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ARTICLE

Orosensory Profiles and Chemical Composition of Black Currant (*Ribes nigrum*) Juice and Fractions of Press Residue

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Fractionation of black currant (*Ribes nigrum*) by juice pressing, four ethanol extractions, ethanol evaporation, and supercritical fluid extraction was studied. Phenolic compounds, sugars, and acids of the fractions were analyzed by high-performance liquid chromatography and gas chromatography. Sensory properties of the fractions were studied using generic descriptive analysis. Most of the sugars and acids were located in the juice, whereas the majority of the phenolic compounds were in the press residue. Ethanol extracted nearly all of the phenolic compounds from the press residue, leaving only fibers and seeds. The juice was dominant in most of the sensory attributes, whereas the extracts were perceived as most astringent. Three flavonol glycosides [kaempferol-3-*O*-(6″-malonyl)glucoside, myricetin-3-*O*-galactoside, and an unknown kaempferol glycoside] were discovered to be the compounds especially contributing to astringency. Ethanol extraction appeared to be an efficient and simple way to isolate phenolic compounds from black currant juice press residue.

KEYWORDS: Black currant; ethanol extraction; astringency; phenolic compounds

INTRODUCTION

The black currant berry (*Ribes nigrum*) is regarded as a natural high-value food raw material and a source of many essential nutritional components (*1*−*4*). It is rich in phenolic compounds such as anthocyanins, flavonols, flavan-3-ols, and phenolic acids (*5*−*7*). The black currant phenolic compounds are potent antioxidants, and they have also been shown to have antimicrobial properties against pathogenic bacteria (*8*). Anthocyanins are known to be responsible for the color of black currants (*9*, *10*). The chemical composition of the black currant depends on variety (*11*−*13*) and seasonal weather conditions (*13*).

In general, the juice of cultivated black currants is used as a raw material in the food industry for drinks and jam production. The press cake and berry seeds are usually discarded as useless byproduct (*14*). Taste and other flavor properties of any food are essential components of product quality. They may also be the main reason for the rejection of foods. Despite the high nutritional value of the black currant, it may be despised because of its sourness and astringency. Both the organic acids and the ratio of sugar and acid components contribute to the intensity of the sour sensation. The American Society for Testing and Materials (ASTM) defines astringency as "the complex sensation due to shrinking, drawing and puckering of epithelium as a result of exposure to the substances such as alums or tannic acids". The compounds contributing to astringency in red currants (*Ribes rubrum*) are reported to be certain phenolic compounds (*15*). The interaction between nonvolatile compounds and berry flavor, and especially the compounds contributing to astringency in the black currant, is still to be investigated.

Most of the studies on black currant composition have been focused on the juice by abandoning the press byproducts. Kapasakalidis et al. (*16*) found that the content of anthocyanins and antioxidant activity were higher in the black currant skin fraction than in the juice when polyphenols were extracted from the pomace with methanol and methanol/water/acetic acid. Berry phenolics dissolve in acetone and methanol better than in water (*17*). The use of proper enzymes releases more phenolic compounds from the press residue to the juice (*7*, *16*). The berry press residue is principally composed of seeds and the polysaccharides of cell walls, but it also contains hydrophobic cuticular polymers, that is, cutin and possibly cutan (*3*). Because of the chemical composition, press residue could also have an important role as a source of insoluble fiber for industrial applications.

The aim of this study was to isolate and identify the compounds contributing to the orosensory properties of black currant juice and skin-rich press residue. Concentrations of phenolic compounds, sugars, and acids in berries, juice, and press residue of black currant together with the location of the compounds in the berry fractions were investigated. In addition, ethanol extraction was applied to isolate the phenolic compounds and to remove the potential astringency-contributing compounds from the berry skin. Our goal was to avoid unnecessary unit operations and to keep the flavor-modifying process as simple and safe as possible for later food industry applications.

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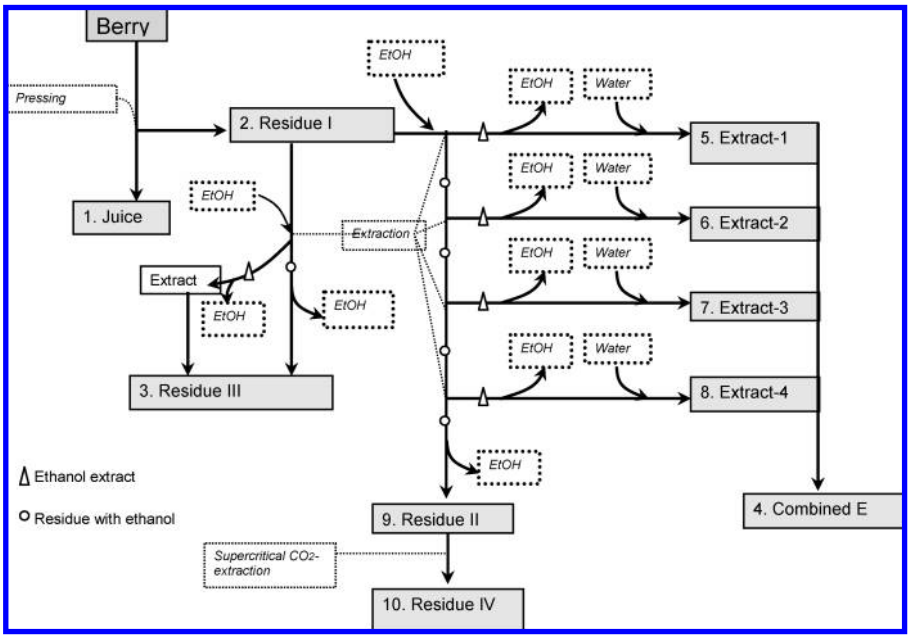

Figure 1. Overview of the processing scheme for black currant fractions (1−10). Ethanol extracts (triangles) were used for chemical analyses. For sensory sample set A, dried extracts were dissolved in water 25 g/L. For sensory sample set B, each of the dried extracts was dissolved in 50 mL of water.

MATERIALS AND METHODS

Berries. The black currant variety 'Mortti' was cultivated in Piikkiö at MTT (Agrifood Research, Finland) and harvested in August 2005. Berries were stored frozen at −20 °C in polyethylene bags to maintain their quality for analyses.

