

Composition and properties of purified phenolics preparations obtained from an extract of industrial blackcurrant (*Ribes nigrum* L.) pomace

By M. SÓJKA^{1*}, S. GUYOT², K. KOŁODZIEJCZYK¹, B. KRÓL¹ and A. BARON²

¹Institute of Chemical Technology of Food, Technical University of Łódź, ul. B. Stefanowskiego 4/10, 90-924 Łódź, Poland

²Unité de Recherches Cidricoles, L'Institut National de la Recherche Agronomique (INRA), Domaine de la Motte, BP 35327, 35653 Le Rheu Cedex, France

(e-mail: droizo@poczta.onet.pl)

(Accepted 31 August 2009)

SUMMARY

An extract obtained from industrial blackcurrant pomace was purified and fractionated on an RP C18 column, resulting in three phenolics preparations of different composition. The phenolics in the preparations were quantified by HPLC, their anti-oxidant capacity was determined, and the preparations were subjected to HPLC-MS analysis in order to identify the bioactive compounds present. The purified preparations selected for study were an anthocyanin-rich preparation, a myricetin and quercetin glycosides-containing preparation, and an aglycon-containing preparation. All three preparations were characterised by having a high content of polyphenols. The anthocyanin-rich preparation was the most concentrated in polyphenols [$> 50\%$ (w/w)], followed by the aglycon-containing preparation [$> 20\%$ (w/w)], and finally the glycosides-containing preparation [$> 9\%$ (w/w)]. The preparations were also characterised by their high anti-oxidant capacity, which was $> 3,100 \mu\text{M TEAC g}^{-1}$ (TEAC = Trolox Equivalent Anti-oxidant Capacity) for all preparations. The HPLC-MS study confirmed that the anthocyanin-rich preparation was composed of the following anthocyanins: delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside, and cyanidin-3-rutinoside. The aglycon preparation contained four aglycons, of which myricetin and quercetin were predominant, and kaempferol and isorhamnetin were present in lower amounts. The glycosides-containing preparation was found to be the most interesting, since it contained myricetin, quercetin, kaempferol, and isorhamnetin glycosides. Myricetin and quercetin galactosides were also detected in blackcurrant extract for the first time. Moreover, the presence of nitrile-containing compounds, two acylated anthocyanins, and one auron-type compound was detected.

Blackcurrant fruit is an important raw material in the production of nectars and drinks (Dmochowska, 2006; Shahidi and Naczka, 2004). These products are characterised by their organoleptic properties (i.e., they have an intensive colour, taste, and aroma) which make them desirable market products (Piry *et al.*, 1995; Brennan *et al.*, 2003).

Phenolics are important secondary metabolites in fruit, with anti-oxidant properties that may be related to health benefits against cardiovascular disease or cancer. Blackcurrant berries are known to be a good source of polyphenolic compounds (Anttonen and Karjalnen, 2006; Maatta-Riihinen *et al.*, 2004). Among the most important flavonoids that occur in blackcurrant fruit are anthocyanins, with a mean content in fresh fruit of approx. 250 mg 100 g⁻¹. Besides anthocyanins, blackcurrants contain glycosides of myricetin, quercetin, and kaempferol, as well as small amounts of isorhamnetin (Anttonen and Karjalnen, 2006). There are many reports which show that these compounds have interesting health-promoting properties (Lee *et al.*, 2007; D'Ischia *et al.*, 2006; Ong and Khoo, 2000; Yan *et al.*, 2002; Kim *et al.*, 2005).

Juice manufacturers can decrease the amount of phenolics compounds, especially during the processes of

enzymatic depectinisation of the fruit mash and pasteurisation of the juice (Shahidi and Naczka, 2004). Some commercial juice-processing enzyme preparations are characterised by having glycosidase activities and can hydrolyse anthocyanin-glycosidic linkages, thereby liberating unstable anthocyanidins (Buchert *et al.*, 2005; Skrede *et al.*, 2000).

Progress in food production technology is improving the nutritional and sensory qualities of products, and increasing the productivity of the processes, but it is also changing the composition of the by-products. Press-cake is the main by-product of fruit juice production. The by-products of plant food processing present a major disposal problem for the industry concerned, but they can also be promising sources of compounds which may be used because of their favourable technological or nutritional properties (Larrauri, 1999; Skrede *et al.*, 2000; Sójka and Król, 2009).

One way to use these by-products is to apply an extraction process which allows one to obtain phenolics extracts and preparations of dietary fibres. Phenolics extracts from such by-products are complex mixtures of phenolics and other substances (e.g., sugars, acids, and pectins). The composition of such phenolics preparations depends both on their origin and on their method of preparation (e.g., the solvent used, extraction time, temperature, etc.). To improve the quality of the extract

*Author for correspondence.

obtained, a purification step must be applied to obtain phenolics extracts in high concentrations, but the efficiency depends on the purifiers or sorbents (selective or non-selective) used.

One of the objectives of the ISAFRUIT Project was to research natural fruit components with health-beneficial properties. High amounts of such substances are present in the extracts obtained during the extraction and purification processes from fruit pomaces. To characterise such products, several analytical methods must be employed.

