



## Polyphenol and vitamin C contents in European commercial blackcurrant juice products

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### ABSTRACT

Vitamin C and polyphenol contents (anthocyanins, proanthocyanidins, phenolic acids and flavonols) were analysed in commercial blackcurrant juice products purchased from various European countries (Finland, Poland, Germany, United Kingdom) using HPLC methods. The aim was to study variation between countries, as well as evaluate the intake of polyphenols from commercial juices. There was significant variation in the contents of polyphenols and vitamin C between countries. Expressed as the ready-to-drink beverages, German, Polish, Finnish and British products averaged anthocyanin contents of 38, 32, 12 and 7.5 mg/2.5 dl, proanthocyanidin contents of 27, 24, 10 and 1.2 mg/2.5 dl, flavonol contents of 16, 15, 5.2 & 1.9 mg/2.5 dl and phenolic acid contents of 12, 8.9, 3.7 and 1.5 mg/2.5 dl, respectively. The mean vitamin C content was highest in British (70 mg/2.5 dl) and lowest in Finnish products (15 mg/2.5 dl). The intake of polyphenols from German and Polish ready-to-drink beverages was clearly higher than that from Finnish, and especially, British beverages.

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### 1. Introduction

Blackcurrants are economically significant horticultural products in Russia, Poland, Germany, Scandinavia, Great Britain, New Zealand and many eastern European countries. The estimated annual world production is around 500,000–600,000 tons. Blackcurrant is primarily grown for juice but the berries are also processed for jams, jellies, teas and other food products. In addition to vitamin C, polyphenol content is a very important quality factor of blackcurrant (Hummer & Barney, 2002).

Polyphenols are secondary plant metabolites, widely present in commonly consumed foods of plant origin, and they are accruing a body of evidence as bioactive components in a wide range of biological systems (Han, Shen, & Lou, 2007; Karjalainen et al., 2009; Scalbert, Manach, Morand, & Rémésy, 2005; Shahidi & Naczki, 1995). The three main types of polyphenols are flavonoids, phenolic acids and tannins, which are powerful antioxidants *in vitro*. These compounds are considered to carry many potential beneficial health effects, e.g. reduction of the risk of cardiovascular diseases (Ghosh & Scheepens, 2009), cancers (Guo, Kong, & Meydani, 2009), neurodegenerative diseases (Joseph, Cole, Head, & Ingram, 2009; Ramassamy, 2006), diabetes (McDougall & Stew-

art, 2005) and osteoporosis (Trzeciakiewicz, Habauzit, & Horcajada, 2009). Such benefits are reported mostly by experimental studies on animals or cultured human cell lines. The conclusive evidence is still limited, and the mechanisms of action remain to be established. In food, polyphenols may contribute to the bitterness, astringency, colour, flavour, and oxidative stability of products.

The four major anthocyanins in blackcurrants have been found to be cyanidin 3-*O*-rutinoside, delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, and delphinidin 3-*O*-glucoside (e.g. Anttonen & Karjalainen, 2006; Pinelo, Landbo, Vikbjerg, & Meyer, 2006), although traces of other anthocyanins have also been detected (Wu, Gu, Prior, & McKay, 2004). Besides anthocyanins, blackcurrants contain phenolic acid derivatives (both hydroxybenzoic and hydroxycinnamic acids), flavonols (glycosides of myricetin, quercetin, kaempferol and isorhamnetin), as well as proanthocyanidins (Anttonen & Karjalainen, 2006; Hellström, Törrönen, & Mattila, 2009; Mattila, Hellström, & Törrönen, 2006; Wu et al., 2004).

Freshly pressed blackcurrant juice is rich in anthocyanins and other polyphenols (Landbo & Meyer, 2004). However, much lower levels of anthocyanins have been found in commercial blackcurrant juice (Hollands et al., 2008; Nielsen, Haren, Magnussen, Dragsted, & Rasmussen, 2003). This may be explained in two ways: first, most commercial blackcurrant juices are diluted and second, the processing of blackcurrant to commercial juices via various treatments, such as crushing, heating, enzyme treatment, pressing,

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pasteurisation, clarification, and filtration, can reduce polyphenol content (Hollands et al., 2008; Iversen, 1999; Koponen, Buchert, Poutanen, & Törrönen, 2008; Landbo & Meyer, 2004; Landbo, Pinelo, Vikbjerg, Let, & Meyer, 2006; Mikkelsen & Poll, 2002). Furthermore, during storage, significant losses of anthocyanins can occur (Iversen, 1999).

The aim of this study, part of the EU-FP7 project BrainHealth-Food (<http://www.uku.fi/brainhealthfood/>), was to analyse the polyphenol contents (anthocyanins, proanthocyanidins, phenolic acids, flavonols) of commercial blackcurrant juices purchased from various European countries, thereby facilitating the evaluation of commercial juices-derived polyphenol intake, and to reveal the variation in the polyphenol content among different juices.

