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ARTICLE

# Analysis of Phenolic Compounds in Six Norwegian Plum Cultivars (*Prunus domestica* L.)

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Six European plum cultivars (*Prunus domestica* L.) grown in Norway have been studied with respect to phenolic composition. Neochlorogenic acid was found to be the most important phenolic acid in all cultivars. Together with other phenolic acids, this compound varied significantly in amount among the cultivars. Cyanidin 3-rutinoside was found to account for >60% of the total anthocyanin content. Minor amounts of flavonols (rutin and quercetin 3-glucoside) were detected in all cultivars. Total antioxidant capacity varied from 814 to 290 $\mu$mol of Trolox 100 $g^{-1}$ of fresh weight. Measurement of total phenolic content in terms of Prussian blue complex formation revealed a method failure of magnitude order compared to results obtained by HPLC. Comparison of the response factors of a range of phenolic compounds obtained upon analysis by the Prussian blue and Folin−Ciocalteu assays revealed that the latter method returned higher yields in terms of gallic acid (GAE).

**KEYWORDS: European plum; phenolic content; chlorogenic acids; anthocyanins; flavonols; Prussian blue assay; Folin−Ciocalteu assay; TEAC; ABTS; HPLC; LCMS**

## INTRODUCTION

The content of phenolic substances in fruit is of valuable interest for a number of reasons: There is a correlation between taste (astringency, bitterness) and content of phenolic compounds (*1*). Second, phenolics seem to play an important role in the natural defense mechanisms in fruit (e.g., antifungal effects) (*2*). In addition, increasing interest in the health benefits of fruits and fruit products is associated with the content of different groups of phenolic compounds with antioxidative effects (*3*). Compared to other fruits, the content of antioxidants in plums is high (*4*). Finally, the color of fruits is an important factor for consumers' preference and resale (*5*).

The most important group of phenolics in European plums (*Prunus domestica* L.) is the hydroxycinnamoylquinic acid esters (*6*−*8*). In particular, three isomers of caffeoylquinic acid (CQA) have been reported to dominate: neochlorogenic acid (3′-CQA), cryptochlorogenic acid (4′-CQA), and chlorogenic acid (5′-CQA). In addition 3′-coumaroylquinic acid occurs at elevated levels. Also, free caffeic acid together with protocatechuic, coumaric, and ferulic acids have been found in these fruits (*9*). Compounds from four classes of flavonoids have hitherto been described from *P. domestica* (L.) cultivars: anthocyanins, flavonols, flavanols, and proanthocyanidins. The 3-rutinoside and 3-glucoside of cyanidin are the major anthocyanins, with smaller amounts of the corresponding peonidin derivatives (*7*, *10*). Analysis of hydrolyzed plum extracts revealed the presence of quercetin, kaempferol, and myricetin in sliding quantities (*9*). Among the flavonols, only rutin (quercetin 3-rutinoside) has to our knowledge been completely characterized from nonhydrolyzed plum extracts by use of nuclear magnetic resonance spectroscopic and mass spectrometric methods (*7*, *10*). Among the flavanols, catechin has been found to account for 4−8% of the total phenolics in some cultivars (*7*, *11*), whereas epicatechin is present in lower amounts (*12*). In addition, two dimeric flavanols, procyanidins B3 and B4, have recently been detected in some cultivars (*12*, *13*).

The major cultivars in Norwegian plum production are *P. domestica* (L.) types. The major European plum cultivars grown in central and eastern Europe are prune types with dark blue oval fruits. In this study cultivars Jubileum and Valor have dark, blue fruits. The fruits of cultivars Avalon, Excalibur, Reeves, and Victoria are yellow with red blush color (*14*). The present paper reports on the analysis of phenolic compounds from six Norwegian-grown plum cultivars (*P. domestica* L.). In addition, we discuss the choice of method with respect to total phenolic measurements in plums and other fruits and vegetables enriched in similar phenolic compounds.