The analytical method most commonly used to determine phenolics is HPLC coupled to UV-DAD (Diode Array Detection; Alonso-Salces *et al.*, 2004; Hakkinen and Auriola, 1998; Sanchez-Rabaneda *et al.*, 2002). DAD makes it possible to detect and classify polyphenols. Thus, the UV-visible spectra of hydroxycinnamates, catechins, dihydrochalcones, flavonols, and anthocyanins are all very different. However, in most cases, this detection method does not allow one to differentiate between components belonging to the same class. When the HPLC system is coupled to a mass spectrometer (MS) detector, the identification and confirmation of structures is made easier and more reliable. MS detection provides determinations of molecular weights, and MS/MS or MS³ analyses give additional information on molecular structures, and is particularly well-adapted for the differentiation of flavonoid glycosides.

Purified blackcurrant preparations (three concentrates) obtained by solid-phase extraction from a raw blackcurrant extract were the subject of this study. The aim of this work was to identify the phenolic compounds (e.g., anthocyanins, myricetin, quercetin, kaempferol, and isorhamnetin glycosides, and their aglycons) which were present in the three purified fractions of a blackcurrant extract. The polyphenol composition and anti-oxidant capacity of each purified preparation were also determined.

MATERIALS AND METHODS

Chemicals

Ultrapure water (Millipore GesmbH., Vienna, Austria) and HPLC gradient-grade methanol (J.T. Baker, Deventer, Holland) were used to prepare all solutions. HPLC gradient-grade acetonitrile and formic acid were purchased from J. T. Baker. Delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, quercetin-3-O-rutinoside, quercetin-3-O-galactoside, quercetin-3-O-glucoside, kaempferol-3-O-rutinoside, kaempferol-3-O-glucoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside, myricetin, quercetin, kaempferol, and isorhamnetin (Extrasynthese, Genay, France) were used as standards for MS spectral comparisons. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals to determine anti-oxidant activities were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Extraction and purification of polyphenolics

Blackcurrant pomace (500 kg) was taken from the concentrated juice production line of the AlpeX

Company (Łczeszycze, Poland) in July 2007. The fresh pomace was dried in an industrial vacuum dryer (Polfarmex Company, Kutno, Poland) at a temperature below 70°C. After drying, the pomace was separated into a seeded fraction (diameter < 2 mm) and a seedless fraction (diameter > 2 mm) by means of a 2.0-mm sieve. The seedless fraction was then separated into two sub-fractions: 2 – 5 mm (F 2–5) and ≥ 5 mm (F 5). Fraction F 5 and fractions resulting from the seeded fraction were used for other research (not described here). The F 2-5 fraction was then subjected to a sequential extraction, as follows: 6 l of water was added to 1.5 kg of the F 2-5 fraction of the pomace and kept at 70°C for 30 min. The extract was then pressed on a laboratory hand-screw press (own-manufacture). Three further extractions were carried out, as previously, but each using 3.5 l water. The water extracts obtained were then pooled and concentrated to a soluble solids content of ca. 40°Brix (digital refractometer PR-32α; Atago, Tokyo, Japan). The water extract obtained was used in other research (not described here), while the sugar- and acid-depleted pomace resulting from the water extraction was then extracted with 45% (v/v) ethanol. This extraction was carried out in a column extractor, with contact time of 1 h, and resulted in 12 l of extract which, after removal of the ethanol, was freeze-dried, resulting in a raw blackcurrant extract, which was later purified as described below.

An aqueous solution of the raw blackcurrant extract [1% (w/v)] was transferred onto a Strata X RP C-18 column (Phenomenex, Torrance, CA, USA), washed with water and 20% (v/v) methanol, and eluted sequentially with 30% to 100% (v/v) methanol, which resulted in three fractions of different composition. The fractions were methanol-depleted and concentrated using a vacuum rotary evaporator at 45°C, then freeze-dried to result in three powdered, concentrated fractions of high purity: F-ACY, an anthocyanins preparation; F-GMQ, a myricetin and quercetin glycosides preparation; and F-AG, an aglycon preparation.

Sample preparation

Samples of the three purified blackcurrant pomace preparations, the raw extract, and known standards were diluted with 50% (v/v) methanol, filtered through PTFE filters (0.45 μm) and introduced into an HPLC system coupled to an electrospray ionisation (ESI) source and an MS detector (Thermo-Finnigan, San Jose, CA, USA). To measure their anti-oxidant activities using the DPPH method, the raw extract, the three purified preparations, and the standards were diluted with 50% (v/v) methanol.

HPLC conditions for quantification

Anthocyanins and other phenolics were determined using a Smartline chromatograph (Knauer, Berlin, Germany) equipped with two pumps. The phenolics preparations were separated on a 150 mm × 4.6 mm i.d., 5 μm, Gemini C18 110A column (Phenomenex) by gradient elution with 10% (v/v) formic acid in water (solvent A) and 50:40:10 (v/v/v) acetonitrile:water:formic acid (solvent B). The column temperature was set to 40°C, the flow rate was 1 ml min⁻¹, and the gradient programme was as follows: 0 – 0.6 min, 12% (v/v) B; 0.6 – 16 min, 12 – 30% (v/v) B; 16 – 20.5 min 30 – 100% (v/v) B.

