meq/L), black mulberry (40.67 meq/L) and apple (47.43 meq/L) were the lowest. The highest
206 value of pH was recorded in white mulberry (6.04), followed by black mulberry (5.04), pear
207 (4.44) and strawberry (4.35). The black currant (2.91) and raspberry (2.96) had the lowest pH
208 values. pH values did not grad cultivars following the same order given by titratable acidity as
209 observed also by Konić-Ristić et al. (2011) in some berry juices.

210 Data of cultivars/landraces grouped per species are shown in Table 2. Apple cultivars
211 ‘Red Chief’ and ‘Golden Delicious’ statistically differed for SSC, pH and TA. ‘Golden
212 Delicious’ showed the highest SSC (13.80 °Brix) and TA (56.20 meq/L) values, although not
213 significantly different from those observed with ‘Gala Brookfield’. Between apricot cultivars,
214 ‘Tonda di Costigliole’ showed the highest values of soluble solids content (14.73 °Brix), while
215 the lowest content was recorded in ‘Goldrich’ (12.07 °Brix). ‘Goldrich’ showed the highest
216 titratable acidity value (359.80 meq/L), followed by ‘Laycot’ (238.83 meq/L) and ‘Tonda di
217 Costigliole’ (175.33 meq/L). The pH values followed on reverse the values of TA. Statistical
218 differences were recorded between highbush blueberry cultivars. ‘Elliot’ showed the highest
219 value of soluble solids content and titratable acidity, 13.27 °Brix and 191.73 meq/L,
220 respectively, whereas ‘Duke’ had the lowest soluble solids content (10.93 °Brix) and did not
221 statistically differ from ‘Bluetta’ for pH and titratable acidity. ‘Ben Lomond’ showed the
222 highest SSC (14.27 °Brix), while ‘Tiben’ recorded the lowest pH value (2.64) among the black
223 currant cultivars. Statistical differences between ‘Hynomaky Rot’ and ‘Hynomaky Gelb’
224 were observed for titratable acidity. The black mulberry ‘Selection2’ had higher SSC (16.27
225 °Brix) and lower TA (33.70 meq/L) than ‘Selection 1’. Nectarine cultivars did not differ
226 significantly for SSC. ‘Big Top’ resulted as the cultivar less acidic with values of pH and
227 titratable acidity of 3.95 and 73.53 meq/L, respectively. No statistical differences were found
228 between pear cultivars for soluble solids content and titratable acidity; however, the pH value
229 of ‘Conference’ fruits resulted the highest. Among Chino-Japanese plum cultivars, ‘Black
230 diamond’ recorded the highest SSC (18.13 °Brix) and pH (3.38), while ‘TC sun’ showed the

231 highest TA value (194.40 meq/L). Significant differences were observed between the European
232 plum cultivars. 'D'Ente 707' showed the highest SSC (21.13 °Brix) and pH (3.66) while
233 'Stanley' recorded the lowest SSC (13.50 °Brix) and TA (111.13 meq/L). No statistical
234 differences was observed between Damask plum landraces for the SSC; instead the 'landrace 1'
235 recorded the lowest pH (3.23) and the highest TA value (225.90 meq/L). Among raspberry
236 cultivars, 'Heritage' had the highest value of soluble solids content (10.53°Brix) and TA value
237 (350.00 meq/L). The 'Heritage' pH value (2.64) was lower than 'Malahat' (3.14) and
238 'Tulameen' ones (3.03). The other cultivars resulted slightly less acidic: these results are
239 similar to those obtained by other authors since fruits of primocane cultivars ('Heritage') are
240 usually more acidic and present higher SSC than floricanes. For strawberry cultivars, 'Maya'
241 showed a lower content in SSC than 'Alba' and 'Arosa'(5.50, 7.47, 7.17 °Brix, respectively),
242 despite the fact that no significant differences were observed. 'Alba' recorded the highest
243 titratable acidity (66.17 meq/L) and the lowest pH value, and showed significant differences
244 with 'Maya'. No significant differences were found between 'Alba' and 'Arosa' for the
245 parameters considered. In general, differences between species were larger than within species
246 but there was also variation among genotypes of the same species. This was often related to
247 ripening season (earlier cultivars tend to have less SSC) and the ratio flesh/seeds present in
248 each genotype.

