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Extraction of Polyphenols from Processed Black Currant (*Ribes nigrum* L.) Residues

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The total phenol and anthocyanin contents of black currant pomace and black currant press residue (BPR) extracts, extracted with formic acid in methanol or with methanol/water/acetic acid, were studied. Anthocyanins and other phenols were identified by means of reversed phase HPLC, and differences between the two plant materials were monitored. In all BPR extracts, phenol levels, determined by the Folin−Ciocalteu method, were 8−9 times higher than in the pomace extracts. Acid hydrolysis liberated a much higher concentration of phenols from the pomace than from the black currant press residue. HPLC analysis revealed that delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-rutinoside were the major anthocyanins and constituted the main phenol class (≈90%) in both types of black currant tissues tested. However, anthocyanins were present in considerably lower amounts in the pomace than in the BPR. In accordance with the total phenol content, the antioxidant activity determined by scavenging of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation, the $ABTS^{\bullet+}$ assay, showed that BPR extracts prepared by solvent extraction exhibited significantly higher (7−10 times) radical scavenging activity than the pomace extracts, and BPR anthocyanins contributed significantly (74 and 77%) to the observed high radical scavenging capacity of the corresponding extracts.

KEYWORDS: Black currant; *Ribes nigrum* L.; pomace; anthocyanins; HPLC; radical scavenging capacity; polyphenols

INTRODUCTION

Black currant (*Ribes nigrum*) is an important plant material for the food industry because of its distinctive color and organoleptic properties. It is cultivated primarily for the production of berries, which are used to prepare juice, liquors, jams, and other food products, believed to be beneficial for human health due to their high antioxidant content (*1*). Black currant antioxidants include an array of phenolic compounds, including phenolic acids and flavonoids (*2*−*4*) with anthocyanins being responsible for the dark color of the berries (*4*). These phenolic substances have been reported to induce health-promoting effects such as antimicrobial and anti-inflammatory action (*5*, *6*), reduced risk of cardiovascular disease (*7*, *8*), inhibition of the oxidation of human low-density lipoproteins (*9*), and anticarcinogenic effects (*10*, *11*). The beneficial effects of phenols have been extensively attributed to their antioxidant properties based on the scavenging of active oxygen species and free radicals (*1*, *12*, *13*). Extracts from black currant have been found to be the second most active radical scavenger among extracts from nine types of berries (*14*).

Solvent extraction of flavonoids and phenolic acids from porous solid fruit material, using liquid solvents, has been the most widely used method in fruit sample preparation. Phenols are generally stable compounds and have been extracted from fresh, dried, or freeze-dried plant material by using aqueous mixtures of methanol, ethanol, and acetone (*15*).

Most of the literature reports focus on black currant fruit and juice, and little work has been devoted to black currant juice byproducts. Although it has been demonstrated that black currant pomace left over from juice production provides a good source of phenolic antioxidants that may possess nutritional benefits (*16*), there is not enough information about the phenolic components in the pomace or the radical scavenging activity of these components.

This study reveals the levels of total phenols and anthocyanins in commercial black currant pomace (i.e., the remaining solid residue after black currant juice has been extracted with the aid of enzymes) and in black currant press residue (BPR) prepared in the laboratory. The aim of this research was also to evaluate black currant pomace as a residue that can be further processed for the extraction of polyphenol antioxidants and most notably anthocyanins. The isolated compounds could be used as natural food additives and colorants. Extractions with methanol/formic acid and with methanol/water/acetic acid were compared to investigate the effect of solvent on the yield and properties of extracts. Methanol was chosen as a good solvent for polyphenol extraction. The radical scavenging activity of all phenolic

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extracts was determined using the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation decolorization assay. The radical scavenging activity of the BPR extracts was compared with that of a model mixture of the anthocyanins present to assess to what extent the anthocyanins could account for the total activity of the extracts.

MATERIALS AND METHODS

Plant Material. *Pomace.* Black currant pomace was kindly supplied frozen by GlaxoSmithKline (GlaxoSmithKline, Coleford, U.K.). The sample contained skin, seeds, and stems. The pomace was freeze-dried, purged with nitrogen, and kept frozen (−18 °C) until further use.

Fruit. Black currant fruit was purchased from a local supermarket. Stems were removed, and the fruit sample was packed under nitrogen and kept frozen (−18 °C) until further treatment.