## MATERIALS AND METHODS

**Plant Material.** Six plum cultivars (*P. domestica* L.) were included in this study. The standard cultivar Victoria is an old English cultivar with yellow fruits with red blush. The five other cultivars are new cultivars recommended for commercial plum growing in Norway. Cultivars Avalon and Excalibur are both bred in the United Kingdom (*15*). The fruits of cv. Avalon are red and ripen under Norwegian growing conditions in late August, 1 week earlier than cv. Victoria. The fruits of cv. Excalibur are yellow with partly red blush. Ripening time is similar to that of cv. Victoria. Cultivar Reeves is a Canadian cultivar (*16*) with rounded large fruits. The color is yellow with a red blush, also ripening at the same time as cv. Victoria. The Swedish cultivar Jubileum has large oval blue fruits

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ripening 1 week later than cv. Victoria (*17*). Cultivar Valor is a Canadian cultivar (*18*). The fruits are oval and dark blue, ripening 2 weeks later than cv. Victoria.

**Growing Conditions.** The plums were grown in the experimental orchard at Bioforsk Vest Ullensvang Research Centre in Western Norway. Orchard management, fertilization, and plant protection were performed as recommended for commercial orchards in the area. The fruits were thinned by hand to 12−15 fruits per meter of branch in mid-June when the fruitlets were approximately 10 mm in diameter. The fruits were picked at a tree-ripe stage, and fruits of uniform size and ripening stage were selected for the analyses. Sampling was performed both in 2007 and in 2008. Soon after picking, the single fruits were frozen and kept at −20 °C until transportation in frozen condition to PlantChem for further analyses.

**Chemicals and Standards.** Chlorogenic acid (5′-caffeoylquinic acid), rutin (quercetin 3-rhamnosylglucoside), naringin, (+)-catechin, (−)-epicatechin, caffeic, ferulic, *p*-hydroxybenzoic, *p*-coumaric, sinapic, gallic, and trifluoroacetic acid (TFA), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium dihydrogenphosphate, sodium hydroxide, sodium chloride, acetonitrile, sodium tungstate, phosphomolybdic acid, hydrochloric acid, orthophosphoric acid, lithium sulfate, bromine, sodium carbonate, and potassium peroxosulfate were obtained from Sigma-Aldrich (Oslo, Norway). Methanol was obtained from Statoil (Stavanger, Norway). Ethyl acetate was purchased from OneMed AS (Oslo, Norway). Quercetin 3-glucoside and cyanidin 3-glucoside were supplied by PlantChem (Klepp, Norway).

**Extraction.** Two plums, each cut in four pieces, were extracted in 100 mL of methanol (0.5% TFA) for 24 h. The plums were finally pressed by hand, and the total volume was adjusted to 150 mL by acidic methanol. The extracts were prepared for HPLC analysis, analysis of total phenolics, and analysis of antioxidants by the Trolox equivalent antioxidant capacity (TEAC) assay.

**Total Phenolics.** The method of Price and Butler (*19*) as modified by Graham (*20*) was used to determine total phenolic content in the extracts. The principle of the assay is based on the reduction of ferric ions by phenolics followed by the generation of a measurable blue complex, ferrous cyanide, $Fe_4[Fe(CN_6)]_3\ (H_2O)_x$, where $14 \leq x \leq 16$. This visible complex is known as Prussian blue. A 20 mM solution of $FeCl_3$ was prepared in 0.10 M HCl, in addition to a 16 mM solution of $K_3Fe(CN)_6$ in pure water. Stabilizer was made by combining 30 mL of distilled water, 10 mL of 85% $H_3PO_4$, and 10 mL of 1% gum arabic. Sample aliquots of 0.1 mL were mixed with 3 mL of destilled water and 1 mL of each of the $FeCl_3$ and $K_3Fe(CN)_6$ solutions. After 15 min at ambient temperature, 5 mL of stabilizer was added to each sample, and absorbance was read at 700 nm (Agilent 8453 UV−vis spectrophotometer, Agilent Technologies). According to Graham's modifications, the method should be less sensitive for color change after the addition of stabilizer. However, some changes were observed, and hence all measurements were done within 1 min after the addition of stabilizer. Absorbance was read at 700 nm, and the results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (FW).