B; 20.5 – 22 min, 100% (v/v) B; 22 – 25 min, 100 – 12% (v/v) B; and 25 – 35 min, 12% (v/v) B. The injection volume was 20 µl. Data were collected using the Eurochrom 2000 Programme (Knauer). Quercetin and myricetin glycosides, and their aglycons, were detected at 360 nm, while anthocyanins were detected at 520 nm. Standard curves using cyanidine-3-rutinoside, rutin, quercetin, myricetin, kaempferol, and isorhamnetin were used for quantification. Cyanidin-3-rutinoside was used to assay for anthocyanins, whereas rutin was used to assay for quercetin and myricetin glycosides.

HPLC-MS conditions for identification

High performance liquid chromatograph (HPLC) coupled to a DAD and an ESI-trap mass spectrometer was used for product identification. The HPLC system was equipped with a SCM1000 membrane solvent degasser (ThermoQuest, San Jose, CA, USA), a binary high pressure gradient pump (1100 Series; Agilent Technologies, Santa Clara, CA, USA), an autosampler, and a column oven (Surveyor Series; Thermo-Finnigan).

The solvents used and the gradient for flavonol separation were as follows: Solvent A, 0.25% (v/v) formic acid in water; solvent B, 85:15 (v/v) acetonitrile:methanol. The gradient programme [time in min + % (v/v) A] used was 0 + 84, 6 + 82, 9 + 82, 14 + 80, 30 + 80, 42 + 74, 50 + 71, 52 + 58, 62 + 58, 66 + 46, 68 + 20, 70 + 20, 72 + 84, and 83 + 84. The flow rates were: 0.4 ml min⁻¹ for 0 – 9 min, followed by a flow-rate gradient increase to 0.6 ml min⁻¹ for 9 – 14 min and 14 – 82 min, followed by a flow-rate gradient decrease to 0.4 ml min⁻¹ for 82 – 83 min.

The solvents used and the gradient for anthocyanin separations were as follow: Solvent A, 0.25% (v/v) formic acid in water; solvent B, 0.25% (v/v) formic acid in acetonitrile. The flow rate was 1 ml min⁻¹. The gradient programme [time min + % (v/v) B] was: 0 + 5, 2 + 5, 32 + 20, 37 + 70, 42 + 70, 45 + 5, and 55 + 5.

A Gemini C18 110A 250 mm × 4.6 mm i.d. 5 µm column (Phenomenex) was used with a pre-column Gemini C18 4 mm × 3 mm Security Guard Cartridges (Phenomenex). The column temperature was 30°C and the injection volume was 10 µl. Chromatographic data were collected using Xcalibur software, Version 1.2

(Thermo-Finnigan). In both conditions, a splitting valve was used to reduce the flow that was introduced into the electrospray source MS detector.

The MS system coupled to the HPLC was an LCQ DECA ion trap mass spectrometer (Thermo-Finnigan) equipped with an ESI source used in the negative mode. Data were collected and processed using Xcalibur software Version 1.2 (Thermo-Finnigan). The source parameters were as follows: ion spray voltage, 4.50 kV; capillary voltage, -23 V; capillary temperature, 240°C; and sheath nitrogen gas flow rate, 80 (arbitrary units). To generate MS/MS or MS³ data, the precursor ions were fragmented by helium gas collision in the ion trap by optimising the collision energy in order to obtain an intensity of the precursor ion close to 10% of the relative scale of the spectrum.

DPPH radical-scavenging activity

DPPH-scavenging activity was determined using the method described by Kim *et al.* (2002). DPPH (60 µM) was dissolved in 80% (v/v) aqueous methanol. Each phenolics extract (0.05 ml) was added to 1.95 ml of the methanolic DPPH solution. The mixture was shaken vigorously and allowed to stand at room temperature, in the dark, for 30 min. The decrease in absorbance of the resulting solution was monitored at 515 nm for 30 min. The control consisted of 0.05 ml of 50% (v/v) aqueous methanol and 1.95 ml DPPH solution. A concentration-response curve was prepared for the absorbance of the DPPH radical at 515 nm, after 30 min, as a function of different Trolox concentrations. The DPPH radical-scavenging activities of the three black currant pomace phenolics extracts were expressed as µM TEAC g⁻¹ (Phenomenex) extract or standard (TEAC, Trolox Equivalent Anti-oxidative Capacity).