Chemicals. Myricetin, quercetin, kaempferol, ferulic acid, caffeic acid, and *p*-coumaric acid were obtained from Sigma (St. Louis, MO). Quercetin-3-*O*-rutinoside (qu-rut), quercetin-3-*O*-galactoside (qu-gal), quercetin-3-*O*-glucoside (qu-glc), kaempferol-3-*O*-glucoside (ka-glc), delphinidin-3-*O*-glucoside (dp-glc), delphinidin-3-*O*-rutinoside (dp-rut), cyanidin-3-*O*-glucoside (cy-glc), and cyanidin-3-*O*-rutinoside (cy-rut) were obtained from Extrasynthese (Genay, France). Sorbitol and tartaric acid were obtained from Merck (Darmstadt, Germany). The acetonitrile, ethyl acetate, methanol, formic acid, potassium hydrochloride, and hydrochloric acid were of HPLC grade or the highest grade available. Activated carbon-filtered tap water was used in preparing the samples for sensory analysis. The ethanol used in sample preparation was 96% ETAX A (Altia, Helsinki, Finland). The reference compounds for sensory analysis are described in **Table 8**.

Berry Fractionation. For one fractionation, a portion of 350 g of frozen berries was gently thawed in a microwave oven [AEG Micromat 725 (1200 W), Nürnberg, Germany] in a 600 mL beaker with 30% power for 4 min. Berries were crushed with a Bamix mixer (Bamix M133, Germany) when half-melted and pressed with a hydraulic juice extractor (Hafico, Germany) until a pressure of 300 kg/cm^3 was reached. Both the juice and the press residue (residue I in the tables) were frozen for further processes and analyses. An overview of the processing scheme for black currant fractionation is presented in **Figure 1**.

EtOH Extraction of Residue I. Residue I was thawed at room temperature before the ethanol extraction. A 100 g batch of residue I was extracted with 200 mL of aqueous ethanol solution in a 600 mL beaker in four successive extractions. Different aqueous ethanol solutions were tested: 96, 90, and 70% ethanol in water. For all experiments, we chose the 90% ethanol solution. Ethanol and residue I (2:1) were first mixed with a Bamix mixer for 1 min and then with a magnetic stirrer for 30 min. The ethanol solution of each batch was filtered (Whatman no. 1, Whatman International Ltd., Maidstone, U.K.) and evaporated with a rotary evaporator (Heidolph VV2000, Heidolph Elektro GmbH & Co. KG, Kelheim, Germany). Residue II was air-dried and stored at room temperature for further use. After the evaporation of ethanol from the four fractions, the extracts were each dissolved in filtered water at a concentration of 25 g/L to get sample set A for sensory analysis (from extracts 1−4). The filtered ethanol extracts were used as such for chemical analyses. In addition, a set of solutions was prepared by dissolving each of the dried extracts of one complete extraction procedure in 50 mL of water to obtain sample set B for sensory analysis.

EtOH Soaking. Residue I was soaked in ethanol (90%) to check the effect of possible reaction products of ethanol on phenolic compounds and taste. Ethanol was evaporated from the filtered extract after 30 min by stirring at room temperature. Dried filtrate was dissolved in filtered water and returned to the once-extracted residue I to create residue III. The volume of water added to the filtrate was chosen to correspond to the moisture of the original residue I.

Supercritical Fluid Extraction of Residue II. Residue II was also extracted with supercritical CO_2 following the pilot scale manufacturing procedure and using the batch process without crushing the seeds (Aromtech Ltd., Tornio, Finland) to produce residue IV.

Dry Matter. For the gravimetric dry matter measurement of berry, juice, and residue I, the samples were kept at 110 °C overnight before weighing. The weights of extracts 1−4 were measured after the evaporation of ethanol.

Spectrophotometry of Total Phenolics. The total phenolic compounds of the extracts were measured using the Folin−Ciocalteu method previously reported for wines (*18*).

Total Anthocyanins. The intensity of the color of the EtOH extracts was analyzed at pH 1.0, set with HCl, by measuring the absorbance at 515 nm. For berry, juice, residue I, and residue II, the intensities of color were analyzed by extracting 5 g of the samples six times with 15 mL of acidic methanol (MeOH/HCl, 99:1). The absorbance of the combined extracts was measured at 515 nm. The total content of anthocyanins (mg/100 g) was quantified using a reference compound mixture prepared according to the proportions of four major anthocyanins (dp-glc, dp-rut, cy-glc, and cy-rut) in black currant.

HPLC-DAD Analyses of Anthocyanins. Anthocyanins of berry, juice, residue I, and residue II were isolated in duplicates according to the method of Buchert et al. (*19*) with minor changes. The samples (5 g) were extracted three times

consecutively with 15 mL of MeOH/HCl (99:1). After 1.5 min of vigorous mixing, the samples were centrifuged for 10 min at 3400*g* (Beckman model J2-21, Beckman Coulter Inc., Fullerton, CA). The three supernatants were combined, and the total volume was set to 50 mL with acidic methanol. EtOH extracts were prepared for analysis by removing solvent from 10 mL of sample with a rotary evaporator, and the dry samples were weighed. The first EtOH sample was diluted to 100 mL of acidified methanol, the second to 40 mL, the third to 20 mL, and the fourth to 10 mL on the basis of the results of total phenolics.