## 2. Materials and methods

### 2.1. Chemicals

The following standards were obtained from Sigma (Sigma-Aldrich Chemie Inc., Steinheim, Germany): protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, gallic acid, syringic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, *tr*-cinnamic acid, chlorogenic acid (5-caffeoylquinic acid), caffeic acid, ferulic acid, (–)-epicatechin, (–)-epicatechin gallate, and (–)-epigallocatechin. (+)-Catechin was purchased from Cayman Chemical (Cayman Chemical Co., Ann Arbor, MI) and sinapic acid from Fluka (Buchs, Switzerland). Procyanidin dimers B1 (epicatechin-(4 $\beta$  → 8)-catechin) and B2 (epicatechin-(4 $\beta$  → 8)-epicatechin) were obtained from PlantChem (PlantChem Co., Sandnes, Norway). Cyanidin 3-*O*-rutinoside, delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, and delphinidin 3-*O*-glucoside were purchased from Polyphenols Laboratories AS, Sandnes, Norway. Myricetin and quercetin-3-*O*-glucoside were purchased from ExtraSynthese, Genay, France. Vitamin C was purchased from Merck KGaA, Darmstadt, Germany. All chromatographic solvents were of HPLC grade and the purity of the reagents used was p.a. or comparable.

### 2.2. Samples

Three commercial blackcurrant juice (or juice concentrate) brands were purchased from Finland, United Kingdom, Poland and Germany in winter and spring, 2009. The juices contained only blackcurrant. Blends with other berries or fruits were not accepted. There were three subsamples for each brand and the sample size was 500–1000 ml. In every country, the subsamples were purchased from three different retail stores, representing different food retail chains. After purchase, the juices were delivered to the MTT laboratory, Finland, bottled in 100 ml bottles and stored in the freezer prior to analysis. Analyses of anthocyanins, phenolic acids, proanthocyanidins and vitamin C were performed at MTT laboratories. Flavonols were analysed at SCRI, UK.

### 2.3. Determination of anthocyanins

The juice sample was prepared by filtering 3 ml through a membrane filter (0.45  $\mu$ m, 25 mm; Pall Gelman Laboratory), discarding ~1 ml of the filtrate and filtering the remainder into 2 ml HPLC ampoules. The analytical HPLC-system consisted of an Agilent 1100 series high-performance liquid chromatograph equipped with a diode array detector. The HPLC pumps, autosampler, column oven, and diode array system were monitored and controlled using an HP Chem Station computer programme. A wavelength of 518 nm was used for quantification. Anthocyanins were separated on a 150  $\times$  4.6 mm i.d., 5  $\mu$ m, Gemini C<sub>18</sub> column with a C<sub>18</sub> guard column. The temperature of the column oven was set at 35 °C. The

mobile phase consisted of 5% formic acid (solvent A) and methanol (solvent B) and the flow rate was 0.6 ml/min. Elution was started at 10% B, followed by a linear gradient to 20% B in 20 min, then isocratic for 10 min, a linear gradient to 35% B in 15 min and, after that, to 80% B in 5 min, isocratic for 5 min and back to the starting point in 3 min. The injection volume was 10  $\mu$ l. All anthocyanins were quantified using an external standard mixture of cyanidin 3-*O*-rutinoside, delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, and delphinidin 3-*O*-glucoside. The samples were analysed in triplicate.

### 2.4. Determination of phenolic acids

Phenolic acids were analysed according to a method modified from Mattila and Kumpulainen (2002). Briefly, a 4 g sample was diluted in 6 ml of a mixture of methanol, containing 2 g/l of butylated hydroxyanisole (BHA) and 10% acetic acid (85:15) and ultrasonicated for 30 min. Next, 12 ml of distilled water containing 1% ascorbic acid and 0.415% EDTA, and 5 ml of 10 M NaOH were placed in the test tube, sealed, and stirred overnight (~16 h) at 20 °C using a magnetic stirrer. The solution was then adjusted to pH 2 with concentrated HCl (37–38%), and the liberated phenolic acids extracted with 15 ml of a mixture of cold diethyl ether and ethyl acetate (1:1), centrifuged at 620g (Rotofix 32, Hettich Zentrifugen, Germany), and the organic layer recovered. The extraction was repeated twice and the organic layers were combined, evaporated, dissolved in 2 ml of methanol, and filtered with a membrane filter (0.45  $\mu$ m, 25 mm; Pall Gelman Laboratory) for HPLC analysis. After alkaline hydrolysis, an acid hydrolysis step was performed on the extraction residue by adding 2.5 ml of concentrated HCl to the test tube and incubating in a water bath at 85 °C for 30 min. The sample was then cooled and adjusted to pH 2 with 6 M NaOH, then prepared for analysis in the same manner as after alkaline hydrolysis.

The same HPLC instrument was used for phenolic acids as for anthocyanins. Phenolic acids were analysed using an Inertsil (GL Sciences, Inc., Japan) ODS-3 (4.0  $\times$  150 mm, 3  $\mu$ m) column with a C<sub>18</sub> guard column. The column oven was set at 35 °C. Gradient elution was employed with a mobile phase consisting of 50 mM H<sub>3</sub>PO<sub>4</sub>, pH 2.5 (solvent A) and acetonitrile (solvent B) as follows: isocratic elution with 5% B from 0 to 5 min; linear gradient from 5% B to 15% B, 5–17 min; linear gradient from 15% B to 20% B, 17–40 min; linear gradient from 20% B to 50% B, 40–60 min; isocratic elution 50% B, 60–65 min; linear gradient from 50% B to 5% B; equilibration at 5% B from 65 to 67 min prior to the next injection. The flow rate was 0.7 ml/min. The wavelengths used for the quantification of phenolic acids with the diode array detector were: 254 nm for protocatechuic acid, *p*-hydroxybenzoic acid and vanillic acid; 280 nm for syringic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, and *E*-cinnamic acid; 329 nm for caffeic acid, ferulic acid, sinapic acid and chlorogenic acid. After HPLC quantification, the results from alkali and acid hydrolysates were combined to represent total phenolic acids. The samples were analysed in triplicate.