RESULTS AND DISCUSSION

Purified blackcurrant concentrates are characterised by having high concentrations of phytochemicals (Table I). The anthocyanin-rich preparation (F-ACY) was distinguished by having the highest concentration of polyphenols [> 50% (w/w)], where delphinidins were the

TABLE I
Composition [% (w/w)] and anti-oxidant activity of purified blackcurrant polyphenol fractions from a raw seedless extract of pomace

Component	Fraction (see text)		
	F-ACY	F-GMQ	F-AG
Myricetin glycosides*	0.0 ± 0.0	4.5 ± 0.1	0.0 ± 0.0
Quercetin glycosides*	0.0 ± 0.0	2.5 ± 0.1	0.0 ± 0.0
Myricetin	0.0 ± 0.0	0.4 ± 0.0	15.4 ± 0.4
Quercetin	0.0 ± 0.0	0.0 ± 0.0	5.6 ± 0.2
Kaempferol	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.0
Isorhamnetin	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0
Delphinidin-3-glucoside**	15.0 ± 0.2	0.2 ± 0.0	0.0 ± 0.0
Delphinidin-3-rutinoside**	17.6 ± 0.3	0.2 ± 0.0	0.0 ± 0.0
Cyanidin-3-glucoside**	5.6 ± 0.1	0.3 ± 0.0	0.0 ± 0.0
Cyanidin-3-rutinoside**	13.3 ± 0.2	0.4 ± 0.1	0.0 ± 0.0
Other anthocyanins**	0.4 ± 0.0	1.1 ± 0.0	0.3 ± 0.0
Total aglycones	0.0 ± 0.0	0.4 ± 0.0	22.6 ± 0.4
Total anthocyanins	51.9 ± 0.9	2.2 ± 0.1	0.3 ± 0.0
Total phyto-compounds	51.9 ± 0.9	9.6 ± 0.2	22.9 ± 0.4
Anti-oxidant activity*** (n = 4)	3,629.7 ± 23.6	3,820.8 ± 36.4	3,168.6 ± 56.7

Values are means ± standard deviations (SD). n = 2.

*The content of glycosides was calculated based on quercetin-3-rutinoside.

**The contents of these substances were calculated based on cyanidin-3-rutinoside.

***Anti-oxidant activity was determined by the DPPH method and is expressed in µM TEAC g⁻¹.

TABLE II
 Identification of anthocyanins in the anthocyanin-rich (F-ACY) fraction from a raw extract of blackcurrant pomace

Compound	RT** (min)	MS [M-H] ⁻ (m/z)	Fragmentation [†] MS/MS (m/z)	UV-visible (nm)	Identification
A1	18.32	463	300, 301	276, 521	Delphinidin-3-glucoside
A2	19.78	609	300, 301	277, 524	Delphinidin-3-rutinoside
A3	20.52	447	285 , 284	280, 518	Cyanidin-3-glucoside
A4	22.02	593	285 , 284	280, 518	Cyanidin-3-rutinoside

[†]The m/z values of the predominant ions are given in bold type.

**RT, retention time (min).

main anthocyanins. The second polyphenol-rich, aglycon-containing preparation (F-AG), had a polyphenol concentration of approx. 20% (w/w), which contained > 15% (w/w) myricetin and approx. 5% (w/w) quercetin. Isorhamnetin and kaempferol were also present in minor amounts.

The glycosides-containing preparation (F-GMQ) was characterised by having a lower polyphenol concentration, with concentrations of myricetin and quercetin glycosides at a level of 7% (w/w). F-GMQ also contained 2% (w/w) acylated anthocyanins, and minor amounts of kaempferol and isorhamnetin glycosides.

The anthocyanin preparation (F-ACY) was characterised by having the highest concentration of phytochemicals, which did not directly correspond to its antioxidant capacity (Table I). The highest antioxidant capacity values were recorded for the flavonol glycosides preparation (F-GMQ), although the concentration of total polyphenols in this preparation was the lowest of the three preparations obtained. The high activity of the F-GMQ preparation may result from the presence of polymeric procyanidins. The relatively low anti-oxidant capacity of the aglycon concentrate (F-AG) was unexpected. The anti-oxidant capacities of all three blackcurrant extract fractions were twice as high as that of the unpurified raw extract ($1,591 \pm 23 \mu\text{M TEAC}$

g^{-1}). The anti-oxidant capacity of all three purified concentrates was lower than that of the pure aglycon standards, which were $10,961 \pm 55$ and $8,615 \pm 99 \mu\text{M TEAC g}^{-1}$ for myricetin and quercetin, respectively. The anti-oxidant capacities of the three preparations obtained were approx. 30-times higher than the capacity of the corresponding raw pomace (Sójka and Król, 2009).

Component identification

Anthocyanin-rich blackcurrant preparation (F-ACY): The results confirmed that delphinidin and cyanidin glucosides and rutinosides were the main anthocyanins present in this preparation. The rutinosides were predominant among the glycosides present. Comparisons of their retention times with available standards using the DAD detector and the MS detector allowed us to determine their characteristic UV-visible spectra and molecular weights. In the case of MS, detection was in the negative mode. Attempts to identify the anthocyanins are summarised in Table II. Many references have confirmed that the anthocyanins listed in Table II were the main colourants in blackcurrant fruit (Slimestad and Solheim, 2002). Our experiments also confirmed that these compounds were the main colourants of industrial blackcurrant pomace, and also of the preparations obtained.