Chemicals and Reagents. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, ±6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin-3-rutinoside (rutin), caffeic acid, gallic acid, and formic acid were purchased from Sigma-Aldrich (Poole, U.K.). Folin−Ciocalteu reagent, sodium carbonate, and hydrochloric acid were purchased from BDH (Lutterworth, U.K.).

Cyanidin-3-rutinoside was generously donated by Dr. Christina Garcia-Viguera (CEBAS-CSIC, Murcia, Spain). All other anthocyanins (in the form of chloride salts) were purchased from Polyphenols Laboratories (Sandnes, Norway).

All solvents were supplied by Rathburn Chemical Co. (Walkerburn, Scotland) and were of HPLC grade.

Sample Preparation. *Pomace.* Freeze-dried black currant pomace was initially milled, in an APEX comminuting mill (type 214, Apex Construction Ltd., London, U.K.), for 30 s and passed through a 0.024 in. aperture sieve. The seeds were removed, and pomace was homogenized in a coffee grinder. Sample with particle size distribution 300−600 μm was obtained by using a two-stage sieving tower with aperture sizes of 300 and 600 μm. Finally, sample was packaged under nitrogen and immediately frozen until required.

Black Currant Press Residue (BPR). Black currant fruit was cut into several pieces and blended until a homogeneous paste was formed. A constant pressure of 5 bar for 2 h was then applied until the juice was removed. The skins comprising the BPR were collected. Sample was freeze-dried, milled, and sieved according to the same procedure used for pomace preparation.

Extraction of Phenols. Each extraction was carried out with 3% formic acid in methanol (solvent A) or with methanol/water/acetic acid (40:55:5) (solvent B). Ground lyophilized plant material (500 mg of pomace or 250 mg of BPR) was accurately weighed. Solvent A or solvent B (25 mL) was added, and the sample was stirred for 10 min. Clear supernatant was collected after centrifugation (4000 rpm, 10 min). The procedure was repeated (four times) with 25 mL of solvent until color was no longer released. Supernatants were combined and taken to dryness (solvent A) or to a very small volume (∼2−3 mL) (solvent B) using a rotary evaporator at 40 °C. The remaining phenolic concentrate was redissolved in either 5 mL of methanol (when solvent A was applied) or 5 mL of water (when solvent B was applied). Extracts were kept in the freezer (−18 °C) until analysis.

Acid Hydrolysis. After phenol extraction, the remaining plant residue was hydrolyzed to efficiently release cell wall-bound phenols and/or the remaining components with antioxidant activity. Anthocyanidins and other phenol groups were identified and quantified by means of high-performance liquid chromatography (HPLC) by comparison with the appropriate external standard.

The method used was similar to the method suggested previously by Hertog et al. (*17*). A 7 mL portion of acidified (hydrochloric acid; 2 M) 60% aqueous methanol was added to each sample and placed in an oven at 90 °C for 90 min exactly. Tubes were allowed to cool, and supernatants were filtered through a 0.45 μm filter. Extracts were kept frozen until analysis.

Analytical Methods. *Determination of Total Phenol Content.* Total phenol content (TPC) was determined according to the Folin−Ciocalteu procedure (*18*). Deionized water (1.8 mL) was added to 0.2 mL of sample appropriately diluted. Folin−Ciocalteu reagent (0.2 mL) was then added, and tubes were shaken vigorously. After 3 min, sodium carbonate solution (0.4 mL of 35% w v^{-1}) was added, along with 1.4 mL of deionized water. Samples were mixed well and left in the dark for 1 h. Absorbance values at 725 nm were converted to gallic acid equivalents (GAE) by using gallic acid as a reference compound.

HPLC Analysis of Anthocyanins, Flavonols, and Hydroxycinnamic Acids. Anthocyanins were analyzed by applying a previously developed RP-HPLC method (*19*).

The HPLC equipment comprised a Dionex ASI-100T automated sample injector, a Dionex P580 pump, and a Dionex PDA-100 photodiode array detector. Chromatograms were recorded and processed by Chromeleon chromatographic software.

Plant extract solution (20 μL) was injected onto a Kromasil reversed phase C-18 column (250 × 4.6 mm i.d., particle size = 5 μm). The temperature of the column was kept at 30 °C during the analysis. The flow rate was set at 1 mL min^{-1}. The mobile phase used was 5% formic acid (v v^{-1}) (solvent A) and methanol (solvent B), using a gradient starting with 15% (B), increasing to 35% (B) at 15 min and to 60% (B) at 30 min, and reaching 95% (B) at 40 min.