### 2.5. Determination of proanthocyanidins

Proanthocyanidins were analysed, after acid-catalysed depolymerisation, by reversed-phase HPLC as free flavan-3-ols (terminal units) and benzylthioethers (extension units) (Hellström & Mattila, 2008). The method used a 150  $\times$  4.0 mm i.d., 3  $\mu$ m, Inertsil ODS-3 column, with a mobile phase consisting of solvent A: 50 mM phosphoric acid (aq.), pH 2.5, adjusted by NaOH and solvent B: acetonitrile. Elution was started isocratically at a flow rate of 0.7 ml/min with 5% B for 5 min, followed by 5–27.5% B over 5–30 min, 27.5–50% B over 30–32 min and 50% B from 32–38 min. Separation

was monitored by diode array detection (DAD;  $\lambda_1 = 270$  nm,  $\lambda_2 = 280$  nm) and fluorescence detection (FLD;  $\lambda_{\text{ex}} = 280$  nm,  $\lambda_{\text{em}} = 323$  nm). External standards derived from authentic compounds were used for the quantification of terminal units (FLD/UVD,  $\lambda = 280$  nm: catechin, epicatechin, epicatechin gallate; UVD,  $\lambda = 270$  nm: epigallocatechin). Epicatechin benzylthioether (FLD/UVD,  $\lambda = 280$  nm) was obtained by thioacidolysis of procyanidin B2 and used as a standard. Other extension units were quantified against epicatechin benzylthioether, using the response factor ratios reported by Vivas et al. (2004). All juice samples were freeze-dried before triplicate analyses.

## 2.6. Determination of flavonols

The juice samples were thawed, prepared in filter vials (Mini-Uniprep units, 0.45  $\mu\text{m}$  pore size, Whatman PLC, Maidstone, UK) and analysed on an LCQ-DECA system, comprising a Surveyor autosampler, pump and photodiode array (PAD) detector and a Thermo mass spectrometer ion trap (Thermo Scientific Ltd., Waltham, MA, USA). The PAD scanned three discrete channels at 280, 365 and 520 nm. The LCQ-DECA was fitted with an electrospray ionisation (ESI) interface and was used in full scan negative mode. The ESI was tuned against quercetin-3-*O*-glucoside. The method used a reversed phase Synergi 4 $\mu$  Hydro C<sub>18</sub> (150  $\times$  4.6 mm, 4  $\mu\text{m}$ ) column (Phenomenex Ltd., Macclesfield, UK) with a gradient of 5% B (0.1% formic acid in acetonitrile) in A (0.1% aqueous formic acid) for 5 min, then a linear gradient to 40% B over 25 min and finally to 100% B over a further 2.5 min which was maintained for a further 5 min. The flow rate was 0.4 ml min<sup>-1</sup>. Flavonols were detected at 365 nm. Flavonols were identified by their MS properties (Anttonen & Karjalainen, 2006; Määttä, Kamal-Eldin, & Törrönen, 2003; Määttä-Riihinen, Kamal-Eldin, Mattila, González-Paramás, & Törrönen, 2004) but quantified as myricetin equivalents against a standard curve. Separation of all flavonol components was not routinely possible, so flavonol-3-*O*-glucoside and galactoside derivatives were quantified as total hexose derivatives. Only flavonol components present in all the juice products were quantified.

## 2.7. Determination of vitamin C

Vitamin C was determined as dehydroascorbic acid according to the method of Speek, Schrijver, and Schreurs (1984). Vitamin C was analysed using a Hewlett Packard 1090 Series HPLC (Waldbronn, Germany) equipped with a fluorescent detector. The analytical column was a Agilent Zorbax Eclipse Plus (100  $\times$  4.6 mm, 3.5  $\mu\text{m}$ , Agilent, Santa Clara, CA, USA) operated at 35 °C at a flow-rate of 0.6 ml/min. The isocratic mobile phase consisted of methanol and 0.08 M phosphate buffer (pH 7.8) at 60:40. Analyses were performed in duplicate. This method for vitamin C was accredited by FINAS (Finnish Accreditation Service).

## 2.8. Statistical analyses

The anthocyanin, phenolic acid, proanthocyanidin, vitamin C and flavonol data were tested statistically by ANOVA (general linear model).

# 3. Results and discussion

## 3.1. General

In Table 1, the purchased commercial blackcurrant brands are listed as codes with the purchasing country, juice content (declared by the producer), dilution instructions recommended by the producer and calculated juice content of the ready-to-drink

**Table 1**

Commercial black currant brands, purchasing country, juice content, instructions for dilution and juice content of the ready-to-drink beverage.