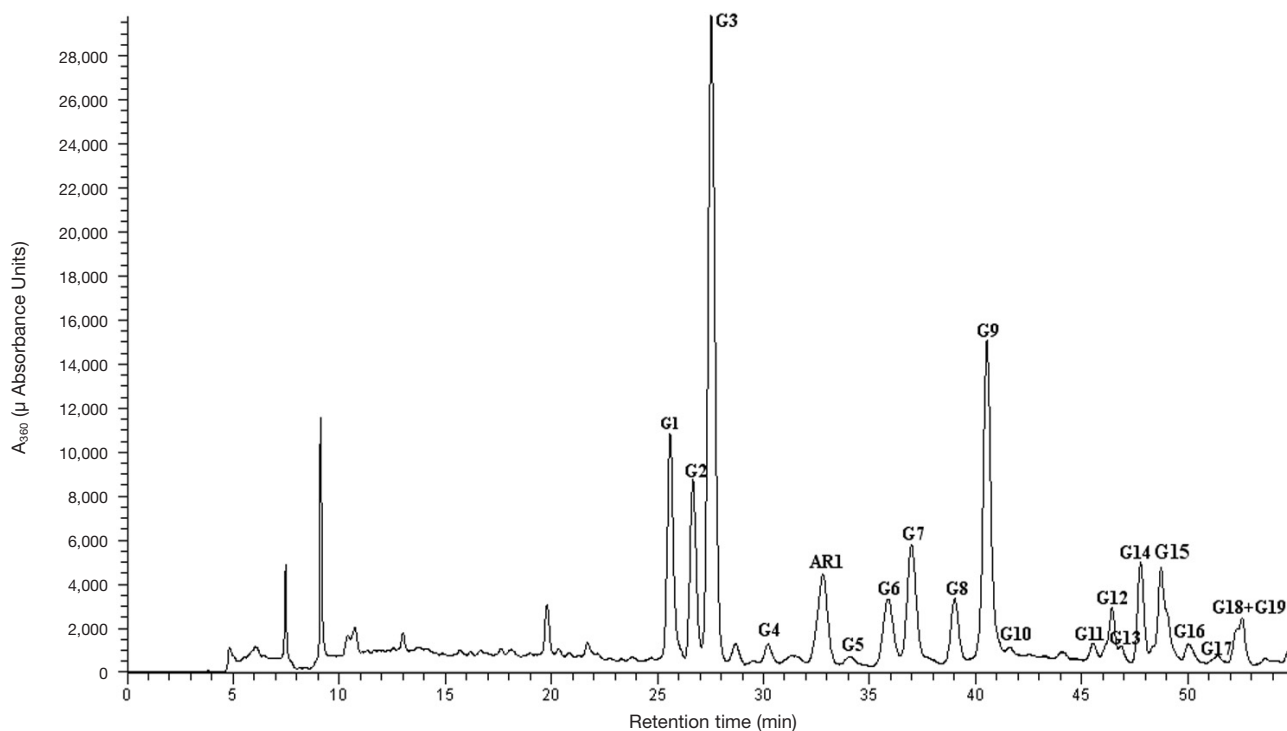


FIG. 1

HPLC chromatogram of the glycosides-containing (F-GMQ) fraction from a raw extract of industrial blackcurrant pomace. Detection was at 360 nm. Peak codes correspond to those in Table III.

Flavonol glycosides and hydroxycinnamic-containing preparation of blackcurrant pomace (F-GMQ): The glycosides-containing preparation was characterised by the diversity of glycosides present (Table III; Figure 1). Our research confirmed that this preparation was composed mainly of myricetin and quercetin glycosides, with minor amounts of kaempferol and isorhamnetin glycosides. Interestingly, this fraction also contained an apigenin glycoside and two nitrile derivatives of *p*-coumaric acid and ferulic acid. The latter showed pseudo-molecular ions [M-H]⁻ at 420 (*m/z*) and 450 (*m/z*) for the *p*-coumaric acid derivative and the ferulic acid derivative, respectively. Considering the “nitrogen rule” in MS, these even-numbered mass signals could be linked to the presence of an uneven number of nitrogen atoms in the molecule. The presence of nitrogen-containing phenolic acids in blackcurrant seeds was described previously (Lu *et al.*, 2002). Therefore, these two compounds were identified as nigrumin-5-*p*-coumarate and nigrumin-5-ferulate, respectively. However, in our case, these compounds were identified in an extract obtained from the seedless fraction of blackcurrant pomace.

Traces of apigenin glycoside were also detected in this blackcurrant preparation by observing the pseudo-molecular ion [M-H]⁻ at 431 (*m/z*), with a major fragment at 269 (*m/z*), in accord with apigenin aglycon. The mass difference between these two signals (i.e., 162 *m/z*) indicated the presence of a hexose moiety. The identity of the molecule still needs to be confirmed due to the lack of availability of apigenin glycoside standards.

Six myricetin glycosides were tentatively identified in the F-GMQ fraction on the basis of their UV-visible and MS features, and also based on published data

(Anttonen and Karjalnen, 2006; Koponen *et al.*, 2008; Maatta *et al.*, 2003). All showed the 316 (*m/z*) fragment in the MS/MS spectrum which accorded with the myricetin aglycon ion. The glucoside was the predominant compound in this series, then the rutinoside, galactoside, and malonylglucoside were present in decreasing amounts. Identification of these compounds was based mostly on their UV-visible spectra, molecular weights, fragmentation patterns, and published data on the chromatographic sequence of glycosides separated under similar HPLC conditions (Koponen *et al.*, 2008). It was surprising that the galactoside, identified on the basis of its retention time and negative mode MS data, was found in our F-GMQ concentrate, as there are no published data on the presence of this compound in blackcurrant. Its presence should be confirmed by further studies, applying other identification methods. Moreover, the two myricetin pentosides probably corresponding to a xyloside and an arabinoside, were present in the extract. These compounds were present in very minor amounts. Their presence, particularly the presence of pentosides, was reported in bilberry juice (Koponen *et al.*, 2008). The presence of hexosides, particularly glucosides and rutinosides, has been shown in blackcurrant seeds and fruits (Koponen *et al.*, 2008; Lu and Foo, 2003).