Detection was at 520, 360, and 320 nm for anthocyanins, flavonols, and hydroxycinnamic acids, respectively.

Quantification and Identification of Anthocyanins and Other Phenols. All phenols and anthocyanins were identified by their retention time and UV spectra compared with reference compounds. Phenols were quantified in three subclasses: anthocyanins (expressed as cyanidin-3-rutinoside equivalents; detected at 520 nm), flavonols (expressed as quercetin-3-rutinoside equivalents; detected at 360 nm), and hydroxycinnamic acids (expressed as caffeic acid equivalents; detected at 320 nm). In the acid-hydrolyzed extracts, anthocyanidins were described as the corresponding anthocyanin equivalent.

The total amounts of anthocyanins, flavonols, and hydroxycinnamates were calculated by the addition of the concentration of individual components, as described by Espin et al. (*13*).

ABTS Radical Scavenging Determination. A scanning spectrophotometer (Lambda 5 UV−vis), linked to a thermostatically controlled water bath and equipped with an automatic sample changer, was used for this study. Data were processed with UV WinLab (Perkin-Elmer) software and recorded automatically on a personal computer.

The method used was the $ABTS^{\bullet+}$ radical cation decolorization assay (*20*). The assay is based on the ability of an antioxidant compound to quench the $ABTS^{\bullet+}$ radical relative to that of a reference antioxidant (e.g., Trolox, a water-soluble vitamin E analogue).

Preparation of $ABTS^{\bullet+}$ Reagent. A stock solution of $ABTS^{\bullet+}$ radical cation was prepared by mixing ABTS solution and potassium persulfate solution, at 7 mM and 2.45 mM final concentrations, respectively. The mixture was maintained in the dark at room temperature for 12−16 h before use. The working $ABTS^{\bullet+}$ solution was produced by dilution in ethanol (1:90 v v^{-1}) of the stock solution to achieve an absorbance value of 0.7 (± 0.02) at 734 nm (at 30 °C).

Procedure. An aliquot (20 μL) of sample, ethanol (blank), or standard (Trolox) was added to $ABTS^{\bullet+}$ working solution (2 mL). Absorbance was measured at 734 nm immediately after the addition and rapid mixing ($A_{t=0}$) and then every minute for 6 min. Readings at $t = 0$ min ($A_{t=0}$) and $t = 5$ min ($A_{t=5}$) of reaction were used to calculate the percentage inhibition values of the extracts according to the following calculation:

$$\%\ \text{inhibition}_{\text{extract}} = (\text{inhibition}_{\text{sample}} - \text{inhibition}_{\text{blank}}) \times 100$$

$$= \left[\frac{(A_{t=0,\text{sample}} \times A_{t=5,\text{blank}}) - (A_{t=5,\text{sample}} \times A_{t=0,\text{blank}})}{A_{t=0,\text{sample}} \times A_{t=0,\text{blank}}}\right] \times 100$$

A standard reference curve was constructed by plotting percent inhibition values against concentration of Trolox. The radical scavenging activity of phenolic extracts was quantified as Trolox equivalents (mmol)/g of freeze-dried plant material.

Anthocyanin Contribution to the Total Radical Scavenging Activity of the Extracts. The contribution of anthocyanins to the radical scavenging activity of BPR extracts was studied by testing the ABTS radical scavenging activity of a model anthocyanin mixture containing

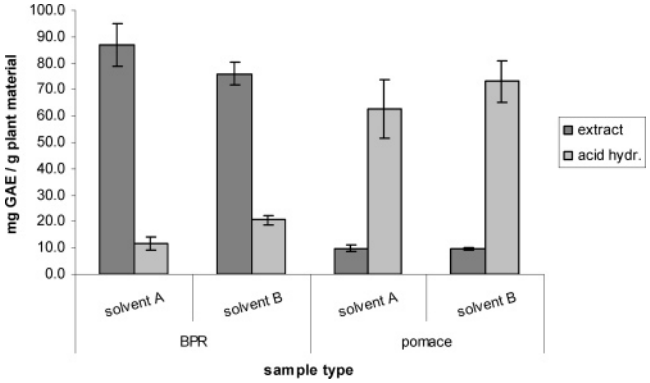

Figure 1. Total phenol determination of plant material by the Folin–Ciocalteu assay.