Brand	Country	Juice content (%)	Instructions for dilution	Juice content of ready-to-drink beverage (%)
F1	Finland	44	1 + 3	11
F2	Finland	100	1 + 4	20
F3	Finland	70	1 + 4	14
G1	Germany	25	1	25
G2	Germany	25	1	25
G3	Germany	35	1	35
P1	Poland	25	1	25
P2	Poland	25	1	25
P3	Poland	25	1	25
UK1	United Kingdom	15	1 + 4	3
UK2	United Kingdom	22	1 + 4	4.4
UK3	United Kingdom	24	1 + 9	2.4

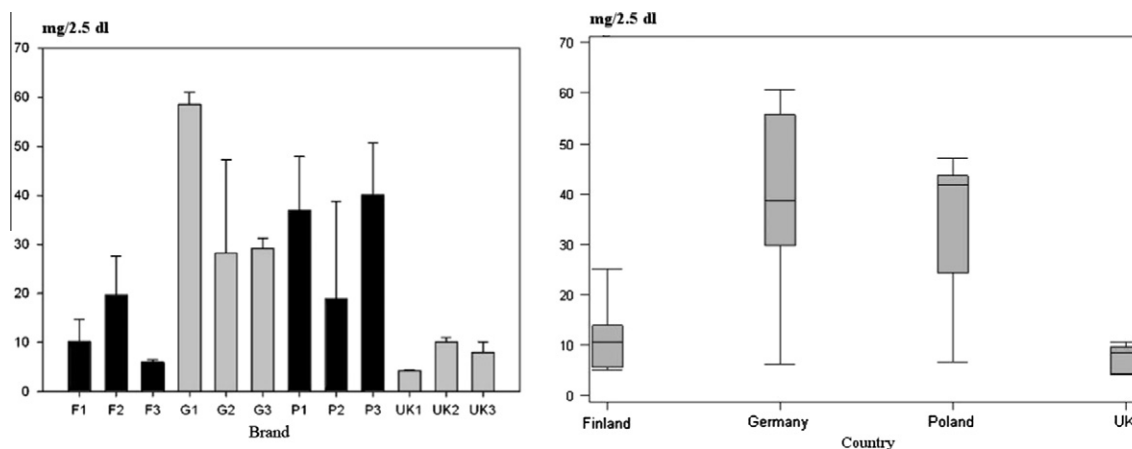
beverage. Concentrates are popular in Finland and the United Kingdom whereas, in Germany and Poland, the most popular blackcurrant juices were ready-to-drink beverages. Heavily diluted juices seem to be especially popular in the United Kingdom. There was over 10-fold variation (from 2.4%; UK3 to 35%; G3) in the juice content of the ready-to-drink beverages.

## 3.2. Anthocyanins

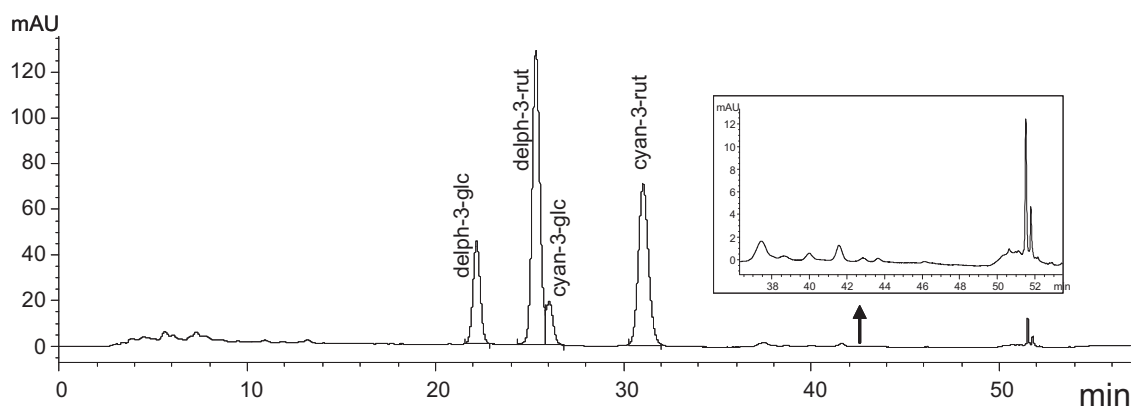
There was 14-fold variation in the anthocyanin content of the 12 analysed European commercial blackcurrant juice products (Fig. 1) from 4.3 (UK1) to 58 mg/2.5 dl (G1) expressed on a ready-to-drink beverage basis. The mean anthocyanin content varied widely between German, Polish, Finnish and British products (38, 32, 12, and 7.5 mg/2.5 dl, respectively,  $p < 0.0001$ ). British and Finnish products had lower levels of anthocyanins than had German and Polish products. In addition, there was significant within-country variation. The variation within brands was significant in many cases. Generally, the juice samples near to their sell-by dates had much lower anthocyanin levels than had fresher ones. Hence, the within-brand variation was probably more directly influenced by different storage stages of the juices rather than different batches. According to Hollands et al. (2008), anthocyanin contents in commercial blackcurrant squash/cordial products ranged from 4.1 to 29.6 mg/100 g FW. Nielsen et al. (2003) analysed 16 commercial blackcurrant juice brands and found a high variation of the total anthocyanin content ranging from 0.14 to 49.2 mg/100 ml. These figures are of the same magnitude as demonstrated in the present study.