The Folin−Ciocalteu method for determination of total phenolics was used in accordance with the description of Waterman and Mole (*21*). To 200 mL of deionized water were added 50 g of $Na_2WO_4$, 6.13 g of $H_3PMo_{12}O_{40}$, 25 mL of concentrated HCl, and 12.5 mL of 85% *o*-$H_3PO_4$, and the solution was refluxed for 10 h. A few drops of $Br_2$ (liq) was added, and the final volume was adjusted to 250 mL. Sample (200 $\mu$L) was vortexed with about 10 mL of destilled water and 1 mL of Folin−Ciocalteu reagent. After 1 min and before 8 min, 3.75 mL of a 20 g/100 mL $Na_2CO_3$ solution was added, and time was recorded as time zero. The volume was made up to 20 mL with distilled water, and the solution was vortexed three to four times during the next 2 h. After exactly 2 h, the absorbance was recorded at 760 nm.

To determine the response of specific phenolics by use of these two methods, several authentic aromatic acids and flavonoids were analyzed. About 10 mg of each compound was accurately weighed and dissolved in 100 mL of methanol. The standards were assayed, and results were calculated as absorbance per mole of sample divided on the absorbance per mole of gallic acid (GAE).

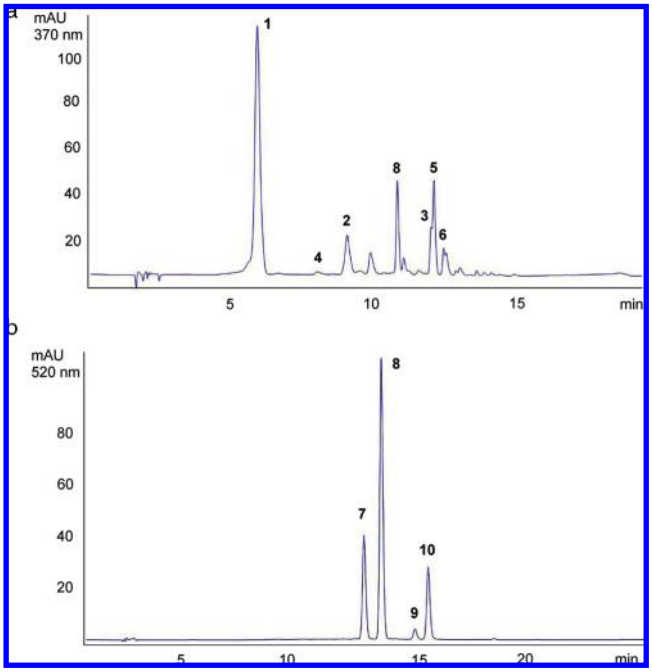


**Figure 1.** HPLC chromatograms of methanolic plum extracts: (**a**) cv. Valor detected at 370 nm; (**b**) anthocyanins in cv. Jubileum detected at 520 nm. For compound identities see **Table 1**.

**TEAC Analyses.** Scavenging capacities of the stable $ABTS^{\bullet}$ radical were measured according to a method slightly modified from that of Re and co-workers (*22*). Phosphate-buffered saline solution (PBS) was prepared by mixing 100 mL of a 100 mM $KH_2PO_4$ buffer (pH 7.4) with 1.5621 g of NaCl (150 mM final concentration). ABTS was dissolved in PBS to give a 7 mM ABTS solution. $ABTS^{\bullet+}$ was produced by the addition of 1.72 mg of potassium peroxosulfate (2.45 mM), and the solution was kept at room temperature for 16 h before use. The solution was diluted with PBS to give an absorbance of 0.70 ± 0.02 at 734 nm. Analyses were performed on an Agilent 8453 UV−vis spectrophotometer, and PBS was used to zero the instrument. Three milliliters of the $ABTS^{\bullet+}$ solution was mixed with 100 $\mu$L of sample and kept at room temperature for exactly 6 min before measurement at 734 nm. Trolox solutions (1−10 mg of Trolox in 100 mL of methanol) were used for calibration.