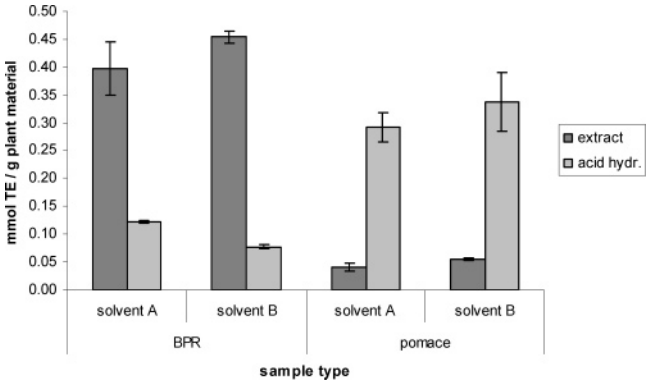

Figure 2. ABTS radical scavenging activity of black currant plant material.

the four major black currant anthocyanins at the concentration of the BPR anthocyanins determined by HPLC.

Statistical Analysis. The statistical program GenStat (6th ed.) was employed to analyze the numerical results by general linear regression. Significance of the responses in different experimental factors was determined by analysis of variance. Significant differences were established at the $P \leq 0.05$ statistical level.

RESULTS

Total Phenol Content. The level of total phenols varied depending on the plant tissue type (BPR or pomace) and different sample treatment methods (solvent extraction/acid hydrolysis, solvent type), ranging from 11.4 to 86.8 mg of GAE/g of material for BPR and from 9.4 to 73 mg of GAE/g of material for pomace (**Figure 1**). In all BPR extracts the concentration of phenols was 8−9 times higher than in the pomace extracts. Acidic hydrolysis after extraction allowed the release of residual phenols. When methanol/formic acid (solvent A) was used for extraction, phenol yields in the pomace extracts after acid hydrolysis were 3.5 times higher than in the BPR residue after acid hydrolysis, and the yield after acid hydrolysis of pomace extract was 5.5 times higher when solvent B (methanol/water/acetic acid) was used.

For solvent system B, phenol yields were higher when extracted before hydrolysis and lower after hydrolysis, whereas the opposite occurred when solvent system A was used (**Figure 1**).

ABTS$^{\bullet+}$ Radical Scavenging Capacity. Radical scavenging capacity varied widely from 0.04 to 0.45 mmol of Trolox equivalents/g of material (**Figure 2**).

The ABTS$^{\bullet+}$ radical scavenging activity was always higher for the BPR solvent extracts (10 times for solvent A and 7.5 times for solvent B) compared to the pomace extracts, but after acid hydrolysis, the residues from the pomace extracts showed higher scavenging activity than those from the BPR extracts (2.5 and 4.5 times), especially when the aqueous solvent mixture (solvent B) was used. Generally, solvent type used for extraction did not affect radical scavenging activity of the extract significantly, but plant type (BPR or pomace) had a significant effect ($P < 0.001$). Hydrolysis released a significant portion of radical scavenging activity in pomace ($P < 0.05$), but in BPR most of the radical scavenging activity was found in the solvent-extracted fraction before hydrolysis (**Figure 2**). The anthocyanins were responsible for most of the high ABTS$^{\bullet+}$ radical scavenging activity of the BPR extracts (**Table 4**).