249

250 **3.2. Total phenolic content (TPC)**

251 The variation of total phenolic content was large among the different species (Table 1).
252 Nectarine, pear, apple and kiwifruit contained small quantities of phenolics (39.89-62.83 mg
253 100 g⁻¹ GAE), followed by apricot and white mulberry fruits (93.72 mg 100 g⁻¹ and 95.91 mg
254 100 g⁻¹, respectively). A significantly higher phenolic content was recorded in strawberry
255 (398.67 mg 100 g⁻¹) and in berry fruits (196.98-362.15 mg 100 g⁻¹). Similar results were
256 reported by Prior et al. (1998) and Moyer et al. (2002). The Damask plum landraces showed an

257 interesting total phenolic content (229.62 mg 100 g⁻¹). Macheix et al. (1990) and Ruiz et al.
258 (2005) reported that berry fruits and stone fruits (peach, nectarine and apricot) contain a wide
259 variety of phenolics including hydroxybenzoic and hydroxycinnamic acid derivatives (phenolic
260 acids), anthocyanins, flavonols, condensed tannins (proanthocyanidins) and hydrolysable
261 tannins. Moreover, Aaby et al. (2007) showed that ellagitannins were, together with
262 anthocyanins, the major class of phenolic compounds in strawberry. Generally, total phenolics
263 were abundant in the highly colored fruits, and in particular, in berry fruits; according to Konić-
264 Ristić et al. (2011), phenolic compounds are abundant in berry fruits and in particular in berries
265 with intense black-purple and red color such as blueberry, black currant, blackberry, strawberry
266 and raspberry, representing a rich source of antioxidants (Moyer et al., 2002). Other coloured
267 fruits, such as plums, are rich in antioxidant components (Gil et al., 2002). In this paper white
268 currant was an exception and showed a high TPC (362.15 mg 100 g⁻¹, respectively).

269 The results and the statistical analysis of data of the different cultivars within the species
270 are shown in Table 2. Among apricot cultivars ‘Goldrich’ contained the highest quantities of
271 phenolics (119.03 mg 100 g⁻¹), followed by ‘Tonda di Costigliole’ (97.77 mg 100 g⁻¹) and
272 ‘Laycot’ (64.33 mg 100 g⁻¹). The black currant cultivar ‘Ben Lomond’ showed the highest TPC
273 (626.84 mg 100 g⁻¹), statistically different from ‘Tiben’ and ‘Titania’ that recorded the lowest
274 total phenolic content (448.33 and 405.03 mg GAE 100 g⁻¹, respectively). The gooseberry
275 cultivar ‘Hynnomaky Gelb’ showed a TPC value (184.03 mg 100 g⁻¹) lower than ‘Hynnomaky
276 Rot’ (209.98 mg 100 g⁻¹), probably due to the different colour of the skin, red in ‘Hynnomaky
277 Rot’ and dark-yellow in the other cultivar. In the black mulberry selections significantly higher
278 phenolic content was observed in ‘Selection 1’ (405.08 mg 100 g⁻¹) respect to ‘Selection 2’
279 (280.76 mg 100 g⁻¹). Among nectarine cultivars, significantly higher phenolic content was
280 recorded in ‘Caldesi 2000’ (72.73 mg 100 g⁻¹), followed by ‘Venus’ (30.53 mg 100 g⁻¹) and
281 ‘Big Top’ (16.40 mg 100 g⁻¹). Chino-Japanese plum ‘Angelino’, a black-purple skinned
282 cultivar, showed a higher phenolic content (237.37 mg 100 g⁻¹) than the two other *P. salicina*

283 cultivars. Among Damask plums the 'Ramassin landrace 2' (264.83 mg 100 g⁻¹) and the
284 'Ramassin landrace 3'(237.03 mg 100) recorded the highest phenolic quantities. Significant
285 differences in TPC among European plums were observed. 'D'Ente 707' with 212.87 mg 100
286 g⁻¹ showed the highest phenolic content, followed by 'Stanley' (172.40 mg 100 g⁻¹) and
287 'President' (140.63 mg 100 g⁻¹). 'Malahat' showed the highest TPC (372.60 100 g⁻¹) between
288 raspberry cultivars, followed by 'Tulameen' (330.00 100 g⁻¹) and 'Heritage' (287.70 100 g⁻¹)
289 with the lowest phenolic content. Among strawberry cultivars, 'Arosa' showed the highest
290 phenolic content (461.46 mg 100 g⁻¹), however no statistical differences with 'Maya' (424.33
291 mg 100 g⁻¹) were observed. 'Alba' recorded the lowest value (310.17 mg 100 g⁻¹) with no
292 significant differences with 'Maya'. No statistical differences were observed between cultivars
293 within apple, blueberry, and pear. Compared to major species, Damask plum landraces
294 recorded the highest TPC and can be considered as a good source of phenolic compounds.