The second largest group of flavonols present in the glycosides-containing preparation F-GMQ were quercetin glycosides. Similar to myricetin, the main quercetin glycoside was a glucoside. Quercetin rutinoside, galactoside, and malonylglucoside were present in lower amounts. The presence of these compounds was confirmed on the basis of available standards showing exactly the same retention times, on

TABLE III
Identification of flavonol glycosides and hydroxycinnamic derivatives in the F-GMQ fraction obtained from a raw extract of blackcurrant pomace

Compound	RT ^{**} (min)	MS [M-H] ⁻ (<i>m/z</i>)	Fragmentation [*] (<i>m/z</i>)	UV-visible(max-nm)	Tentative identification
Hydroxycinnamic acid derivatives					
H1A	20.00	179	135	HCA	Caffeic acid
H1B	20.00	449	287 , 259	HCA	Unknown
H2	28.80	393	196, 137	HCA (310)	Unknown
H3	46.63	420	163	HCA (314)	Nigrumin-5- <i>p</i> -coumarate
H4	49.27	450	193	HCA (246, 328)	Nigrumin-5-ferulate
Flavonol glycosides					
G1	25.80	625	316 , 287, 271	FL (246, 355)	Myricetin-rutinoside
G2	26.88	479	316 , 287, 271	FL (248, 358)	Myricetin-galactoside
G3	27.73	479	316 , 287, 271	FL (251, 356)	Myricetin-glucoside
G4	30.42	449	316 , 271	FL (245, 358)	Myricetin-xyloside
G5	34.25	449	316 , 271	FL	Myricetin-arabinoside
G6	36.07	565	521, 479, 316	FL (248, 354)	Myricetin-malonylglucoside
G7	37.18	609	301 , 271, 255	FL (254, 353)	Quercetin-rutinoside
G8	39.22	463	301 , 271, 243	FL (246, 353)	Quercetin-galactoside
G9	40.73	463	301, 271, 243	FL (256, 356)	Quercetin-glucoside
G10	41.83	479	317 , 461	FL	Unknown
G11	45.73	433	301 , 300, 271, 255	FL	Quercetin-pentoside
G12	46.63	593	285 , 255	FL	Kaempferol-rutinoside
G13	47.07	623	315 , 300, 271, 255	FL	Isorhamnetin-rutinoside
G14	47.98	549	505, 300 , 271, 255	FL (248, 353)	Quercetin-malonylglucoside
G15	48.95	447	285 , 255	FL (246, 346)	Kaempferol-glucoside
G16	50.23	477	315 , 271	FL	Isorhamnetin-glucoside
G17	51.62	477	314 , 299	FL	Unknown
G18	52.53	317	271, 179 , 151	FL	Myricetin
G19	52.77	431	269 , 241	FL	Apigenin-hexoside
Aurons					
AR1	33.00	447	436, 327, 285	AUR (325, 406)	Aureudisin-glucoside
Anthocyanins					
ACY1	31.65	609	463, 301, 300	ACY (280, 530)	Delphinidin-3-O-(6''-coumaroylglucoside)
ACY2	40.20	593	447, 285 , 294	ACY (282, 522)	Cyanidin-3-O-(6''-coumaroylglucoside)

*The *m/z* values of the predominant ions are given in bold type.

**RT, retention time (min).

TABLE IV
 Identification of compounds in the aglycon-containing preparation (F-AG) fraction obtained from a raw extract of blackcurrant pomace

Compound	RT ^{**} (min)	MS [M-H] ⁻ (<i>m/z</i>)	Fragmentation [*] MS/MS (<i>m/z</i>)	UV-visible (nm)	Identification
AG1	52.58	317	299, 271, 179 , 151	253, 373	Myricetin
AG2	59.77	301	273, 257, 179 , 151	255, 371	Quercetin
AG3	66.22	285	257 , 241	265, 366	Kaempferol
AG4	67.37	315	300 , 271, 258	250, 369	Isorhamnetin

^{*}The *m/z* values of the predominant ions are given in bold type.