Anthocyanins were analyzed with a modified method of Määttä et al. (*9*). Prior to HPLC analysis, the samples were filtered through a 0.45 μm syringe filter (Spartan 13, Whatman GmbH, Dassel, Germany). The HPLC-DAD system was a Shimadzu LC-10AVP (Shimadzu, Kyoto, Japan) with an LC-10AT pump, an SIL-10A autosampler, and an SPD-M10AVP diode array detector linked to the SCL-M10AVP data handling station. Samples were separated on a 250 × 4.60 mm i.d., 5 μm Phenomenex Prodigy RP-18 ODS-3 column (Torrance, CA) with a 30 × 4.60 mm i.d., 5 μm Phenomenex Prodigy precolumn. The analysis of anthocyanins was performed using 5% formic acid as solvent A and acetonitrile as solvent B with the following gradient: 0−5 min, 5−10% B; 5−10 min, 10% B; 10−25 min, 10−40% B; 25−35 min, 40−90% B; 35−40 min, 90−5% B; 40−45 min, 5% B. The flow rate of the mobile phase was 1.0 mL/min. Anthocyanins were detected at 520 nm. Quantitative analysis was carried out after the identification of the peaks using the four major anthocyanins of black currant as external standards for detected compounds. For berry, juice, residue I, and residue II the concentrations were expressed in milligrams per 100 g of fresh weight and for the EtOH extracts, in milligrams per 100 mL of water-diluted dry extract. Concentrations were also expressed as fractions of 1 kg of berry. Additionally, the total content (CR) of each anthocyanin was calculated mathematically according to four consecutive extracts, forming a descending curve using Origin 8 software (Originlab Corp., Northampton, MA). The equation of the descending curve ($y = A \times e^{(-x/B)} + C$) was chosen for simplicity and to adjust R^2 values. A (amplitude) and B (decay constant) were constant numbers calculated by the software, and C (offset) was set to zero.

HPLC-DAD Analyses of Other Phenolic Compounds. Flavonols, their aglycons, and hydroxycinnamic acid derivatives were analyzed in duplicate according to a modified method of Määttä et al. (*9*). Frozen berry, juice, residue I, and residue II were thawed and weighed (ca. 5 g accuracy) for analysis. Ten milliliters of the EtOH fraction (20 mL of the fourth fraction) was evaporated with a rotary evaporator. The weighed residues were diluted in 5 mL of water. The extraction of flavonols was performed at room temperature with 10 mL of ethyl acetate four consecutive times. The samples were mixed vigorously for 1.5 min and centrifuged for 10 min at 3400*g*. For the EtOH extracts, the first batch of solvent was added through the evaporation bottle to extract the phenolic compounds remaining. The four extracts were combined, and ethyl acetate was removed using a rotary evaporator. The flavonol fractions were diluted in 3 mL of MeOH. Half of the sample was filtered and analyzed for the flavonols and hydroxycinnamic acid conjugates, and the other half was hydrolyzed with 0.6 M HCl for 50 min in a boiling water bath for the analysis of flavonol aglycons and hydroxycinnamic acids.

Analyses were done using the same Shimadzu HPLC-DAD apparatus as described above and using 1% formic acid as solvent A and acetonitrile as solvent B with a gradient as follows: 0−20 min, 5−30% B; 20−30 min, 30−90% B; 30−35 min, 90−5% B; 35−40 min, 5% B. Flavonols and their aglycons were detected at 360 nm and hydroxycinnamic acids and their conjugates at 320 nm. Quantitative analysis was carried out using qu-rut, qu-glc, qu-gal, and ka-glc as the external standards for these compounds and qu-glc for other identified flavonol glycosides. Myricetin, quercetin, and kaempferol were used as the external standards for flavonol aglycons and ferulic acid, caffeic acid, and *p*-coumaric acid for hydroxycinnamic acid derivatives. For berry, juice, residue I, and residue II, the concentrations were expressed in milligrams per 100 g of fresh weight, and for extracts 1−4 in milligrams per 100 mL of the water-diluted dry extract. Concentrations were also expressed as fractions of 1 kg of berry. Additionally, the total content of each compound was calculated using Origin 8.

Identification of Phenolic Compounds by UPLC-MS. HPLC conditions were as described above, and the apparatus was an Acquity Ultra Performance LC (Waters, Milford, MA) interfaced to a Waters Quattro Premier quadruple mass spectrometer. ESI-MS analysis for anthocyanins was performed in positive ion mode using a capillary voltage of 3.5 kV, a cone voltage of 40 V, and an extractor voltage of 3 V and for flavonols using a capillary voltage of 5 kV, a cone voltage of 20 V, and an extractor voltage of 3 V. In both cases, the source temperature was 120 °C and the desolvation temperature was 300 °C. In the MS analysis (full scan), data were acquired over a mass range of m/z 250−700. The UV−vis spectra, reference times, reference compounds listed under Chemicals, and mass spectra, as well as literature data (*7*, *9*, *10*, *12*, *20*−*22*), were used for identification.

Analyses of Sugars and Acids. Sugars and acids were analyzed in duplicate by gas chromatography as trimethylsilyl (TMS) derivatives of berry, juice, residue I, and extract 1 according to the method applied by Kallio et al. and Tiitinen et al. (*23*, *24*). Fifty grams of berries was pressed and centrifuged (3400*g*, 10 min, room temperature). Fifty grams of residue I was soaked in 100 mL of water, mixed for 1 min with a Bamix mixer, stirred for 30 min, and filtered. Extract 1, representing a 50 mL portion of evaporated EtOH solution, was diluted in water (1:40, w/v).

Sensory Evaluation. General guidelines for the selection, training, and monitoring of assessors (*25*) were used. Sensory descriptive profiling was applied with 15 voluntary panelists, of whom 8 were women and 7 were men (ages 21−57 years). The assessors were selected according to their willingness, good health (self-reported), and availability. The ability of assessors to recognize different taste samples (sweet, salty, sour, bitter, and water) and to rank the taste solutions according to bitterness were tested (0.02, 0.04, 0.08, and 0.14% caffeine). In addition, a triangle test was used to test the sensitivity of the assessors with blank black currant juice and citric acid-spiked juice. The descriptors were generated following ISO/DIS standard (*26*) during four independent sessions. During the training sessions the descriptors were created, and the assessors were familiarized with the usage of the attributes, the intensity scale, and the Compusense-*five* data collection software (version 4.6, Compusense, Guelph, ON, Canada).

Intensities of the attributes were rated on a line scale anchored from 0 (none) to 10 (very strong) with the help of the references (**Table 8**), and each assessor evaluated all of the samples in triplicate during separate sessions. All attributes of a given sample were evaluated at a session in unrandomized order. Sensory evaluations consisted of two parts with three parallel sample evaluation sessions. In the first part, residue I, juice, residue II, residue III, and a combination of the four ethanol extracts (combined extract) were evaluated. Juice and the combined ethanol extract were liquid, and the other three samples were solid. In the second part, the samples comprised the four consecutive ethanol extracts. Before tasting them, ethanol was evaporated and the dried samples were diluted in water (25 g/L) to obtain extracts 1−4 (sample set A). In addition, the panel evaluated the intensity of extracts in a constant volume (each dried extract was dissolved in 50 mL of water, sample set B) using ordinal scaling (*27*). The diluted extracts were rank-ordered from the strongest intensity (value = 1) to the weakest intensity (value = 4). To find out the effect of SFE technology, we used the triangle test (*28*) to evaluate the difference between residue II and residue IV (12 assessors, 3 replicates, $n = 36$).