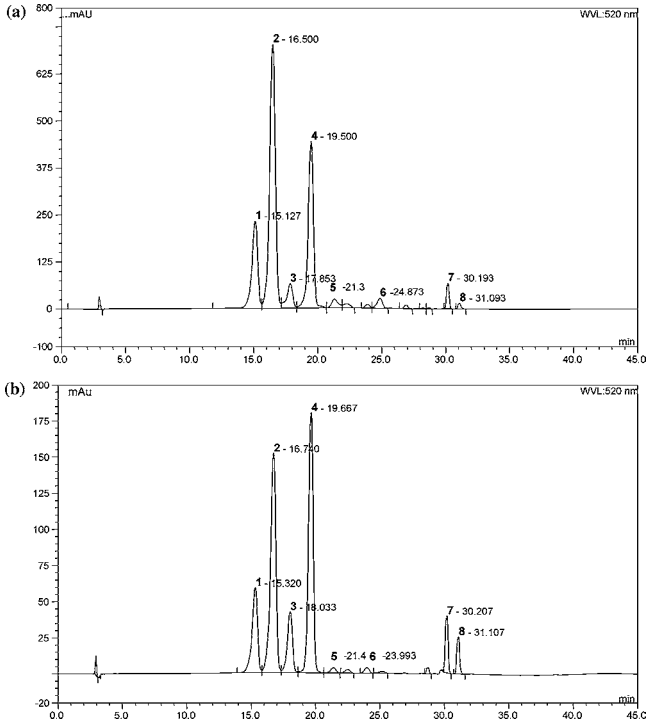

Figure 3. HPLC chromatograms of (**a**) BPR and (**b**) black currant pomace at 520 nm. For peak identity refer to **Table 1**.

Table 1. Anthocyanins in Black Currant Plant Material[a] Identified by HPLC[b]

sample treatment	peak	retention time (min)	identity
solvent extracted	1	15.3 (15.1)	delphinidin-3-glucoside
	2	16.7 (16.5)	delphinidin-3-rutinoside
	3	18.0 (17.9)	cyanidin-3-glucoside
	4	19.7 (19.5)	cyanidin-3-rutinoside
	5[c]	21.4 (21.3)	*petunidin-3-rutinoside*
	6[c]	24.0 (24.9)	*peonidin-3-rutinoside*
	7[c]	30.2 (30.2)	*delphinidin-acylated*
	8[c]	31.1 (31.1)	*cyanidin-acylated*
after acid hydrolysis	1′	24.4 (24.3)	delphinidin
	3′	27.9 (27.8)	cyanidin

[a] The term "plant material" refers to both BPR and pomace. Retention times for BPR are given in parentheses. [b] Chromatograms and peak manifestation were identical for either type (A or B) of solvent used. [c] Compounds identified by reference to the literature (peaks 5–8) constitute a diminutive (<3%) anthocyanin fraction. The identity of these compounds was not confirmed, although identified by other researchers (*21*, *28*, *30*).

Identification and Quantification of Anthocyanins. The anthocyanin concentrations in all extracts were determined. Flavonols and hydroxycinnamic acids were also quantified.

HPLC analysis (**Figure 3**) showed that the plant materials tested (BPR and pomace) were similar in the relative concentra-

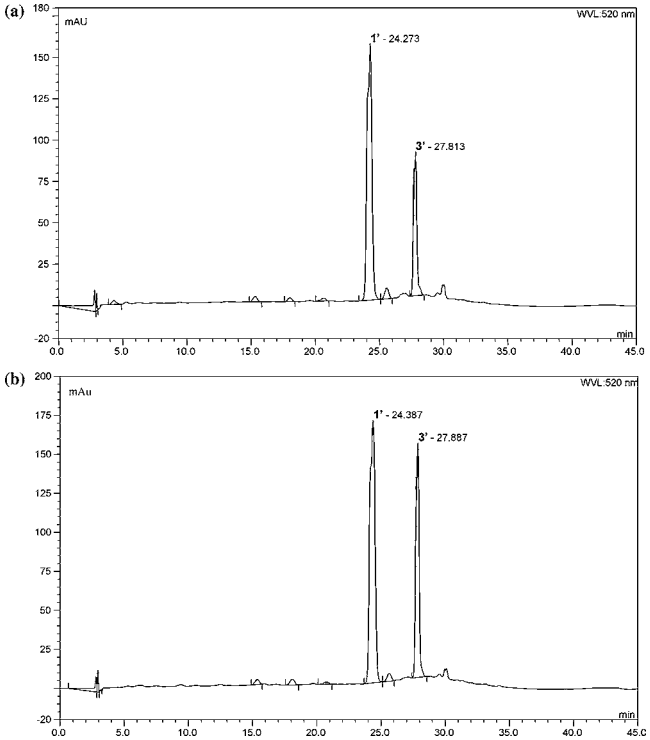

Figure 4. HPLC chromatograms of acid-hydrolyzed (**a**) BPR and (**b**) black currant pomace at 520 nm. For peak identity refer to **Table 1**.

tion of the anthocyanin components (**Table 1**). Peaks 1−4 were identified as delphinidin-3-glucoside (Dp-3-glu), delphinidin-3-rutinoside (Dp-3-rut), cyanidin-3-glucoside (Cy-3-glu), and cyanidin-3-rutinoside (Cy-3-rut) anthocyanin compounds, respectively. Additionally, four minor anthocyanins (peaks 5−8) were detected but could not be unequivocally identified, as standard compounds were not available. However, the data of Slimestad and Solheim (*21*) indicate that peaks 5 and 6 correspond to petunidin-3-rutinoside and peonidin-3-rutinoside, whereas peaks 7 and 8 correspond to delphinidin-3-(6″-coumaroylglucoside) and cyanidin-3-(6″-coumaroylglucoside) acylated compounds, respectively.