**HPLC.** A liquid chromatography system (Agilent 1100 system, Agilent Technologies) was used for the analysis of individual phenolics. Separation took place over an Eclipse XDB-C8 (4.6 × 150 mm, 5 $\mu$m) column (Agilent Technologies) by use of a binary solvent system consisting of (A) 0.05% TFA in water and (B) 0.05% TFA in acetonitrile. The gradient (percent B in A) was linear from 5 to 10 in 5 min, from 10 to 25 for the next 5 min, from 25 to 85 in 6 min, and from 85 to 5 in 2 min, and finally the column was reconditioned with 5% in 2 min. All HPLC samples were filtered through a 13 mm syringe filter (Nylon 0.45 $\mu$m, VWR International) prior to injection. The flow rate was 0.8 mL $min^{-1}$, 10 $\mu$L samples were injected on the column, and separation took place at 30 °C. Phenolic acids were recorded at 320 nm and flavonols at 370 nm, whereas anthocyanins were recorded at 520 nm. Standard curves of chlorogenic acid, rutin, and cyanidin 3-glucoside were used for quantification of compounds belonging to phenolic acids, flavonols, and anthocyanins, respectively. Analysis of flavanols was performed using the same column and solvent systems as described above. An isocratic elution with 100% A was kept for 10 min. Then a linear gradient (percent B in A) was followed from 0 to 100 B in 26 min and from 100 to 5 for the next 2 min, and finally the column was reconditioned with 100% A in 2 min. The flow rate was set to 0.5 mL $min^{-1}$. Detection was made by use of a fluorescence detector (HP 1046A) with excitation at 276 nm and emission at 316 nm.

**Mass Spectrometry.** The individual peaks were further characterized by liquid chromatography coupled with mass spectrometry (LCMS) using an Acquity UPLC (Waters) connected to a QTOF micro (Waters) with Lockspray mass calibration at Stavanger University Hospital. Reversed phase separations were achieved on a custom-made 0.5×225 mm HotSep

column (G&T Septech, Norway) packed with PLRP-S particles (Polymer Laboratories, U.K.) of 3 $\mu$m diameter and 1000 Å pore size. The mobile phase gradient was a mixture of (A) 0.1% formic acid and (B) acetonitrile, starting with 5% B for 2 min and then a linear increase to 60% B in 15 min. The flow rate was 20 mL min$^{-1}$. Electrospray ionization in the positive mode (ESI+) was used with a capillary voltage at 3 kV and a cone voltage at 35 V.

**Statistics.** The results were subjected to analysis of variance using the GLM procedure from the SAS statistical computer program (version 9.1). The SNK test was used to determine significant differences between cultivars and years. Correlations between parameters were calculated using the CORR procedure in SAS.

## RESULTS AND DISCUSSION

**Phenolic Compounds.** Fruits from four cultivars of the European plum, *P. domestica* L., sampled at Ullensvang, Norway, in 2007 and 2008, were analyzed with respect to their content of total phenolics, antioxidant capacities, and content of specific phenolic compounds. In addition, another two cultivars were analyzed in 2008. The compounds (**1**−**10**) were characterized and assigned according to their chromatographic and spectral behaviors and were found to belong to three groups of phenolic compounds: phenolic acids, flavonols, and anthocyanins (**Figure 1**; **Table 1**).

Compound **2** occurred with the same retention value as authentic chlorogenic acid (5′-CQA) and had an identical absorbance spectrum. Mass spectrometric analysis revealed a fragment ion corresponding to caffeoyl ($m/z$ 163.024), whereas the pseudomolecular ion was found to be $m/z$ 355.053. The compound was thus assigned to be 5′-CQA. Compound **1** had similar UV absorbance and mass spectral features as authentic chlorogenic acid, and the different retention values of both **1** and **3** from that of **2** were thus used to assign the compounds to be neochlorogenic acid (**1**, 3′-CQA) and cryptochlorogenic acid (**3**, 4′-CQA) in accordance with the results of Nakatani and co-workers (*8*). Another compound (**4**) with similar chromatographic behavior but with an absorbance maximum in accordance with that of coumaric acid was tentatively assigned as coumaroylquinic acid, in agreement with reports on 3′-coumaroylquinic acid (*7*).