Table 1. Distribution of the Dry Matter and Phenolic Compounds in Black Currant Berry and Its Fractions[a].

fraction	fresh wt (g)	dry matter (g)	total phenolic compd (mg)	total anthocyanins (mg)	anthocyanins HPLC (mg)	flavonol glycosides HPLC (mg)	flavonol aglycons HPLC (mg)	hydroxycinnamic acids HPLC (mg)
berry	1000	170	−	3400	4100	100	30	60
juice	760	80	−	700	500	30	10	20
residue I	240	90	−	2900	3200	70	20	40
extract 1		18	2600	1300	1400	80	20	30
extract 2		10	1200	800	800	50	10	20
extract 3		5	400	300	200	10	5	10
extract 4		3	200	100	100	5	2	3
residue II		55	−	100	30	*	*	*
residue IV		55	−	−	−	−	−	−
SFE extract		0	−	−	−	−	−	−

[a] Juice and residue I are the fractions of the whole berry. Extracts 1−4 and residue II are the fractions of residue I. Residue IV and SFE extract are the fractions of residue II. *, below the detection limit of S/N > 3; −, analysis was not made.

All of the sensory analyses were performed at the sensory laboratory in accordance with ISO standard 8589-1988 (*29*).

Statistical Analyses. Differences between samples were analyzed by a one-way analysis of variance (ANOVA) together with Tukey's *t* test and the Tamhane test ($p < 0.05$). The results from ranking test (sample set B) were analyzed with the Friedman test ($p < 0.001$). To interpret the results for the nine sensory attributes, principal component analysis (PCA) was applied. To find the relationships between the two data matrices, the partial least-squares regression (PLS) method was applied for standardized data. *X*-variables (predictors) were the chemical compounds, and *Y*-variables (responses) were the sensory properties. Cross-validation was used to estimate the number of principal components for a statistically reliable model. Statistical analyses were performed using SPSS14.0 (SPSS Inc. H, Chicago, IL), SAS 6.11 (SAS Institute Inc., Cary, NC), and Unscrambler 9.8 (Camo Process AS, Oslo, Norway).

RESULTS AND DISCUSSION

Fractionation of the Berries. The unit operations in the fractionation of black currant berries were juice pressing, ethanol extraction of the press residue, evaporation of ethanol under reduced pressure, and extraction of the residue by supercritical CO_2. The whole process is illustrated in **Figure 1**, and the process recoveries of each fraction are presented in **Table 1**. The yield of press cake in the juice extraction (residue I) was 24 ± 4% ($n = 39$) with the dry matter content of residue I being 38%. Four consecutive extractions of residue I were carried out to investigate the extractability of different phenolic compounds and to compare the sensory properties of the fractions obtained (extracts 1−4). Thus, 100 g of residue I was extracted with a total of 800 mL of 90% ethanol. The dry matter of extract 1 was highest at 18 g, representing 1 kg of berries, whereas the weight of extract 4 was just 3 g (**Table 1**). The sum of the dry matter of extracts 1−4 and residue II was equivalent to the dry matter of residue I. On the basis of gravimetric measurement, supercritical CO_2 extraction did not remove a significant amount of components (< 1%) from residue II.

Extraction by 90% ethanol was applied to isolate fractions containing especially the phenolic compounds from residue I (*14*, *30*), even though acidified ethanol is known to be a more effective solvent (*31*). On the basis of earlier investigations (*14*, *30*), methanol would also have been more effective than ethanol. In this study the goal was, however, to keep the process safe and applicable to food processing. The dilution of ethanol improved the solubility of phenolic compounds (*14*, *31*), and the recoveries obtained in descending order of increasing ethanol concentrations were 70, 90, and 96%. The 90% EtOH was chosen as a compromise due to sufficient yield and proper evaporation time of the solvent after extraction. All of the ethanol was removed from the extracts because of the sensory evaluation and tasting of the fractions.

Total Phenolics and Anthocyanins. The total content of the phenolic compounds was measured in extracts 1−4 with the Folin−Ciocalteau method, which is known to be approximate and indicative only. As shown in **Table 1**, the first ethanol extraction had already released the majority of the phenolics from residue I. This difference, when compared to the following steps, was enhanced by the more dilute ethanol due to the high water content of residue I (data not presented). Six consecutive MeOH−HCl extractions were enough to isolate 99% of the anthocyanins from berry, juice, residue I, and residue II. Anthocyanins were clearly concentrated in the fraction residue I. After four consecutive extractions, the content of total anthocyanins in residue II was < 4% of those in the extracts 1−4, showing clearly > 95% extraction yield.

Identification of Phenolic Compounds. Figure 2 shows HPLC-DAD chromatograms of black currant fractions of the four different groups of phenolic compounds. Identification of the compounds was based on chromatographic and mass spectral information, reference compounds, and literature (*7*, *9*, *10*, *12*, *20*−*22*). A total of 30 phenolic compounds identified are listed in **Table 2**. Identification was considered to be unambiguous in the case of the match with the data of a defined reference compound. Because the identification was based on spectra, chromatographic retention, and the literature only, we considered the identification to be tentative. All of the compounds listed were found in every sample except residue II, in which the content of some of the compounds was less than the detection threshold.