The samples that were acid hydrolyzed contained peaks 1′ and 3′ that correspond to the anthocyanidins delphinidin and cyanidin, respectively (**Figure 4**).

BPR had the highest anthocyanin contents (35 and 37 mg g^{-1} of freeze-dried material extracted with solvents A and B, respectively), whereas the concentrations of anthocyanins in pomace were ≈7 times lower (**Table 2**). Acid-hydrolyzed BPR contained very low amounts of anthocyanins, almost half of the amount found in the acid-hydrolyzed pomace extracts (7 and 3 mg g^{-1}).

Flavonol derivatives were present at higher concentrations (3.16 and 4.67 mg g^{-1}) in BPR, but in pomace levels were 5.5−8 times lower. No flavonol compounds were detected in the acid-hydrolyzed extracts, but this is most probably due to efficient solvent extraction prior to hydrolysis.

Hydroxycinnamic acids (HCA) were found in low amounts in BPR (0.61 mg g^{-1} for solvent A and 1.2 mg g^{-1} for solvent B), but even lower amounts (∼7 times lower) were identified in pomace. Interestingly, high levels of HCA were detected in the acid-hydrolyzed pomace extracts.

Statistical analysis showed that the yields of HCA and flavonols depended in a similar way on processing variables for the two types of plant material. All combinations of treatment, solvent, and plant type affected the yield significantly

Table 2. Anthocyanin and Other Phenols[a] in Black Currant Plant Material[b]

	phenol group		
sample type	anthocyanins	HCA	flavonols
BPR (A)	35 ± 4.2	0.60 ± 0.01	3.16 ± 0.14
BPR (A) acid hydr	3 ± 1.4	0.27 ± 0	0 ± 0
BPR (B)	37 ± 2.1	1.20 ± 0.06	4.67 ± 0.16
BPR (B) acid hydr	2 ± 0.70	0.25 ± 0.01	0 ± 0
pom (A)	5 ± 1.41	0.09 ± 0.02	0.57 ± 0.01
pom (A) acid hydr	7 ± 0.70	0.41 ± 0.01	0 ± 0
pom (B)	5 ± 0	0.17 ± 0.01	0.57 ± 0.01
pom (B) acid hydr	3 ± 1.41	0.37 ± 0.01	0 ± 0

[a] Results expressed as milligrams of phenolic equivalent per gram of freeze-dried material (mean ± SD of duplicate assays). Phenolic equivalent relative to cyanidin-3-rutinoside for anthocyanins, caffeic acid for HCA (hydroxycinnamic acids), and quercetin-3-rutinoside for flavonols. [b] Black currant plant material refers to either BPR (black currant press residue) or pom (pomace). Letter A or B denotes solvent system used for extraction.

(where treatment = solvent extraction or acid hydrolysis). For anthocyanins, the combination of treatment and plant type was highly significant ($P < 0.001$).

To fully elucidate the anthocyanin profile of the tested plant materials, the anthocyanin composition, expressed as a percentage, was calculated (**Table 3**).

As can be seen in **Table 3**, Dp-3-glu and Dp-3-rut were present at a lower percentage of the total anthocyanins in pomace than in BPR, whereas the two major cyanidin glycosides represented a smaller portion of the total anthocyanin content in BPR. In addition, all major anthocyanins were more efficiently extracted with the aqueous solvent mixture (solvent B). Acylated anthocyanins were present at a higher relative concentration in the pomace (except for the acylated delphinidin extracted with solvent B) and especially in the pomace sample extracted with solvent A (4.4 and 3%, respectively).

The relative amounts of each anthocyanin component extracted from the tested plant materials were Dp-3-rut > Cy-3-rut > Dp-3-glu > Cy-3-glu for BPR and Cy-3-rut ≥ Dp-3-rut > Dp-3-glu > Cy-3-glu for pomace (**Table 3**).