^{**}RT, retention time (min).

their UV-visible data, mass spectra, and fragmentation patterns. For the malonylglucoside, identification was based on published data (Anttonen and Karjalnen, 2006; Koponen *et al.*, 2008; Maatta *et al.*, 2003), UV-visible spectra, mass spectra, and fragmentation patterns. Quercetin galactoside, like the galactoside of myricetin, was thus identified in blackcurrant extract for the first time. The presence of one quercetin pentoside in a significantly minor amount was also detected. Moreover, two kaempferol glycosides, more precisely a glucoside and a rutinoside, were unambiguously identified in the F-GMQ concentrate by comparison of the analytical data (i.e., retention time, MS and UV-visible spectra) with commercial standards. For isorhamnetin, the presence of a glucoside and a rutinoside was also confirmed using the same procedure. No kaempferol or isorhamnetin galactosides were found. Similar to myricetin glycosides, the glycosides of quercetin, kaempferol, and isorhamnetin have previously been identified in the fruits and seeds of blackcurrant (Koponen *et al.*, 2008; Lu and Foo, 2003; Maatta *et al.*, 2003). In addition, the presence of an auron derivative in the F-GMQ preparation was suspected. The molecular weight of the pseudo-molecule [M-H]⁻ of this compound was 447 (*m/z*) and its fragmentation gave 285 (*m/z*), the same as kaempferol hexoside. Nevertheless, the theoretical MS³ fragmentation for kaempferol is different from the above. Additionally, the value of the UV-visible spectrum maximum at *ca.* 400 nm, corresponded to the value characteristic for an auron. The presence of this compound was confirmed by Lu and Foo (2002), who identified it as auresidin. Auresidin was identified in both the post-extraction residues of seeds (Lu and Foo, 2002) and in whole fruit (Anttonen and Karjalnen, 2006). Two acylated anthocyanins were also tentatively identified in the F-GMQ concentrate. The UV-visible spectra of both compounds were characterised by having an additional maximum at 310–320 nm, which was linked to the presence of hydroxycinnamic acid derivatives. Both compounds gave 146 (*m/z*) and 163 (*m/z*) fragments which were linked to the loss of the *p*-coumaric acid and hexoside moieties, respectively. Similar results were presented by Slimestad and Solheim (2002) for fruit of the blackcurrant 'Titania'. These authors identified these compounds as delphinidin 3-O-(6''-coumaroylglucoside) and cyanidin 3-O-(6''-coumaroylglucoside).

Aglycon-containing preparation of blackcurrant pomace (F-AG): The aglycon-containing preparation was characterised by the presence of four aglycons

(Table IV), among which, myricetin and quercetin were predominant. Kaempferol and isorhamnetin were present in minor concentrations. The UV-visible and MS spectra allowed these compounds to be identified. All identifications were confirmed by comparisons with available standards. The molecular weights of the pseudo-molecules [M-H]⁻ were 317 (*m/z*) for myricetin, 301 (*m/z*) for quercetin, 285 (*m/z*) for kaempferol, and 315 (*m/z*) for isorhamnetin. The identification data for these compounds are given in Table IV. The presence of these compounds was closely linked to the juice preparation process, especially to the enzyme treatment and heat process used, where glycosides are hydrolysed. Minor amounts of myricetin and quercetin aglycons present in blackcurrant and bilberry juices have been reported by Koponen *et al.* (2008).

CONCLUSIONS

Undoubtedly, blackcurrant pomace is a useful raw material for the preparation of extracts that are highly concentrated in phenolics. Raw extract could be separated efficiently into three fractions with different polyphenol compositions: an anthocyanin, a flavonol-glycoside, and an aglycon-containing fraction. Concentrates prepared by freeze-drying the separate fractions were valuable products, characterised by having a high purity, high polyphenol concentrations, and high anti-oxidant activities. The glycosides-containing concentrate (F-GMQ) was found to be the most interesting, since it had the highest anti-oxidant capacity, despite having the lowest polyphenol concentration, and contained myricetin, quercetin, kaempferol, and isorhamnetin glycosides, nitrile-containing compounds, acylated anthocyanins, and one auron. The presence of a myricetin hexoside other than a glucoside (tentatively identified as a galactoside), quercetin galactoside, and apigenin hexoside were detected for the first time in a blackcurrant extract. Considering the health-beneficial properties of these compounds, blackcurrant pomace as well as its extracts and preparations could be used as functional food additives.

The ISAFRUIT Project is funded by the European Commission under Thematic Priority 5 – Food Quality and Safety of the 6th Framework Programme of RTD (Contract No. FP6-FOOD-CT-2006-016279).

Disclaimer: Opinions expressed in this publication may not be regarded as stating an official position of the European Commission.