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

298

299 **3.3. Total anthocyanin content (TAnC)**

300 Significant differences in anthocyanin content were recorder in the different species and
301 genotypes (Table 1). Black mulberry contained the highest anthocyanin content (341.53 mg
302 100 g⁻¹ cyanidin-3-glucoside fw), followed by black currant (224.79 mg 100 g⁻¹ fw), blueberry
303 (222.74 mg 100 g⁻¹ fw) and blackberry cultivars (106.40 mg 100 g⁻¹ fw). Berry species with
304 light skin colour, such as white mulberry, white currant, gooseberry, raspberry, and the fruits
305 belonging to the major species contained a lower amount of anthocyanins (0.03-44.43 mg 100
306 g⁻¹ fw), as expected. Määttä et al. (2003) observed that delphinidin 3-*O*-glucoside, delphinidin
307 3-*O*-rutinoside, cyaniding 3-*O*-glucoside and cyaniding 3-*O*-rutinoside represent the 4 major
308 anthocyanins present in black currant with 97% of the total content. Although the white currant

309 showed a low anthocyanin value, the high value of the phenolic content was probably due to
310 hydroxycinnamic acid derivatives (caffeic acid hexose derivative, *p*-coumaric acid 4-*O*-
311 glucoside, *p*-coumaroylglucose and hexoses, feruloylhexose and ferulic acid hexose
312 derivatives) and flavonol glycosides (rutin, quercetin 3-*O*-glucoside and quercetin hexoside-
313 malonate) as reported by Määttä et al. (2003). The values of anthocyanin content in the
314 analyzed berry fruits (blueberry, raspberry, blackberry and gooseberry cultivars in particular)
315 are consistent with anthocyanin amounts presented by other authors (Wang and Lin, 2000;
316 Prior et al., 1998; Moyer et al. 2002; Pantelidis et al., 2007). Among major fruits, strawberry
317 and plum cultivars recorded the highest total anthocyanin content (44.43 mg 100 g⁻¹ fw and
318 13.70-33.38 mg 100 g⁻¹ fw, respectively). Similar results for anthocyanin content were reported
319 by others researchers, both in strawberry (Rababah et al., 2011; Wang et al., 2000) and plum
320 (Usenik et al., 2009). A direct correlation existed between the levels of anthocyanins and the
321 fruit skin and flesh colour. Anthocyanins are responsible for the red, purple and blue colour of
322 the fruits, and they have been shown to have potent antioxidant activities (Baliga et al., 2011).
323 In particular, the anthocyanins are abundant in brightly coloured berry fruits (Shukitt-Hale et
324 al., 2008) and represent one of the most dominant classes of bioactive compounds in berries
325 (Clifford, 2000).

326 Usenik et al. (2009) reported that in plum cultivars the total anthocyanin content varied in
327 relation to cultivars and ripening stage. Present results indicate that a great variability exists in
328 anthocyanin content among cultivars of the same species (Table 2). The concentration of
329 anthocyanins is clearly related to the colour of the fruit. Significantly higher anthocyanin
330 content was recorded in *Ribes grossularia* L., between cultivar ‘Hynnomaky Rot’ (5.99 mg 100
331 g⁻¹ fw), with a red fruit skin, and the cultivar ‘Hynnomaky Gelb’ (0.44 mg 100 g⁻¹ fw), with a
332 dark-yellow skin. The Chino-Japanese plums ‘Black diamond’ and ‘Angeleno’, with a black-
333 purple skin, showed a higher TAnC than ‘TC sun’ characterized by a yellow-orange skin, 47.93
334 mg 100 g⁻¹ fw, 43.53 mg 100 g⁻¹ fw and 8.67 mg 100 g⁻¹ fw, respectively. The violet Damask

335 plum ‘Ramassin landrace 3’ recorded the highest anthocyanin content 50.63 mg 100 g⁻¹ fw,
336 followed by ‘Ramassin landrace 2’ (29.27 mg 100 g⁻¹ fw) and ‘Ramassin landrace 1’ (19.77 mg
337 100 g⁻¹ fw) with a blu skin colour. Among European plums the cultivar ‘Stanley’, characterised
338 by a dark blue-violet skin colour, showed a higher TAnC (19.77 mg 100 g⁻¹ fw) than ‘D’Ente
339 707’ (14.70 mg 100 g⁻¹ fw) and ‘President’ (6.63 mg 100 g⁻¹ fw), that are red-violet skinned
340 cultivars.