Two flavonols (**5** and **6**) were detected in all samples, **5** being the major compound (**Figure 1**). This compound coeluted on HPLC with authentic rutin, and its UV and MS features were also in agreement with rutin. Another compound revealed an absorbance spectrum identical to that of rutin and had a molecular mass 146 amu below that of rutin, indicating the absence of the rhamnose unit. Upon cochromatography with an authentic compound, **6** was assigned to be quercetin 3-glucoside (isoquercitrin). Whereas rutin has been reported to occur in this species previously (*7*, *10*), this is to our knowledge the first report of isoquercitrin from *P. domestica* L. Although the number of reports on flavonols in plums (*P. domestica* L.) is scarce, several flavonols have been detected in, for example, *Prunus salicina*: quercetin pentoxyl hexoside, quercetin glucoside, quercetin rutinoside, quercetin pentosyl pentoside, quercetin xyloside, and quercetin rhamnoside (*23*). In the present work four anthocyanins (**7**−**10**) were characterized (**Table 1**). The two most frequent ones were found to be the 3-rutinoside and 3-glucoside of cyanidin, whereas the corresponding peonidin glycosides were found to occur irregularly. The presence of these four structures is in agreement with earlier descriptions (*24*). Interestingly, neither catechin nor epicatechin was detected in any sample even when using the HPLC-fluorescence method. However, some additional minor peaks appeared in the chromatograms by use of this detection method, but these compounds were not further investigated. The absence of flavanols (even in peel samples) of Norwegian grown plums is not in accordance with reports from other countries (*7*, *11*−*13*), and this discrepancy has to be further studied.

**Cultivar Variations.** With respect to concentration levels, neochlorogenic acid was found to be the major phenolic compound

**Table 1.** Chromatographic and Spectral Characteristics of Chlorogenic Acids and Flavonoids in Norwegian Plum Cultivars (*Prunus domestica* L.)

| | compound[a] | $t_R$, min | $\lambda_{max}$, nm | $[M + H]^+$ | fragment ions |
|---|---|---|---|---|---|
| **1** | 3′-CQA | 6.30 | 326 | 355.053 | 163.024 |
| **2** | 5′-CQA | 9.21 | 326 | 355.053 | 163.024 |
| **3** | 4′-CQA[b] | 12.03 | 327 | | |
| **4** | 3′-CoQA[b] | 8.34 | 311 | | |
| **5** | quercetin 3-rutinoside | 12.16 | 355, 258 | 611.208 | 303.057; 465.081; 633.136 |
| **6** | quercetin 3-glucoside | 12.57 | 354, 258 | 465.145 | 303.057; 487.014 |
| **7** | cyanidin 3-glucoside | 12.34 | 517, 280 | 449.142 | 287.004 |
| **8** | cyanidin 3-rutinoside | 13.10 | 518, 281 | 595.270 | 287.195; 449.206 |
| **9** | peonidin 3-glucoside | 14.62 | 519 | | |
| **10** | peonidin 3-rutinoside | 15.21 | 520, 274 | 609.231 | 301.082; 463.105 |

[a] 3′-CQA, 3′-caffeoylquinic acid (neochlorogenic acid); 5′-CQA, 5′-caffeoylquinic acid (chlorogenic acid); 4′-CQA, 4′-caffeoylquinic acid (cryptochlorogenic acid); 3′-CoQA, 3′-hydroxycoumaroyl quinic acid. [b] Assignment of 3′-CoQA and 4′-CQA was done according to retention values and UV spectra in agreement with the results of Nakatani and co-workers (*8*).