Anthocyanins delphinidin-3-*O*-glucoside (peak 1), delphinidin-3-*O*-rutinoside (peak 2), cyanidin-3-*O*-glucoside (peak 3), and cyanidin-3-*O*-rutinoside (peak 4) were identified on the basis of reference compounds, UV−vis spectra, mass spectra, and the literature. Delphinidin and cyanidin were the two major anthocyanidins, commonly known in black currant (*6*, *10*). There were also other, minor anthocyanins detectable in the chromatograms (**Figure 2A**),

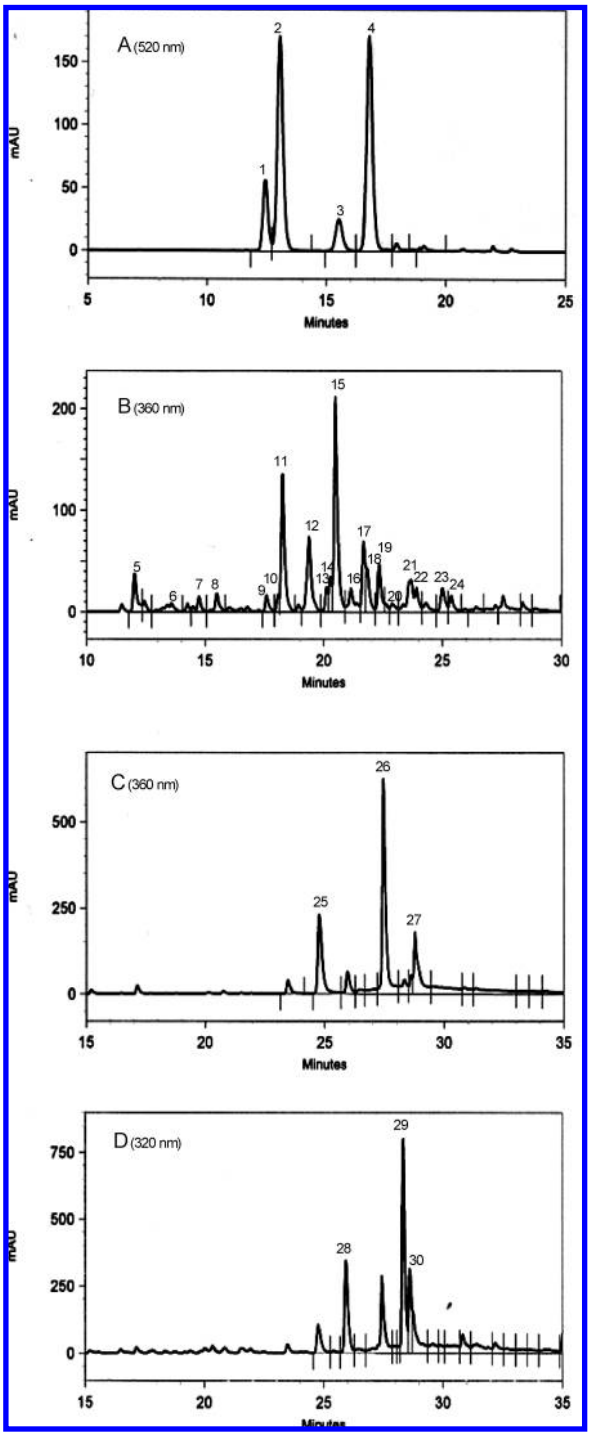

Figure 2. HPLC-DAD chromatograms of anthocyanins (**A**, 520 nm), flavonol glycosides and hydroxycinnamic acid conjugates (**B**, 360 nm, hydroxycinnamic acid conjugates quantified at 320 nm), flavonols (**C**, 360 nm), and hydroxycinnamic acids (**D** 320 nm) in ethanol-extracted skin fraction of black currant (extract 1). Numbers of peaks refer to **Table 2**.

but their concentrations were too low (S/N < 3) for detailed identification. These minor peaks most likely included rutinosides and glucosides of petunidin, peonidin, and pelargonidin as well as coumaroylglucosides of delphinidin and cyanidin.

A total of 14 flavonol glycosides were detected (**Figure 2B**). Quercetin-3-*O*-galactoside (peak 14, **Figure 2B**) eluted just before quercetin-3-*O*-glucoside (peak 15), as recognized with the reference compounds. Galactosides and glucosides have the same molar masses and mass spectra, and they differ according to their retention times only. Analogously, myricetin galactoside (peak 10) was assumed to elute before myricetin glucoside (peak 11). Myricetin rutinoside (peak 9) and kaempferol rutinoside (peak 16) were also identified in our samples, as defined in earlier investigations (*7*, *20*, *22*). Peak 18 was quercetin arabinoside according to the mother ion *m*/*z* 435 and fragment *m*/*z* 303, which due to the mass difference *m*/*z* 132 indicates the existence of arabinose. According to the retention pattern and literature references, all of the compounds **9**, **10**, **11**, **16**, and **18** were all highly evidently 3-*O*-glycosides. Quercetin-3-*O*-rutinoside (peak 12) coeluted with myricetin-3-*O*-arabinoside and myricetin-3-*O*-(6″-malonyl)glucoside. Myricetin-3-*O*-arabinoside was detected in our samples in trace amounts only. Malonylglucosides of myricetin, quercetin, and keampferol (peaks 12, 17, and 24) have previously been reported in black currant (*7*, *20*, *22*). Peak 23 in **Table 2** was identified as a kaempferol glycoside according to the fragment ion *m*/*z* 287 (100% intensity). Isorhamnetin glycosides have previously been reported in black currants (*7*, *22*), but they were detectable in our samples in trace amounts only.

Hydroxycinnamic acid conjugates (**Figure 2B**) were identified by comparing their UV−vis and mass spectra with published data (*20*, *22*). Among peaks 13 and 20−22 there are hexose derivatives of caffeic acid, ferulic acid, and *p*-coumaric acid, where the sugar moiety may be glucose. By comparison with the literature, peaks 5−8 may be caffeoylglucose, *p*-coumaroylglucose, *p*-coumaroylquinic acid, and feruloylglucose respectively (*20*, *22*). The identification of hydrolyzed flavonol aglycons (**Figure 2C**, myricetin, quercetin, kaempferol) and free hydroxycinnamic acids (**Figure 2D**, ferulic acid, *p*-coumaric acid, caffeic acid) was based on the comparison of retention times and UV−vis spectra of the reference compounds. Other phenolic compounds such as flavan-3-ols and their polymers and oligomers as well as aurones (auresidin glucoside) were tentatively detected with the support of data previously reported (*7*, *20*, *22*), but the compounds existed in trace amounts only.