When the analytical results were calculated to correspond to 100% juice, the anthocyanin content varied from 17 mg/100 ml (F3) to 133 mg/100 ml (UK3), with a mean value of  $58 \pm 33$  mg/100 ml. The next highest contents were observed in G1 (94 mg/100 ml), UK2 (92 mg/100 ml) and P3 (64 mg/100 ml), whereas the next lowest levels of anthocyanins were observed in P2 (30 mg/100 ml), G3 (33 mg/100 ml) and F1 (37 mg/100 ml). According to Landbo and Meyer (2004), freshly pressed blackcurrant juice can contain 134–322 mg anthocyanins/100 ml. The commercial products analysed in this study indicate much lower levels of anthocyanin when re-calculated to correspond to 100% juice. Finnish juice processing habits, in particular, seem to incur substantial anthocyanin loss.

Delphinidin-3-rutinoside was the major anthocyanin in all juices, followed by cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside, respectively. This is in accordance with earlier data for blackcurrant (Anttonen & Karjalainen, 2006; Hollands et al., 2008; Slimestad & Solheim, 2002; Wu et al., 2004). HPLC-DAD ( $\lambda = 518$  nm) chromatograms of most juices had small



**Fig. 1.** Anthocyanin contents of blackcurrant juice products purchased from different countries. F1–F3: Finnish juice brands; G1–G3: German juice brands; P1–P3: Polish juice brands; UK1–UK3: British juice brands. Values are expressed in mg/2.5 dl of ready-to-drink beverage.



**Fig. 2.** A typical HPLC-chromatogram of the anthocyanin analysis of black currant juice. Delph-3-glc: delphinidin-3-glucoside, delph-3-rut: delphinidin-3-rutinoside, cyan-3-glc: cyanidin-3-glucoside, cyan-3-rut: cyanidin-3-rutinoside.

additional peaks with anthocyanidin-like spectral maxima (Fig. 2), indicating the presence of some minor anthocyanins, in accordance with Wu et al. (2004). However, they were not identified or quantified in this study.

### 3.3. Phenolic acids

The phenolic acid contents (Fig. 3) varied nearly as much as in the case of anthocyanins, and the products with the lowest (1.0 mg/2.5 dl; UK1) and highest (13 mg/2.5 dl; G1) contents were the same as for anthocyanins. There was statistical significance in between-country variation ( $p < 0.001$ ). The mean contents of phenolic acids were 12, 8.9, 3.7 and 1.5 mg/2.5 dl of ready-to-drink beverage in German, Polish, Finnish and British juices, respectively. Similarly to anthocyanins, Finnish and British juices had lower levels of phenolic acids than had Polish and German juices.

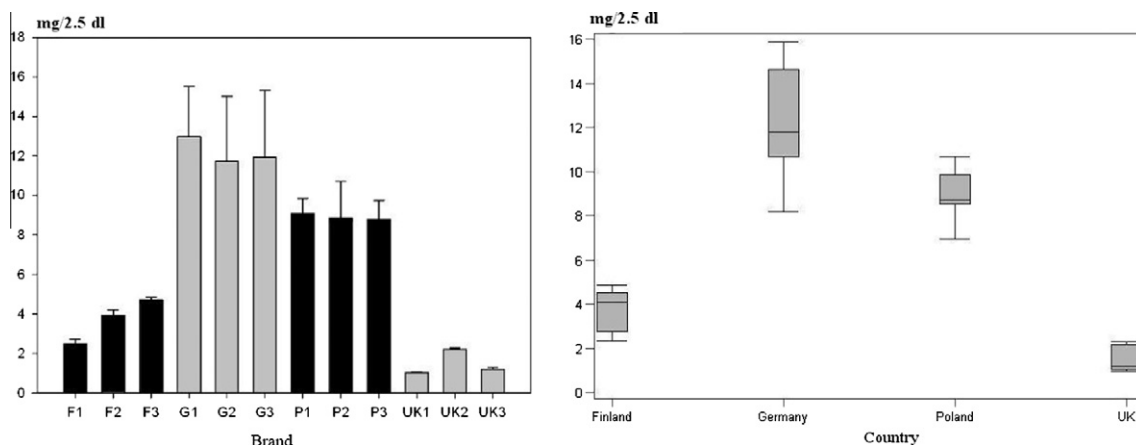
When the phenolic acid contents of the various brands were calculated to correspond to 100% juice, the variation was lower than in the case of anthocyanins. Phenolic acid contents varied from 7.9 (F2) to 21 (G1) mg/100 ml, the mean value being  $15 \pm 4.2$  mg/100 ml. The next highest contents were observed in UK2 (21 mg/100 ml) and UK3 (20 mg/100 ml). Hence, the three best juice sources were the same as in the case of anthocyanins. The lowest levels of phenolic acids were observed in F2 (7.9 mg/100 ml), F1 (9.0 mg/100 ml), F3, G3, and UK1 (14 mg/100 ml).