341 The data indicated statistical differences also in genotypes of the same species with similar
342 colour of the skin, such as for *Ribes nigrum* L., cv ‘Tiben’ (271.33 mg 100 g⁻¹ fw) and ‘Titania’
343 (184.10 mg 100 g⁻¹ fw), and for *Morus nigra* L. ‘Selection 1’ and ‘Selection 2’.

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

348

349 **3.4 Total antioxidant capacity (FRAP)**

350 The values of total antioxidant capacity, expressed as FRAP, are shown in Table 1. The
351 results showed large variations between the different species. In general berries, and in
352 particular the white currant cultivar. ‘Werdavia’ (85.97 mmol Fe²⁺/kg fw) and the blackberry
353 cv. ‘Navaho’ (79.17 mmol Fe²⁺/kg fw) presented the highest antioxidant capacity. The high
354 antioxidant capacity of berry fruits, according to the literature (Carlsen et al., 2010; Kalt et al.,
355 1999), is likely due the high content of phenolic acids and flavonoids, such as anthocyanins, in
356 fruits with dark skin (Macheix et al., 1990; Pantelidis et al., 2007) which have demonstrated a
357 high antioxidant activity (Pellegrini et al., 2003; Shukitt-Hale et al., 2008; Beccaro et al., 2006).

358 Among the fruits belonging to major species, strawberry had the highest total antioxidant
359 capacity (40.84 mmol Fe²⁺/kg fw), in agreement with Szeto et al. (2002). Strawberries were
360 also higher in antioxidant capacity than other berry fruit such as gooseberry and raspberry, in

361 agreement with Kalt et al. (1999). No statistical differences were found between apple, apricot,
362 nectarine, pear and plum. On the contrary, plums and Damask plums had the highest FRAP
363 values; in particular the 'Ramassin' landraces had 17.88 mmol Fe²⁺/kg fw against the 13.02
364 mmol Fe²⁺/kg fw of Chino-Japanese plums and 12.12 mmol Fe²⁺/kg fw of European plums.
365 This data is in agreement with those reported by Pellegrini et al. (2003), Szeto et al. (2002) and
366 Gil et al. (2002) that described a higher antioxidant activity of plums in comparison to other
367 stone fruits, in particular nectarines. Among major fruit species kiwifruit recorded an
368 antioxidant capacity similar to those observed for the others peeled fruits with values in
369 agreement with Szeto et al. (2002).

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

373 'Elliot' had the highest total antioxidant capacity between blueberry cultivars (71.23
374 mmol Fe²⁺/kg fw), followed by 'Duke' (47.83 mmol Fe²⁺/kg fw) and 'Bluetta' with the lowest
375 value (29.30 mmol Fe²⁺/kg fw). Significant differences in FRAP were observed in black
376 currants. 'Tiben' and 'Ben Lomond' had the highest antioxidant capacity (79.60 and 68.53
377 mmol Fe²⁺/kg fw, respectively). The gooseberry cultivar 'Hynnomaky Rot' showed twice as
378 much antioxidant capacity than 'Hynnomaky Gelb' (24.07 mmol Fe²⁺/kg fw and 12.76 mmol
379 Fe²⁺/kg fw, respectively). Among black mulberry cultivars, 'Selection 1' recorded the highest
380 FRAP (77.15 mmol Fe²⁺/kg fw). Between nectarine cultivars, 'Caldesi 2000' showed the
381 highest FRAP value (12.73 mmol Fe²⁺/kg fw) despite the fact that no statistical differences
382 were observed with 'Big Top' (9.83 mmol Fe²⁺/kg fw). 'Venus' had the lowest total antioxidant
383 capacity (5.57 mmol Fe²⁺/kg fw) without statistical differences with 'Big Top'. 'Ramassin
384 landrace 2' showed the highest FRAP (27.73 mmol Fe²⁺/kg fw), twice as much as the other
385 plum cultivars (11.63-13.80 mmol Fe²⁺/kg fw). Among strawberry cultivars, 'Arosa' showed
386 the highest FRAP (74.97 mmol Fe²⁺/kg fw), statistically different from 'Alba' (24.03 mmol

387 $\text{Fe}^{2+}/\text{kg fw}$) and 'Maya' (23.53 $\text{mmol Fe}^{2+}/\text{kg fw}$). These differences are related principally to
388 the colour of the flesh and in particular of the skin, where the highest content of anthocyanins
389 and carotenoids is concentrated, and with genotype and the size of the fruit (skin/flesh ratio)
390 (Howard et al., 2003).