Anthocyanin Profiles. The main phenolics of each fraction were anthocyanins. The distribution of the major single anthocyanins in various fractions and sensory samples is shown in **Table 3** and the corresponding sum of the anthocyanins in **Table 1**. Over 80% of colorants remained in the residue (residue I) after fractionation, and less than one-fifth was obtained in the juice. After four ethanol extractions, <1% of anthocyanins remained in residue II. Delphinidin-3-*O*-rutinoside was the major anthocyanin throughout the fractions; from 1 kg of berries, 16−1600 mg of the compound was distributed in single fractions. The order of abundance was dp-rut, cy-rut, dp-gluc, cy-gluc, in each of the four extracts, despite the high standard deviations. Total content of rutinosides was higher than total glucosides, as also reported previously (*10*, *21*). The calculated theoretical values (CR) of each anthocyanin in residue I are shown in **Table 3**. These calculated values are very similar to measured values. On the basis of a total of 20 calculated extractions, the first four extractions isolated approximately 98% of each compound (**Figure 3**).

For the sensory evaluations, isocratic water solutions of each dried extract, extracts 1−4, were prepared (25 g/L water) on the basis of gravimetric determination of the extraction yields (**Table 3**). The proportions of anthocyanins in the sensory samples had a continuously decreasing trend without any exception from extracts 1−4 due to higher ethanol solubility of anthocyanins than the solubility of

Table 2. Identification of Phenolic Compounds[a] in Black Currant

			UPLC-MS[b]		HPLC-DAD		
no.	code	compound	$[M + H]^+$ (*m*/*z*)	fragment ions (*m*/*z*)	ref[c]	HPLC[d]	lit.[e]
1	dp-glc	delphinidin-3-*O*-glucoside	465	303	x	x	9, 10, 12, 20, 21
2	dp-rut	delphinidin-3-*O*-rutinoside	611	303, 465	x	x	9, 10, 12, 20, 21
3	cy-glc	cyanidin-3-*O*-glucoside	449	287	x	x	9, 10, 12, 20, 21
4	cy-rut	cyanidin-3-*O*-rutinoside	595	287, 449	x	x	9, 10, 12, 20, 21
5	caff5	caffeic acid sugar conjugate	365			x	9, 20
6	coum6	*p*-coumaric acid sugar conjugate	349			x	9, 20
7	coum7	*p*-coumaric acid sugar conjugate	349			x	9, 20
8	fer8	ferulic acid sugar conjugate	379			x	9, 20
9	my-rut	myricetin-3-*O*-rutinoside	627	319, 481		x	7, 9, 20, 22
10	my-gal	myricetin-3-*O*- galactoside	481	319		x	7
11	my-glc	myricetin-3-*O*-glucoside	481	319		x	7, 9, 20, 22
12	qu-rut	quercetin-3-*O*-rutinoside	611	303, 465	x	x	7, 9, 20, 22
		myricetin-3-*O*-arabinoside	451	319			7
		myricetin-3-*O*-(6″-malonyl)glucoside	567	319			7, 9, 20, 22
13	caff13	ferulic acid sugar conjugate	379			x	9, 20
14	qu-gal	quercetin-3-*O*-galactoside	465	303	x	x	7
15	qu-glc	quercetin-3-*O*-glucoside	465	303	x	x	7, 9, 20, 22
16	ka-rut	kaempferol-3-*O*-rutinoside	595	287, 449		x	7, 9, 20, 22
17	qu-mal	quercetin-3-*O*-(6″-malonyl)glucoside	551	303		x	7, 9, 20, 22
18	qu-ara	quercetin-3-*O*-arabinoside	435	303		x	7
19	ka-glc	kaempferol-3-*O*-glucoside	449	287	x	x	7, 9, 20, 22
20	coum20	*p*-coumaric acid sugar conjugate	349			x	9, 20
21	fer21	ferulic acid sugar conjugate	452			x	9, 20
22	caff22	caffeic acid sugar conjugate	520			x	9, 20
23	ka-gly	unknown kaempferol glycoside	597	287, 432, 474		x	
24	ka-mal	kaempferol-3-*O*-(6″-malonyl)glucoside	535	287		x	7, 9, 20
25	my	myricetin			x	x	6
26	qu	quercetin			x	x	6
27	ka	kaempferol			x	x	6
28	fe	ferulic acid			x	x	6
29	co	*p*-coumaric acid			x	x	6
30	ca	caffeic acid			x	x	6

[a] Numbering and codes of the peaks are used throughout in figures and other tables. [b] Mass spectral comparison, positive ion mode. [c] Retention time and UV−vis spectrum compared to reference compound. [d] UV−vis spectra of the analytes. [e] Literature cited (*6*, *7*, *9*, *10*, *12*, *20*−*22*).

Table 3. Total Phenolics, Total Anthocyanins, and Individual Anthocyanins[a] in Sensory Samples[b] and in Fractions[c]

	berry	juice	residue I	CR[d]	extract 1	extract 2	extract 3	extract 4	residue II
				Sensory Samples (Milligrams per 100 g)					
totphen					360	310	210	190	
totant	340	90	1200		180	190	160	90	160
dp-glc	61 ± 3.7 a	7.2 ± 0.3 b	210 ± 12 c		41 ± 19 ad	31 ± 1.4 bd	16 ± 4.0 bd	15 ± 0.6 bd	9.7 ± 0.5 b
dp-rut	220 ± 13 a	42 ± 0.7 b	670 ± 32 c		150 ± 67 ad	110 ± 4.9 bd	53 ± 13 bd	48 ± 2.1 bd	28 ± 1.1 b
cy-glc	22 ± 2.5 a	*	110 ± 10		21 ± 9.6 a	14 ± 0.7 a	6.4 ± 1.6 a	5.4 ± 0.6 a	*
cy-rut	110 ± 6.6 a	21 ± 0.5 b	340 ± 20 c		77 ± 35 ab	55 ± 2.5 bd	26 ± 6.5 bd	24 ± 1.2 bd	9.9 ± 0.3 b
				Fractions (Milligrams per Kilogram of Berry)					
dp-glc	610 ± 37 a	55 ± 2.0 a	510 ± 30 a	490	300 ± 140 a	120 ± 5.8 a	32 ± 8.0 a	17 ± 0.8 a	5.4 ± 0.2 a
dp-rut	2200 ± 130 b	320 ± 5.6 b	1600 ± 76 b	1700	1100 ± 480 a	420 ± 20 b	110 ± 95 b	58 ± 16 b	16 ± 0.6 b
cy-glc	220 ± 25 c	*	270 ± 24 c	230	150 ± 69 a	57 ± 2.7 c	13 ± 3.1 a	6.4 ± 0.7 c	*
cy-rut	1100 ± 66 d	160 ± 3.6 c	810 ± 50 d	880	560 ± 260 a	220 ± 10 d	53 ± 13 ab	28 ± 1.4 d	5.5 ± 0.2 a