Phenolic acid content and composition in blackcurrant juices has attracted limited attention. In addition, various methodologies

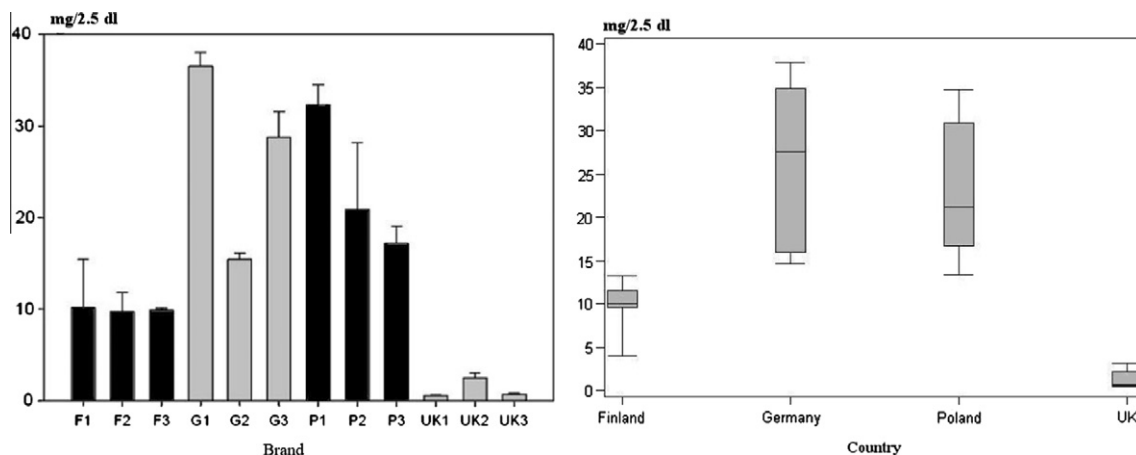
have been used to analyse phenolic acids which has led to divergent compositional values. In this study, both alkaline and acid hydrolysis were used to liberate phenolic acids from their compounds and then quantify them as aglycones. Pinelo et al. (2006) quantified phenolic acids in raw blackcurrant juice, as intact compounds, at 177 mg/l. This total matches quite well with our results for 100% juice. However, there was some dissimilarity in the composition of phenolic acids. In this study, gallic acid and *p*-coumaric acid were the most abundant phenolic acids in samples, followed by caffeic acid. In the study of Pinelo et al. (2006), neochlorogenic acid was the dominant compound in raw juice, followed by *p*-coumaroyl quinic acid, and gallic acid was not detected. On the other hand, Russell, Labat, Scobbie, Duncan, and Duthie (2009) analysed whole berries as phenolic acid aglycones and found the same major phenolic acids as in our study. However, we could not detect gallic acid at all in some brands (e.g. F2, F3 and UK2). In addition, the phenolic acid profiles in UK3 and UK2 had unexpectedly high proportions of sinapic acid.

### 3.4. Proanthocyanidins

Proanthocyanidin contents (Fig. 4) varied widely between different brands. In ready-to-drink juices, the highest proanthocyanidin content (37 mg/2.5 dl) was found in G1 and the lowest (0.53 mg/2.5 dl) in UK1. The same juices were also the richest and the poorest in anthocyanins and in phenolic acids. There was statistically significant between-country variation ( $p < 0.0001$ ) in



**Fig. 3.** Phenolic acid contents of blackcurrant juice products purchased from different countries. F1–F3: Finnish juice brands; G1–G3: German juice brands; P1–P3: Polish juice brands; UK1–UK3: British juice brands. Values are expressed in mg/2.5 dl of ready-to-drink beverage.



**Fig. 4.** Proanthocyanidin contents of blackcurrant juice products purchased from different countries. F1–F3: Finnish juice brands; G1–G3: German juice brands; P1–P3: Polish juice brands; UK1–UK3: British juice brands. Values are expressed in mg/2.5 dl of ready-to-drink beverage.

proanthocyanidin levels indicating that, on average, the contents in German (27 mg/2.5 dl) and Polish (24 mg/2.5 dl) products were higher than Finnish (10 mg/2.5 dl) and British (1.2 mg/2.5 dl) products. Similar results were obtained for anthocyanins and phenolic acids. Remarkable within-brand variation was found in two juices (F1 and P2) but the general variation within brands was fairly modest.

When the proanthocyanidin results of various brands were calculated to correspond to 100% juice, the highest (59 mg/100 ml) and lowest (7.3 mg/100 ml) levels were found in G1 and UK1, respectively. Other juices rich in proanthocyanidins were P1 (52 mg/100 ml), F1 (37 mg/100 ml) and P2 (33 mg/100 ml) while UK3 (11 mg/100 ml) and F2 (16 mg/100 ml) had rather low proanthocyanidin contents.