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

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

413 upper right part of the plot the berry fruits high in phenolics, anthocyanins and FRAP were
414 placed.

415

416 **Conclusion**

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

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

431 Including cultivation and post harvest considerations, the berry species with higher
432 chances of a larger direct use is highbush bluberry. Damask plum showed intermediate
433 properties between berry and major species and their consumption should be encouraged. In
434 Piedmont, Damask plums are sold in local markets being frequently found in small orchards
435 and family gardens; they are consumed during summer mostly as fresh fruit but are also
436 processed for the production of ice-creams. The intake of phenolics and antioxidants from
437 seasonal fruit during autumn and winter would be potentially lower than in summer, due to the

438 type of local fruit available (apple, pears, kiwifruit) but in the global market the offer of fruit
439 and vegetables is large enough to provide products from other areas, such as citrus fruits or
440 berry fruits from the Southern hemisphere, that can help the needs.

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

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553

554 **Tables**

555

556 **Table 1.**

557 **Soluble solids content (SSC; °Brix), titratable acidity (TA; meq L⁻¹), pH, total phenolic content (TPC; mg GAE**
 558 **100 g⁻¹ fw), total anthocyanin content (TAnC; mg cyanidin-3-glucoside equivalents 100 g⁻¹ fw) and total**
 559 **antioxidant capacity (FRAP; mmol Fe²⁺ kg⁻¹ fw) in the studied species.**

Species	SSC	pH	TA	TPC	TAnC	FRAP
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Apple (<i>Malus x domestica</i> Borkh.)	13.24cd	3.79d	47.43gh	56.84g	0.03d	9.43e
Apricot (<i>Prunus armeniaca</i> L.)	13.63cd	3.41defg	257.99bc	93.72fg	0.99d	11.11e
Blackberry (<i>Rubus ulmifolius</i> L.)	12.53cde	3.27efghi	148.77ef	257.47cde	106.40c	79.17ab
Highbush blueberry (<i>Vaccinium corymbosum</i> L.)	11.90cdef	3.38defgh	129.74efg	320.39bcd	222.74b	49.45c
Black currant (<i>Ribes nigrum</i> L.)	13.89bc	2.91i	177.02cdef	493.39a	224.79b	58.43bc
White currant (<i>Ribes rubrum</i> L.)	10.73def	3.05ghi	361.27a	362.15b	0.93d	85.97a
Gooseberry (<i>Ribes grossularia</i> L.)	13.57cd	3.07fghi	280.93ab	196.98ef	3.20d	18.40de
Kiwifruit (<i>Actinidia deliciosa</i> Chev.)	11.77cdef	3.43defg	181.33cde	62.83g	-	8.50e
Black mulberry (<i>Morus nigra</i> L.)	14.47bc	5.04b	40.67gh	342.92bc	341.53a	56.50bc
White mulberry (<i>Morus alba</i> L.)	21.60a	6.04a	21.90h	95.91fg	1.99d	11.51e
Nectarine [<i>Prunus persica</i> (L.) Batsch]	9.15fg	3.66de	91.15fgh	39.88g	6.22d	9.38e
Pear (<i>Pirus communis</i> L.)	14.41bc	4.44c	34.48h	46.18g	1.79d	8.07e
Chino-Japanese plum (<i>Prunus salicina</i> Lindl.)	14.81bc	3.27efghi	166.84def	176.61ef	33.38cd	13.02e
European plum (<i>Prunus domestica</i> L.)	16.73b	3.48def	159.58ef	175.30ef	13.70d	12.12e
Damask plum (<i>Prunus insititia</i> L.)	14.82bc	3.43defg	153.19ef	229.62de	33.11cd	17.88de
Raspberry (<i>Rubus idaeus</i> L.)	9.70efg	2.96hi	249.84bcd	321.04bcd	40.09cd	12.92e
Strawberry (<i>Fragaria x ananassa</i> Duch.)	6.71g	4.35c	53.09gh	398.67ab	44.43cd	40.84cd

Means followed by the same letter in a column are not significantly different at $p = 0.05$ (Tukey's test).