[a] Names refer to **Table 2**; *, below the detection limit of S/N > 3. [b] Extracts are sample set A (diluted in water, 25 g/L). Significant differences between samples in each compound (top section) based on Tukey's test ($p < 0.05$) are marked with letters a−e. [c] Contents in each original fraction (Table**1**). Significant differences between compounds (bottom section) in each fraction based on Tukey's test ($p < 0.05$) are marked with letters a−h. [d] Calculated theoretical values of residue I (Figure**3**).

extracted compounds on average. However, deviations in repeated samples and analyses were so high that there were hardly any statistical differences among extracts 1−4.

Profiles of Other Phenolic Compounds. The flavonol glycosides and hydroxycinnamic acid glyco-conjugates in the process fractions and in the sensory samples are shown in **Tables 4** and **5**. A total of eight derivatives of hydroxycinnamic acids were included in the phenolic profiles despite their tentative identification. Abbreviation and numbering of the compounds are as in **Table 2**. Theoretical values of flavonol glycosides and hydroxycinnamic acid conjugates in residue I are shown in **Tables 4** and **5**. Values were calculated

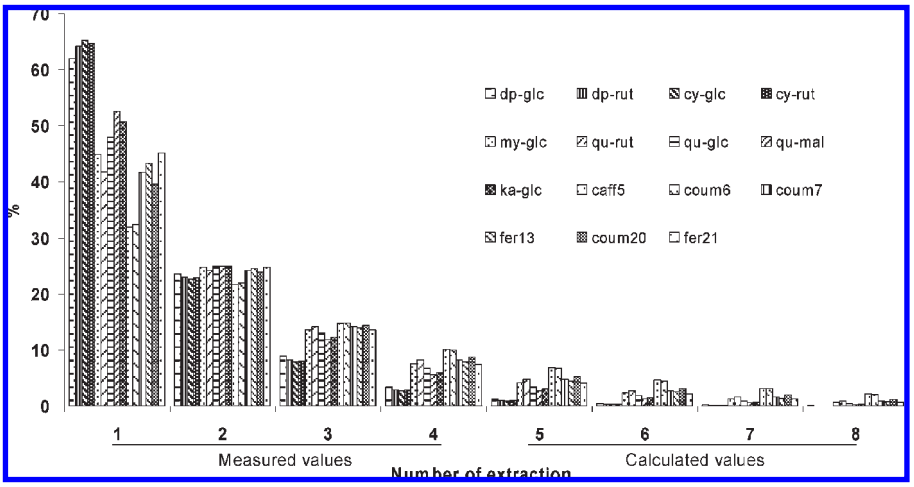

Figure 3. Relative proportions of each compound measured in all four extracts: anthocyanins (R^2 values varied from 0.9927 to 0.9944), flavonol glycosides (R^2 values from 0.8745 to 0.9737), and hydroxycinnamic acid conjugates (R^2 values from 0.7899 to 0.9576).

Table 4. Flavonol Glycosides[a] in Sensory Samples[b] and in Fractions[c]

	berry	juice	residue I	CR[d]	extract 1	extract 2	extract 3	extract 4	residue II
					Sensory Samples (Milligrams per 100 g)				
my-rut	*	*	1.5 ± 0.0 a		0.2 ± 0.0 b	0.4 ± 0.0 c	*	*	*
my-gal	*	*	*		0.1 ± 0.0 a	*	*	*	*
my-glc	1.7 ± 0.0 ab	0.9 ± 0.0 c	5.7 ± 0.2 d		1.8 ± 0.1 ab	2.2 ± 0.2 a	1.6 ± 0.1 b	1.1 ± 0.0 c	*
qu-rut	1.1 ± 0.1 ab	0.6 ± 0.0 c	3.8 ± 0.2 d		1.3 ± 0.1 ab	1.8 ± 0.1 e	1.4 ± 0.1 ae	0.9 ± 0.1 bc	*
qu-gal	*	*	0.6 ± 0.1 a		0.2 ± 0.0 b	0.2 ± 0.0 b	*	*	*
qu-glc	3.1 ± 0.0 a	1.6 ± 0.0 b	7.2 ± 0.1 c		2.8 ± 0.1 ad	3.0 ± 0.4 a	2.3 ± 0.1 de	1.8 ± 0.0 be	*
ka-rut	0.5 ± 0.0 a	0.3 ± 0.0 b	1.6 ± 0.0 c		0.5 ± 0.0 a	0.6 ± 0.1 a	*	*	*
qu-mal	0.5 ± 0.0 a	0.3 ± 0.0 b	3.5 ± 0.0 c		0.7 ± 0.0 d	0.7 ± 0.1 d	0.5 ± 0.0 a	0.3 ± 0.0 ab	*
qu-ara	0.3 ± 0.0 a	*	1.7 ± 0.0 b		0.5 ± 0.0 a	0.5 ± 0.1 a	*	*	*
ka-glc	0.6 ± 0.0 a	0.3 ± 0.0 b	1.7 ± 0.0 c		0.6 ± 0.0 a	0.6 ± 0.1 a	0.4 ± 0.0 b	0.3 ± 0.0 b	*
ka-gly	*	*	*		0.4 ± 0.0 a	0.3 ± 0.0 a	*	*	*
ka-mal	*	*	*		0.2 ± 0.0 a	*	*	*	*
					Fractions (Milligrams per Kilogram of Berry)				
my-rut	*	*	3.7 ± 0.0 a		1.4 ± 0.1 ab	1.4 ± 0.1 a	*	*	*
my-gal	*	*	*		1.1 ± 0.0 a	*	*	*	*
my-glc	17 ± 0.3 a	6.7 ± 0.0 a	14 ± 0.6 b	29	13 ± 0.6 c	8.9 ± 0.9 b	3.2 ± 0.2 a	1.3 ± 0.0 a	*
qu-rut	11 ± 1.0 b	4.5 ± 0.1 b	9.1 ± 0.4 c	24	9.4 ± 0.5 d	7.3 ± 0.6 b	2.8 ± 0.2 a	1.1 ± 0.1 b	*
qu-gal	*	*	1.4 ± 0.1 d		1.8 ± 0.1 ab	0.7 ± 0.1 a	*	*	*
qu-glc	31 ± 0.3 c	12 ± 0.1 c	17 ± 0.1 e	43	20 ± 0.8 e	12 ± 1.4 c	4.7 ± 0.2 b	2.2 ± 0.0 c	*
ka-rut	5.0 ± 0.1 d	1.9 ± 0.0 d	3.9 ± 0.1 a		3.4 ± 0.2 fg	2.2 ± 0 0.2 a	*	*	*
qu-mal	4.6 ± 0.1 de	2.0 ± 0.0 d	8.4 ± 0.1 c	10	5.3 ± 0.1 h	3.0 ± 0.4 a	0.9 ± 0.1 c	0.4 ± 0.0 d	*
qu-ara	2.8 ± 0.0 e	*	4.1 ± 0.1 a		3.9 ± 0.3 fg	2.0 ± 0.2 a	*	*	*
ka-glc	6.2 ± 0.4 d	2.5 ± 0.0 e	4.1 ± 0.1 a	8.9	4.5 ± 0.2 gh	2.6 ± 0.3 a	0.9 ± 0.0 c	0.4 ± 0.0 d	*
ka-gly	*	*	*		2.6 ± 0.1 bf	1.2 ± 0.1 a	*	*	*
ka-mal	*	*	*		1.7 ± 0.0 ab	*	*	*	*