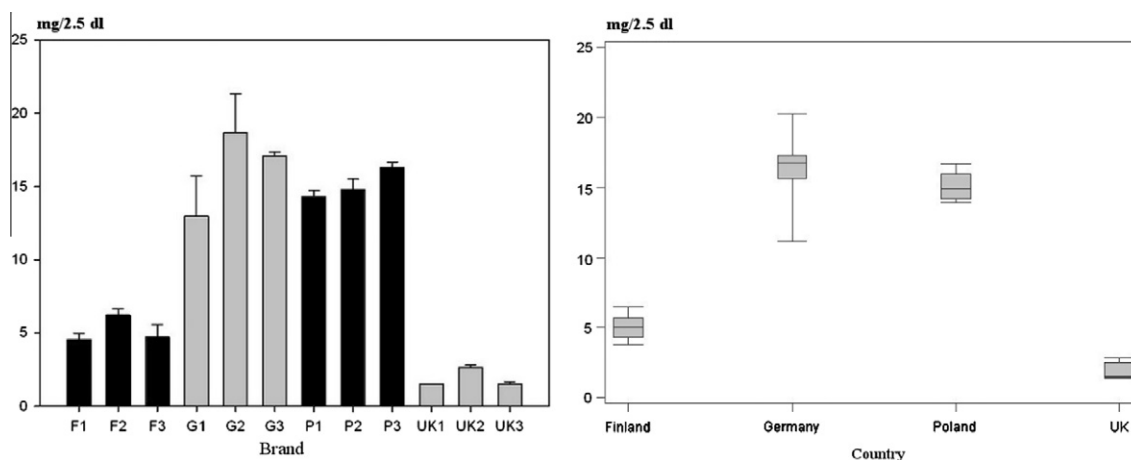
Proanthocyanidins in all samples were always mixtures of procyanidins and prodelphinidins; thus, they were constituted of both (epi)catechin and (epi)gallocatechin subunits. In most cases, (epi)gallocatechins (i.e. prodelphinidins) dominated and made up over 60% of the structural flavan-3-ols. However, UK2 and UK1 had significantly lower prodelphinidin proportions, at 46% and 51%, respectively. The average degree of polymerisation of proanthocyanidins was normally between 10 and 16. However, in UK1, it was only 6.1.

Data on proanthocyanidin contents in blackcurrant juices is largely lacking and only two reports could be found, both dealing

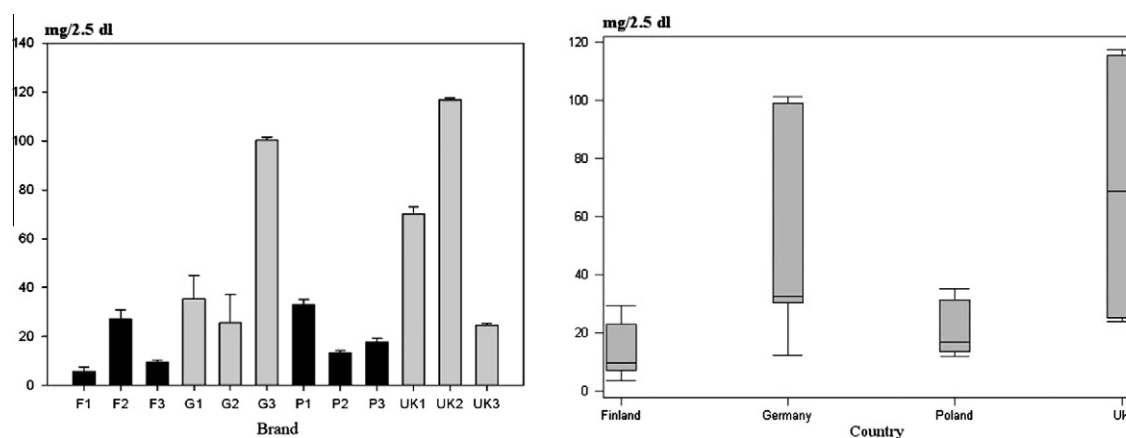
with 100% blackcurrant juices prepared on a laboratory scale (Bagger-Jørgensen & Meyer, 2004; Oszmianski & Wojdyło, 2009). In the study of Oszmianski and Wojdyło (2009), the proanthocyanidin content determined by thioacidolysis was 59.2 mg/100 ml in fresh blackcurrant juice and this decreased during six months of storage to 36.3 mg/100 ml (at 4 °C) and 17.5 mg/l (at 30 °C). The average degree of polymerisation varied from 6.5 to 13.4 (Oszmianski & Wojdyło, 2009). These results are in good agreement with the present study. Bagger-Jørgensen and Meyer (2004) reported proanthocyanidin contents as percentual proportions of total polyphenols determined by the colorimetric Folin–Ciocalteu procedure. Proanthocyanidins represented 19.5–46.7% of total polyphenols in freshly made blackcurrant juices which matches rather well with our results, where ca. 30% of polyphenols were proanthocyanidins in most juices. However, in British juices, the proportion was significantly lower (approx. 10%).

### 3.5. Flavonols

There was 12-fold variation in flavonol content in the 12 European commercial blackcurrant juice brands (Fig. 5) expressed as ready-to-drink beverage. UK1 turned out to be the poorest (1.5 mg/2.5 dl) source of flavonols, as seen in the case of anthocyanin, phenolic acid and proanthocyanidin contents. However, the highest levels were found in G2 (19 mg/2.5 dl). Similar to other



**Fig. 5.** Flavonol contents of blackcurrant juice products purchased from different countries. F1–F3: Finnish juice brands; G1–G3: German juice brands; P1–P3: Polish juice brands; UK1–UK3: British juice brands. Values are expressed in mg/2.5 dl of ready-to-drink beverage.