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563 **Table 2.**

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

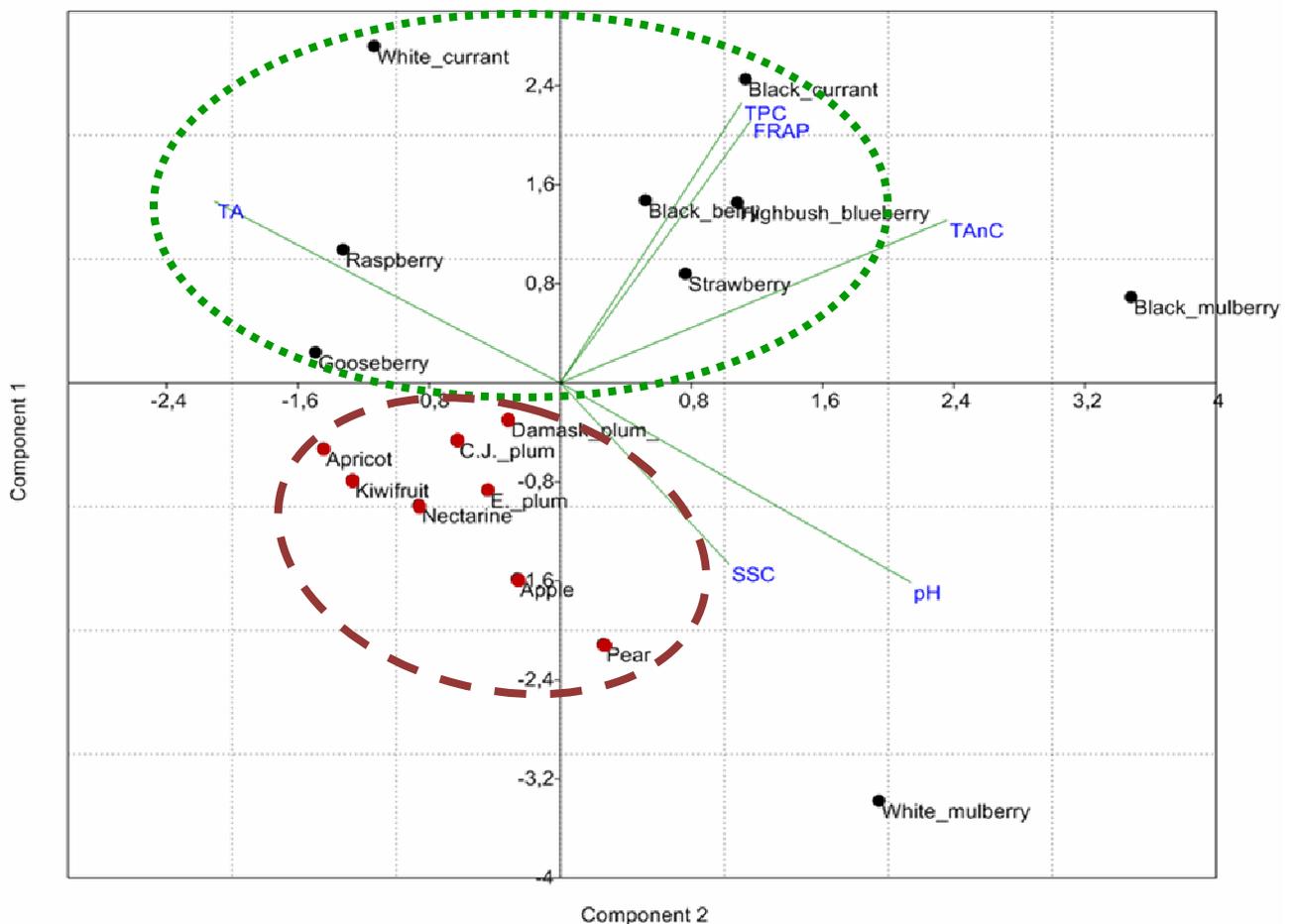
Species	Cultivar/landrace	Skin colour	SSC	pH	TA	TPC	TAnC	FRAP
Apple	Gala Brookfield	red striped	13.00b	3.75ab	50.60a	57.07	0.03	9.27
	Red Chief	red	12.93b	4.08a	35.50b	63.57	0.03	0.47
	Golden Delicious	yellow-orange	13.80a	3.53b	56.20a	49.80	0.03	8.30
Apricot	Tonda di Costigliole	yellow-orange	14.73a	3.72a	175.33c	97.77ab	1.03	0.46
	Goldrich	orange	12.07c	3.09c	359.80a	119.03a	0.57	11.57
	Laycot	orange-red	14.10b	3.43b	238.83b	64.33b	1.37	11.10
Blackberry	Navaho	black	12.53	3.27	148.77	257.47	106.40	79.17
Highbush blueberry	Bluetta	blue	11.67b	3.41ab	90.43b	348.87	249.13	29.30c
	Duke	light blue	10.93c	3.55a	107.07b	311.37	200.90	47.83b
	Elliott	light blue	13.27a	3.17b	191.73a	300.93	218.20	71.23a
Black currant	Ben Lomond	black	14.27a	3.07a	162.37	626.84a	218.93ab	68.53a
	Tiben	black	13.73ab	2.64b	216.97	448.32b	271.33a	79.60a
	Titania	black	13.67b	3.03a	151.74	405.03b	184.10b	27.17b
White currant	Werdavia	yellow-white	10.73	3.05	361.27	362.15	0.06	85.97
Gooseberry	Hynnomaky Gelb	dark yellow	13.80	3.07	253.34b	184.03b	0.44b	12.76b
	Hynnomaky Rot	dark red	13.33	3.06	308.53a	209.98a	5.99a	24.07a
Kiwifruit	Hayward	brown	11.77	3.43	181.33	62.83	-	8.50
Black mulberry	Selection 1	black	12.67b	5.01	47.63a	570.29a	486.15a	77.15a
	Selection 2	black	16.27a	5.06	33.70b	280.76b	196.89b	35.85b
White mulberry	Selection 3	pinkish white	21.60	6.04	21.90	95.91	1.99	11.51
Nectarine	Caldesi 2000	green-red	9.13	3.53b	104.60a	72.73a	2.90	12.73a
	Big top	yellow-red	9.33	3.95a	73.53b	16.40b	6.97	9.83ab
	Venus	yellow-red	9.00	3.50b	95.33ab	30.53b	8.80	5.57b