In addition, the "major" anthocyanins (i.e., delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-rutinoside, and cyanidin-3-glucoside) represented >90% of the total anthocyanin content.

Results (**Table 2**) also show that anthocyanins were the major phenol class present in the black currant plant residues (87−95% of the total phenol content determined by HPLC). Flavonol derivatives comprised up to 10% of the total phenol content, but were not recovered from the acid-hydrolyzed samples. Hydroxycinnamates were present in quite low amounts, but significant portions were present at 5.3−10.6% of total phenols in the acid-hydrolyzed extracts. Again, total phenol content was high for BPR samples except for the samples that had been acid-hydrolyzed. Pomace extracts had considerably lower total phenol content. Solvent type used for extraction did not affect phenol yields extensively.

To conclude, a comparison of **Figure 1** and **Table 2** indicates that the levels of total phenols measured by the Folin−Ciocalteu procedure were considerably higher (1.7−21 times) than the levels determined by HPLC, especially for the extracts obtained after acid hydrolysis.

DISCUSSION

Differences in sample treatment (solvent extraction or acid hydrolysis) and the difference between the two tested black

Table 3. Anthocyanin Fraction as Percentage of Total Anthocyanin Content[a]

	anthocyanin						
sample	Dp-3-glu[b]	Dp-3-rut	Cy-3-glu	Cy-3-rut	Dp-acylated[c]	Cy-acylated	other
BPR (A)	15.8	46.2	4.5	27.1	2.1	0.5	3.8
BPR (B)	16.9	49.4	4.3	27.4	1.6	0.3	0.3
pomace (A)	12.6	31.4	8.4	33.4	4.4	3	6.8
pomace (B)	12.8	35.2	8.4	36.2	1.4	0.8	5.2

[a] For total anthocyanin content see **Table 2**. [b] Dp, delphinidin; Cy, cyanidin; glu, glucoside; rut, rutinoside. [c] The two acylated anthocyanins correspond to compounds delphinidin-3-(6″-coumaroylglucoside) and cyanidin-3-(6″-coumaroylglucoside), whereas "other" mostly refers to petunidin-3-rutinoside and peonidin-3-rutinoside (see *21*).

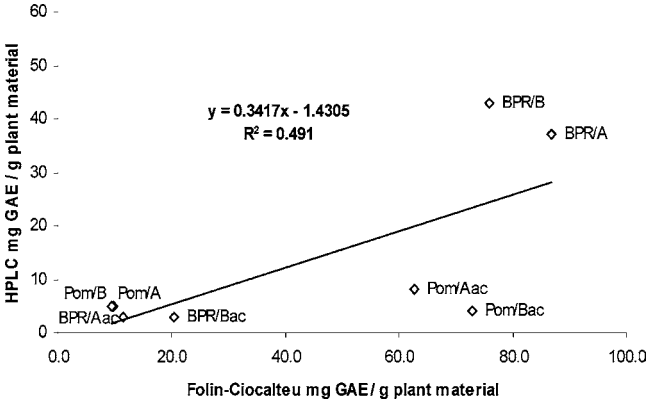

Figure 5. Correlation for total phenols measured by HPLC to total phenols measured by the Folin–Ciocalteu procedure.

currant plant samples were the main factors contributing to the variation in results.

These treatments account for the difference in the levels of total phenols and the radical scavenging activity of the extracts. Statistical analysis showed a strong dependence of yield on treatment and plant type, which was a consequence of the high phenol content and high radical scavenging activity found in the solvent-extracted BPR, which contrasted with the higher phenol content and radical scavenging activity after acidic hydrolysis for the pomace. These results indicate that BPR was a plant material rich in phenols, easily extracted from the plant cells, leaving only a small portion behind. In contrast, the pomace had noticeably low "free" phenol content but "cell wall-bound" phenols were present at relatively high concentrations. In plant cells, phenolic compounds are deposited mainly in the cell wall, where lignin and other compounds (ferulic acid esters, flavonoids) accumulate, and in the vacuoles (*22*, *23*). It appears that the commercial juice-making procedure efficiently releases anthocyanins from black currant tissue (*24*), but there was also a considerable amount of phenols associated with or bound to the pomace cell wall. A major difference between the commercial and laboratory extraction processes is in the use of plant cell wall degrading enzymes in the commercial process. It appears likely that the commercial extraction process caused some hydrolysis of flavonoids to the more reactive aglycones, which bound to the cell wall. When water was incorporated into the extraction solvent, a lower concentration of phenols was measured in the BPR after acid hydrolysis. This difference occurred because flavonoid glycosides are polar and more efficiently extracted with a water-containing solvent.