**Fig. 6.** Vitamin C contents of blackcurrant juices purchased from different countries. F1–F3: Finnish juice brands; G1–G3: German juice brands; P1–P3: Polish juice brands; UK1–UK3: British juice brands. Values are expressed in mg/2.5 dl of ready-to-drink beverage.

polyphenols, there was statistically significant between-country variation ( $p = 0.003$ ), indicating that mean contents in German (16 mg/2.5 dl) and Polish (15 mg/2.5 dl) juices were higher than they were in Finnish (5.2 mg/2.5 dl) and British (1.9 mg/2.5 ml) ones. Within-country variation was evident for all the countries but more so for Germany and the UK. Substantial within-brand variation was found only for G1 and G2.

When the flavonol results of various brands were calculated to correspond to 100% juice, flavonol contents varied from 12 (F2) to 30 (G2) mg/100 ml. The next highest contents were observed in P3 (26 mg/100 ml), UK3 (25 mg/100 ml) and UK2 (24 mg/100 ml). The next lowest levels of flavonols were observed in other Finnish juices (F3: 14 mg/100 ml; F1: 16 mg/100 ml).

The flavonol composition of the black currant juice products was similar to those in previous reports (e.g. Anttonen & Karjalainen, 2006; Koponen et al., 2008; Määttä et al., 2003) and was predominantly composed of rutinoside and hexose (glucoside and galactoside) derivatives of myricetin, quercetin and kaempferol. As expected, due to inter-species and environmental variation in phenolic composition of black currants used in the products, other flavonols, such as isorhamnetin derivatives, (Koponen et al., 2008) were detected in some juice products. However, these were mainly detected in products with higher % juice contents and the quantification focused on flavonol components common to all products. The aglycones, myricetin and quercetin, were detected in all juice

products (Koponen et al., 2008) but quercetin co-eluted with unknown peaks and could not be satisfactorily quantified. The relatively high content of myricetin compared to its glycosylated derivatives may be caused by the use of glycosidase-rich enzyme preparations to facilitate juice production (Landbo & Meyer, 2004) and/or other processing methods.

Differences in methodology make comparison of flavonol content with previous work difficult but the values are in the same overall range as those reported previously (Anttonen & Karjalainen, 2006; Koponen et al., 2008; Määttä et al., 2003).

### 3.6. Vitamin C

Vitamin C levels (Fig. 6) varied widely between products from 6.6 (F1) to 117 (UK2) mg/2.5 dl of ready-to-drink beverage. There was statistically significant between-country variation ( $p = 0.0002$ ) with the lowest and highest levels of vitamin C determined in Finnish products (15 mg/2.5 dl) and UK (70 mg/2.5 dl), respectively. The variation was highest in German products. However, two British products (UK1 and UK2) are known to be fortified with vitamin C, and the vitamin C levels declared by the producers are 45 and 120 mg/2.5 dl of ready-to-drink beverage, respectively. These contents were quite well in accordance with the values obtained in this study (UK1: 70 mg/2.5 dl and UK2: 117 mg/2.5 dl). One producer from Germany and one from Poland declared natural

vitamin C contents in their products as G3 = 75 mg/2.5 dl and P2 = 25 mg/2.5 dl, respectively. The analysed results for these products were 100 and 13 mg/2.5 dl, respectively. In addition, F2 and UK3 contained ascorbic acid to serve as an antioxidant but the content was not declared.

When the vitamin C results of various juice products were calculated to correspond to 100% juice, there was a huge variation, because many of the brands (UK1, UK2, UK3, F2) were fortified with this vitamin. In unfortified products, vitamin C content varied from 17 (F1) to 115 (G3) mg/100 ml. Zheng, Yang, Tuomasjukka, Ou, and Kallio (2009) reported ascorbic acid contents of 60–190 mg/100 ml in black currant juices made from three cultivars and grown in two different locations. However, it is difficult to compare these results with the commercial juices due to the totally different processing methods of the juices. It is known that vitamin C content declines during juice processing, for example during pasteurisation (Iversen, 1999).

#### 4. Conclusions

German (94.0 mg/2.5 dl) and Polish (79.5 mg/2.5 dl) ready-to-drink juice beverages had clearly higher levels of total polyphenols (the sum of flavonols, anthocyanins, phenolic acids and proanthocyanidins) than had Finnish (30.7 mg/2.5 dl) and British (12.1 mg/2.5 dl) beverages. This order was also followed for each individual polyphenol group. The intake of polyphenols from German and Polish juice products was moderately high when compared, for example, to the daily intake of polyphenols by Finnish adults [estimated at 863 ± 415 mg (Ovaskainen et al., 2008)]. On the other hand, British juice products had very low levels of polyphenols, mainly because they were heavily diluted. Anthocyanins were the main polyphenol group in all juice products; ca. 40–60% of the total polyphenol content was anthocyanins. Vitamin C levels varied widely between juice brands. All British products were fortified with vitamin C and therefore had the highest levels.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.foodchem.2011.01.129](https://doi.org/10.1016/j.foodchem.2011.01.129).

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