Pear	William	light green-yellow	13.37	4.29b	34.30	47.93	2.70b	1.27
	Abate fetel	yellow-red	14.47	4.25b	33.70	60.30	8.67a	2.57
	Conference	green-yellow bronze	15.40	4.79a	35.43	30.33	12.83a	1.53
Chino-Japanese plum	Angeleno	black-purple	13.20b	3.18b	159.37b	237.37a	43.53a	13.40
	TC sun	yellow-orange	13.10b	3.24b	194.40a	143.27b	8.67b	13.80
	Black diamond	black-purple	18.13a	3.38a	146.77b	149.20b	47.93a	11.87
Damask plum	Ramassin landrace1	blu	15.40	3.54a	97.33c	187.00b	19.43c	12.80b
	Ramassin landrace2	violet	14.27	3.51a	136.33b	264.83a	29.27b	27.73a
	Ramassin landrace3	violet	14.80	3.23b	225.90a	237.03a	50.63a	13.10b
European plum	Stanley	dark blue-violet	13.50c	3.51b	111.13b	172.40ab	19.77a	12.17
	President	red-violet	15.57b	3.27c	201.20a	140.63b	6.63b	12.57

	D' Ente 707	red-violet	21.13a	3.66a	166.46a	212.87a	14.70ab	11.63
Raspberry	Heritage	light red	10.53a	2.64c	350.00a	287.70b	62.50a	14.27
	Malahat	medium red	8.47c	3.14a	215.57b	372.60a	21.90b	0.56
	Tulameen	medium red	9.80b	3.03b	179.07b	330.00ab	25.63b	11.33
Strawberry	Alba	bright red	7.47	4.10b	66.17a	310.17b	46.80	24.03b
	Arosa	bright red	7.17	4.28b	55.47ab	461.46a	37.07	74.97a
	Maya	bright red	5.50	4.66a	37.63b	424.33ab	49.43	23.53b

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

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571 Figures



572

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Figure 1

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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).

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C.J._plum (Chino-Japanese plum); E._plum (European plum).

- 578 ● Major species
- 579 ● Berry species
- 580