Total phenol content determined according to the Folin−Ciocalteu procedure showed a very poor correlation ($R^2 = 0.49$) with the total phenol concentrations calculated by HPLC (**Figure 5**). Several researchers (*18*, *24*, *25*, *26*) noted that the Folin−Ciocalteu determination is subject to interference from other chemical components present in the extract, including sugars and ascorbic acid. The high reducing sugar level of the extracts

Table 4. Contribution[a] of Anthocyanin Content to the Radical Scavenging Activity

extract	radical scavenging activity	contribution of anthocyanins to radical scavenging activity (%)
BPR (A)	0.397 ± 0.049	77
BPR (B)	0.454 ± 0.011	74

[a] Based on calculations derived by testing an anthocyanin model solution (1200 μM) comprising all of the major black currant anthocyanins. Results are expressed as millimoles of Trolox equivalents per gram of freeze-dried fruit (mean ± SD of duplicate determinations).

can increase the apparent total phenol concentration determined by using the Folin−Ciocalteu procedure. The effect can be reduced by applying standard correction factors (*25*) or by applying solid-phase extraction of the liquid matrices (*27*). This approach was not followed in this study because sugar removal with solid-phase extraction can reduce the amount of total phenols by 5−11% (*15*). Nevertheless, the correlation between total phenol measurement (determined with the Folin−Ciocalteu method) and the radical scavenging activity (**Figures 1** and **2**) suggests that the extracts may have contained phenolic compounds, such as catechins and hydroxybenzoic acids, that were not detected by the HPLC method.

The HPLC analysis revealed that the main phenol class present in the tested black currant plant material was anthocyanins followed by flavonols and hydroxycinnamic acids (**Table 2**). The yield of HCA and flavonols varied significantly for combinations of treatment, solvent, and plant type. The difference in anthocyanin content in BPR before and after hydrolysis was relatively large, whereas in pomace the corresponding values were rather close and much lower. These results clearly demonstrate that BPR contains a high amount of anthocyanins, which can be simply extracted with the proposed solvent extraction procedures, but the phenols in the pomace are not well extracted by solvent extraction. Other phenol classes, such as hydroxybenzoic acids and flavan-3-ols (catechins), detected in black currant extracts by other researchers (*2*, *15*) were not detected in this study. The different HPLC method, degree of sample purity, and very low amounts of these phenolics (*22*) may account for this result.

In general, the high anthocyanin content of BPR was mainly responsible for the high radical scavenging activity of the extracts indicated in **Table 4**. In this study, the radical scavenging activity of BPR extracts was only slightly higher than that of a model mixture of anthocyanins at a concentration approximating that in the BPR extracts. According to Kahkonen and Heinonen (*28*), anthocyanins and their aglycones are powerful antioxidants and exhibit strong radical scavenging activities. The strong $ABTS^{\bullet+}$ radical scavenging properties of polyphenols and most notably anthocyanins were also reported by other investigators (*29*). However, nonphenolic components

present in black currants, such as ascorbic acid, might contribute to the radical scavenging activity of the isolated extracts.

Finally, the relative anthocyanin composition of pomace was similar to that of BPR. Four main anthocyanins, that is, delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside, and cyanidin-3-rutinoside, were detected in extracts from both black currant residues. These findings agree well with other published data (*21*, *30*, *31*). In addition, four minor peaks were also detected, but their structure could not be confirmed (**Figure 3**) because appropriate standards were not available. The relative concentration of the different anthocyanins in BPR (i.e., Dp-3-rut > Cy-3-rut > Dp-3-glu > Cy-3-glu) is similar to that reported in an earlier study (*31*), but in this study Cy-3-rut was more concentrated in the pomace (Cy-3-rut ≥ Dp-3-rut > Dp-3-glu > Cy-3-glu). Anthocyanin profiles are used to verify the authenticity of fruit products (*32*), but these results indicate that fruit treatment and extraction procedures affect the anthocyanins, because pomace is an enzyme-treated black currant byproduct and presents qualitative differences compared to BPR and other products.

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