**Table 2.** Total Phenolic Content (TPC) and Antioxidant Capacity (TEAC) Together with Content of Specific Phenolic Compounds (**1**−**10**) in Six Cultivars of European Plums (*Prunus domestica* L.) in 2008[a]

| | | | | mg 100 g$^{-1}$ of FW | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| cultivar | $n$ | TPC (mg of GAE 100 g$^{-1}$ of FW) | TEAC ($\mu$mol 100 g$^{-1}$ of FW) | **1**−**3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| Avalon | 15 | 27.8 b | 465 b | 77 c | 10 e | 0.37 ab | 0.08 ab | 0.50 a | 1.15 b | 0.01 a | 0.09 c |
| Excalibur | 15 | 19.7 a | 315 a | 51 b | 2 a | 0.49 abc | 0.19 b | 0.15 a | 0.29 a | 0.00 a | 0.00 a |
| Reeves | 12 | 18.4 a | 290 a | 28 a | 4 b | 0.12 a | 0.02 a | 0.10 a | 0.41 a | 0.00 a | 0.04 ab |
| Victoria | 8 | 44.3 c | 676 c | 137 d | 6 c | 0.71 bc | 0.11 ab | 0.21 a | 0.46 a | 0.00 a | 0.05 b |
| Jubileum | 3 | 41.2 c | 814 d | 124 d | 8 d | 0.95 c | 0.11 ab | 3.19 b | 6.19 c | 0.39 c | 0.00 a |
| Valor | 3 | 42.0 c | 747 cd | 158 e | 15 f | 0.81 bc | 0.14 ab | 4.14 c | 6.38 c | 0.05 b | 0.16 d |
| *P* value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.004 | <0.0001 |

[a] Means with the same letter are not significantly different.

**Table 3.** Total Phenolic Content (TPC) and Antioxidant Capacity (TEAC) Together with Content of Specific Phenolic Compounds (**1**−**10**) in Four Cultivars of European Plums (*Prunus domestica* L.) from Two Years

| | | | | mg 100 g$^{-1}$ of FW | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | TPC (mg of GAE 100 g$^{-1}$ of FW) | TEAC ($\mu$mol 100 g$^{-1}$ of FW) | **1**−**3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| cultivar | | | | | | | | | | | |
| Avalon | 30 | 19.5 b[b] | 393 b | 35 a | 33 b | 0.66 b | 0.10 b | 0.60 b[a] | 0.71 b | 0.02 b | 0.05 c |
| Excalibur | 30 | 13.7 a | 272 a | 29 a | 20 a | 0.61 b | 0.15 b | 0.28 a | 0.23 a | 0.00 a | 0.00 a |
| Reeves | 24 | 12.6 a | 246 a | 16 a | 14 a | 0.21 a | 0.03 a | 0.21 a | 0.23 a | 0.02 b | 0.02 b |
| Victoria | 12 | 31.1 c | 585 c | 97 b | 12 a | 1.18 c | 0.12 b | 0.22 a | 0.30 a | 0.01 ab | 0.03 b |
| *P* value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.00 | <0.0001 |
| year | | | | | | | | | | | |
| 2007 | 48 | 9.6 a | 281 a | 41 | 2 a | 0.80 b | 0.09 | 0.26 a | 0.16 a | 0.02 a | 0.00 a |
| 2008 | 48 | 25.2 b | 404 b | 31 | 41 b | 0.38 a | 0.10 | 0.45 b | 0.61 b | 0.00 b | 0.05 b |
| *P* value | | <0.0001 | <0.0001 | ns[c] (0.077) | <0.0001 | <0.0001 | ns[c] (0.57) | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

[a] The upper part of the table represents mean values for each cultivar for two years, whereas the lower part reports on the year-to-year variation as mean values of all four cultivars. [b] Means with the same letter are not significantly different. [c] ns, not significantly different at the 5% level.