REFERENCES

- ALONSO-SALCES, R. M., NDJOKO, K., QUEIROZ, E. F., IOSET, J. R., HOSTEITMANN, K., BERUETTA, L. A., GALLO, B. and VICENTE, F. (2004). On-line characterization of apple polyphenols by liquid chromatography coupled with mass spectrometry and ultraviolet absorbance detection. *Journal of Chromatography A*, **1046**, 89–100.
- ANTTONEN, M. J. and KARJALAINEN, R. O. (2006). High-performance liquid chromatography analysis of black currant (*Ribes nigrum* L.) fruit phenolics grown either conventionally or organically. *Journal of Agricultural and Food Chemistry*, **54**, 7530–7538.
- BRENNAN, R. M., HUNTER, E. A. and MUIR, D. D. (2003). Relative effects on cultivar, heat-treatment and sucrose content on the sensory properties of blackcurrant juice. *Food Research International*, **36**, 1015–1020.
- BUCHERT, J., KOPONEN, J. M., SUUTARINEN, M., MUSTRANATA, A., LILLE, M., TORRONEN, R. and POUTANEN, K. (2005). Effect of enzyme-aided pressing on anthocyanins yield and profiles in bilberry and blackcurrant juices. *Journal of the Science of Food and Agriculture*, **85**, 2548–2556.
- D'ISCHIA, M., PANZELLA, L., NAPOLITANO, P. and NAPOLITANO, M. (2006). The chemical basis of the anti-nitrosating action of polyphenolic cancer chemopreventive agents. *Current Medicinal Chemistry*, **13**, 3133–3144.
- DMOCHOWSKA, H. (2008). *Statistical Yearbook of Agriculture and Rural Areas 2008*. Central Statistical Office, Zakład Wydawnictw Statystycznych, Warsaw, Poland. 288–289.
- HAKKINEN, S. and AURIOLA, S. (1998). High-performance liquid chromatography with electrospray ionization mass spectrometry and diode array ultraviolet detection in the identification of flavonol aglycones and glycosides in berries. *Journal of Chromatography A*, **829**, 91–100.
- KIM, D. O., LEE, K. W., LEE, H. J. and LEE, C. Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *Journal of Agricultural and Food Chemistry*, **50**, 3713–3717.
- KIM, D. O., HEO, H. J., KIM, Y. K., YANG, H. S. and LEE, C. Y. (2005). Sweet and sour cherry phenolics and their protective effects on neuronal cells. *Journal of Agricultural and Food Chemistry*, **53**, 9921–9927.
- KOPONEN, J. M., HAPPONEN, A. M., AURIOLA, S., KONTKANEN, H., BUCHERT, J., POUTANEN, K. S. and YORRONEN, R., (2008). Characterization and fate of blackcurrant and bilberry flavonols in enzyme-aided processing. *Journal of Agricultural and Food Chemistry*, **56**, 3136–3144.
- LARRAURI, J. A. (1999). New approaches in the preparation of high dietary fibre powders from fruit by-products. *Trends in Food Science and Technology*, **10**, 3–8.
- LEE, E. R., KANG, G. H. and CHO, S. G. (2007). Effect of flavonoids on human health: old subjects but new challenges. *Recent Patents on Biotechnology*, **1**, 139–150.
- LU, Y. and FOO, L. Y., (2003). Polyphenolic constituents of blackcurrant seed residue. *Food Chemistry*, **80**, 71–76.
- LU, Y., FOO, L. Y. and WONG, H. (2002). Nigrumin-5-*p*-coumarate and nigrumin-5-ferulate, two unusual nitrile-containing metabolites from black currant (*Ribes nigrum*) seed. *Phytochemistry*, **59**, 465–468.
- MAATTA, K. R., KAMAL-ELDIN, A. and TORRONEN, A. R. (2003). High-performance chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: *Ribes* species. *Journal of Agricultural and Food Chemistry*, **51**, 6736–6744.
- MAATTA-RIIHINEN, K. R., KAMAL-ELDIN, A., MATTILA, P., GONZALEZ-PARAMAS, M. and TORRONEN, R. (2004). Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *Journal of Agricultural and Food Chemistry*, **52**, 4477–4486.
- ONG, K. C. and KHOO, H. E. (2000). Effects of myricetin on glycemia and glycogen metabolism in diabetic rats. *Life Sciences*, **67**, 1695–1705.
- PIRY, J., PRIBELA, A., DURCANSKA, J. and FARKAS, P. (1995). Fractionation of volatiles from blackcurrant (*Ribes nigrum* L.) by different extractive methods. *Food Chemistry*, **54**, 73–77.
- SANCHEZ-RABANEDA, F., JAUREGUI, O., LAMUELA-RAVENTOS, R. M., VILADOMAT, F., BASTIDA, J. and CODINA, C. (2004). Qualitative analysis of phenolic compounds in apple pomace using liquid chromatography coupled to mass spectrometry in tandem mode. *Rapid Communications in Mass Spectrometry*, **18**, 553–563.
- SHAHIDI, F. and NACZK, M. (2004). *Phenolics in Food and Nutraceuticals*. CRC Press Inc., Boca Raton, FL, USA. 136–141.
- SKREDE, G. and WROSTLAND, R. E. (2002). Flavonoids from berries and grapes. In: *Functional Foods*. (Shi, J., Mazza, G. and Maguer, M. L., Eds.). CRC Press Inc., Boca Raton, FL, USA. 71–133.
- SKREDE, G., WROSTLAND, R. E. and DURST, R. W. (2000). Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *Journal of Food Science*, **65**, 357–364.
- SLIMESTAD, R. and SOLHEIM, H. (2002). Anthocyanins from black currant (*Ribes nigrum* L.). *Journal of Agricultural and Food Chemistry*, **50**, 3228–3231.
- SÓJKA, M. and KRÓL, B. (2009). Composition of industrial seedless black currant pomace. *European Food Research and Technology*, **228**, 597–605.
- YAN, X., MURPHY, B. T., HAMMOND, G. B., VINSON, J. A. and NETO, C. C. (2002). Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *Journal of Agricultural and Food Chemistry*, **50**, 5844–5849.