in all cultivars (**Figure 1**; **Table 2**). Its concentration ranged from 23 mg 100$^{-1}$ g of FW in cv. Reeves to 153 mg 100$^{-1}$ g of FW in cv. Valor. A strong correlation was found with respect to the concentration of all the different phenolic acids in the six cultivars. The total content of phenolic acids was found to be 32 mg 100$^{-1}$ in cv. Reeves and 173 mg 100$^{-1}$ g in cv. Valor. The same two cultivars were found to be the outermost ones with respect to both flavonol and anthocyanin contents. Cultivar Reeves was found to contain 0.2 mg of flavonols and 0.7 mg of anthocyanins, whereas cv. Valor contained 1.0 and 10.7 mg 100$^{-1}$ g of flavonols and anthocyanins, respectively (**Table 2**). The level of flavonols was lower than that of anthocyanins in most cultivars. At least 50% of the total flavonol content was addressed to rutin, whereas cyanidin 3-rutinoside was found to be the major anthocyanin, responsible for 60% of the total anthocyanin content. The ability to quench free radicals was determined by use of the TEAC assay. The values ranged from 290 $\mu$mol 100 g$^{-1}$ of FW in cv. Reeves to 814 $\mu$mol for cv. Jubileum (**Table 2**). The TEAC values closely correlated with the amount of phenolics in the plums ($r = 0.97$).

Among the six cultivars analyzed, cvs. Jubileum and Valor had the highest contents of most measured compounds. Because the fruits of these cultivars are dark blue, the high contents of anthocyanins were expected. These cultivars also had high content of colorless chlorogenic acids and accordingly high TEAC assay values. Cultivar Victoria had high contents of chlorogenic acids compared to the other cultivars with yellow-red fruits. Hence, the TEAC values of cv. Victoria were higher than the TEAC values of cvs. Avalon, Excalibur, and Reeves.

Phenolic compounds contribute to the taste of bitterness or astringency in fruits (*1*). Of these plum cultivars included in this investigation, the fruits of cv. Reeves have not a very strong taste, whereas fruits of cv. Avalon are known for their very good and strong plum flavor (*25*). Even though the contents of all phenolic compounds measured in this study were lower in cv. Reeves than in cv. Avalon (not statistically different for some compounds), further studies including sensory analysis are needed to draw any conclusions on the possible correlations between contents of phenolic compounds and flavor of various plum cultivars.

**Year-to-Year Variation.** Analysis of total phenolic content (TPC), TEAC, and content of 3′-CQA revealed similar differences between the four cultivars in both 2007 and 2008 (**Table 3**). The values were found to be higher in the 2008 samples. Also with respect to the chlorogenic acid isomers the cultivar differences were found to be similar for both years. The amounts were higher in 2007, although this difference was not significant ($p > 0.05$). Content of anthocyanins was found to be significantly higher in 2008, although peonidin 3-glucoside occurred at higher values in 2007. Plums of cvs. Jubileum and Valor contained the highest amounts of anthocyanins in total (**Table 3**). Peonidin 3-rutinoside did not occur in cv. Jubileum.

Previous studies have found that hydroxycinnamates are present only as esters in this species and account for up to 90% of the total phenolic content (*7*). Neochlorogenic acid has been found to predominate in other cultivars (4.9−80.7 mg 100$^{-1}$ g of FW), followed by chlorogenic acid (3.1−14.4 mg 100$^{-1}$ g of FW) and 3′-coumaroylquinic acid (1.0 mg 100$^{-1}$ g of FW) (*7*, *9*). Catechin has been reported to account for 4−8% of the total phenolics, whereas flavonols, mainly rutin, accounted for 2−3%. Anthocyanins accounted for 4−9% of the total phenolic compounds in a red cultivar. The content of flavonoids in a Bulgarian cultivar was found to be 136.2 mg of catechin equivalents 100$^{-1}$ g of FW as determined by an aluminum chloride colorimetric assay for total flavonoid determination (*11*). The mean flavonoid content of two Croatian cultivars was found to be 113 mg 100$^{-1}$ g of FW (*26*). However, both of these measurements include the content of flavanols and condensed tannins, which have not been detected in the present investigation. (+)-Catechin alone has been found at levels from 1.3 to 3.9 mg 100$^{-1}$ g of FW in different cultivars of yellow plums together with varying levels of procyanidins B3 and B4 (*13*).

**Assay for Total Phenolics.** Total phenolic content (TPC) of the plum cultivars was found in the range of 18−44 mg GAE 100 g$^{-1}$ of FW (**Table 2**). However, the HPLC determinations of specific compounds revealed 32−143 mg of total phenolic acids 100 g$^{-1}$ of FW alone. Converted to GAE units, this corresponds to 16−76 mg of GAE 100 g$^{-1}$ of FW. In addition to the acids is the content of anthocyanins, flavonols, and other phenolics. It thus follows that the Prussian blue method seems to underestimate TPC in the samples. A closer inspection of this method versus different pure phenolics was therefore performed. The response factors of phenolic acids together with those of catechin, naringin, and rutin were determined on a molar-to-molar ratio (**Table 4**). *p*-Benzoic acid gave hardly any detectable Prussian blue complex. *p*-Coumaric, caffeic, and chlorogenic acids revealed response factors lower than 50% of gallic acid.

**Table 4.** Molar Response Factors for Authentic Compounds Measured as Gallic Acid Equivalents (GAE) by Use of the Prussian Blue Assay (PB) and the Folin−Ciocalteu Assay (F-C) for Determination of Total Phenolics (See Materials and Methods)

| | response factors | |
|---|---|---|
| compound | PB | F-C |
| gallic | 1.00 | 1.00 |
| *p*-benzoic acid | 0.03 | 0.39 |
| *p*-coumaric acid | 0.20 | 0.78 |
| caffeic acid | 0.37 | 1.03 |
| ferullic acid | 0.61 | 0.92 |
| sinapic acid | 0.63 | 1.02 |
| chlorogenic acid | 0.43 | 1.26 |
| syringic | 0.42 | 0.60 |
| (+)-catechin | 1.19 | 2.04 |
| naringin | 0.20 | 0.86 |
| rutin hydrate | 0.59 | 2.02 |

Ferulic and sinapic acids together with rutin responded 59−63% to that of gallic acid, whereas (+)-catechin was overestimated (111% GA).

Similar disagreements with the present case have been reported by use of a Folin−Ciocalteu-based method. The content of total polyphenols in Polish plums was found to be 160−300 mg 100 $g^{-1}$ by use of a Folin−Ciocalteu assay, whereas the specific measurement of anthocyanins and flavanols alone (phenolic acids excluded!) revealed contents of these compounds at 1833 and 914 mg 100 $g^{-1}$, respectively (*13*). Moreover, the mean phenolic content of two Croatian plum cultivars was found to be 203 mg of GAE 100 $g^{-1}$ of FW, whereas the content of flavonoids alone was found to be as much as 113 mg of GAE 100 $g^{-1}$ of FW. Both analyses were performed by use of Folin−Ciocalteu assays (*26*). By use of HPLC quantification the present investigation clearly demonstrates that flavonoids contribute to a lower extent to TPC in plums compared to chlorogenic acids.

Elsewhere, TPC in different cultivars of *P. domestica* L. have been reported to be within the range of 110−300 mg of GAE 100 $g^{-1}$ of FW (*7*, *11*, *13*, *26*, *27*). These values have also been obtained by use of the Folin−Ciocalteu method or variations thereof (*28*). On the basis of the variation in content of TPC in plums found in the literature, a comparison was set up between the response factors obtained by use of the Prussian blue assay and those obtained by use of a Folin−Ciocalteu assay (**Table 4**) (see Material and Methods). For most compounds the response factor measured as GAE was found to be 1.5−2.5 times higher when measured by the Folin−Ciocalteu assay (**Table 4**). Chlorogenic acid and rutin gave nearly 3 times the yields compared to those obtained by the Prussian blue assay, whereas the response of benzoic acid was found to be 13 times higher.

Hence, a Folin−Ciocalteu assay might be a preferred method of choice upon analysis of such phenolic compounds. However, literature data and the present demonstration imply that the use of both Prussian blue and Folin−Ciocalteu methods should be carefully evaluated before being used to determine total phenolic contents. Moreover, a reference compound should represent the analyte(s) in a satisfactory way with respect to determination of the actual concentration level of phenolics. The present inspection reveals that gallic acid accounts for only 43% of the actual content of chlorogenic acid when measured in the Prussian blue assay (**Table 4**). In this case, chlorogenic acid itself would probably be a better reference choice than gallic acid. The ability to make a straightforward comparison with literature data would, however, then disappear.

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