[a] Compounds refer to **Table 2**; *, below the detection limit of S/N > 3. [b] Extracts are sample set A (diluted in water, 25 g/L). Significant differences between samples in each compound (top section) based on Tukey's test ($p < 0.05$) are marked with letters a−e. [c] Contents in each original fraction (Table**1**). Significant differences between compounds (bottom section; flavonol glycosides and hydroxycinnamic acid conjugates compared separately) in each fraction based on Tukey's test ($p < 0.05$) are marked with letters a−h. [d] Calculated theoretical values of residue I (Figure**3**).

for compounds that were measurable in all four ethanol extracts. Quantification is based on calculated values. The difference between calculated and measured values could be explained by extraction efficiency of ethyl acetate. The systematically decreasing trend of concentrations in the ethanol fractions is unambiguous without any exception. Again, among the 20 phenolic compounds in the isocratic samples prepared for sensory analysis, the concentrations were typically highest in extract 2. In the case of a caffeic acid sugar conjugate (compound **5**) and a coumaric acid sugar conjugate (compound **20**), the concentrations even in extract 3 exceeded those of extract 1 (**Table 5**). These trends differ from the behavior of the anthocyanin glycosides (**Table 2**) and are clear evidence of the selectivity of the ethanol extraction process. The first ethanol extraction also dissolves other components, not analyzed in this project, more effectively than flavonols and hydroxycinnamic acid derivatives. Four extractions isolated 88−94% of the five flavonol glycosides and 79−91% of six hydroxycinnamic acids (**Figure 3**). The results illustrate the difference in extractability among

Table 5. Hydroxycinnamic Acid Conjugates[a] in Sensory Samples[b] and in Fractions[c]

	berry	juice	residue I	CR[d]	extract 1	extract 2	extract 3	extract 4	residue II
				Sensory Samples (Milligrams per 100 g)					
caff5	0.9 ± 0.0 a	0.5 ± 0.0 b	2.7 ± 0.0 c		0.5 ± 0.0 b	0.7 ± 0.0 d	0.8 ± 0.0 d	0.6 ± 0.0 e	*
coum6	0.7 ± 0.0 a	0.4 ± 0.0 b	1.8 ± 0.0 c		0.4 ± 0.0 bd	0.7 ± 0.1 a	0.7 ± 0.0 a	0.5 ± 0.0 d	*
coum7	0.7 ± 0.0 a	0.3 ± 0.0 b	1.8 ± 0.0 c		0.4 ± 0.1 bd	0.5 ± 0.0 d	0.5 ± 0.0 d	0.4 ± 0.0 d	*
fer8	0.6 ± 0.0 a	0.3 ± 0.0 b	1.7 ± 0.0 c		0.3 ± 0.0 b	0.4 ± 0.0 ab	0.4	*	*
fer13	0.4 ± 0.0 ad	0.2 ± 0.0 b	1.3 ± 0.0 c		0.4 ± 0.0 d	0.4 ± 0.0 a	0.4 ± 0.0 ad	0.3 ± 0.0 e	*
coum20	0.8 ± 0.0 a	0.4 ± 0.0 b	2.4 ± 0.1 c		0.7 ± 0.0 d	0.9 ± 0.1 a	0.9 ± 0.0 a	0.6 ± 0.0 d	*
fer21	0.7 ± 0.0 ad	0.4 ± 0.0 b	2.8 ± 0.1 c		0.6 ± 0.0 ad	0.7 ± 0.1 a	0.6 ± 0.0 d	0.4 ± 0.0 b	*
caff22	0.2 ± 0.0 a	0.1	0.9 ± 0.0 b		0.2 ± 0.0 a	0.3 ± 0.0 a	*	*	*
				Fractions (Milligrams per Kilogram of Berry)					
caff5	9.4 ± 0.0 a	3.8 ± 0.1 a	6.5 ± 0.0 a	11	3.2 ± 0.2 a	3.0 ± 0.1 ab	1.6 ± 0.1 ab	0.7 ± 0.0 ab	*
coum6	7.4 ± 0.1 b	3.0 ± 0.1 b	4.2 ± 0.1 bc	10	3.1 ± 0.0 ab	2.8 ± 0.3 b	1.5 ± 0.0 b	0.7 ± 0.0 b	*
coum7	7.0 ± 0.2 b	2.7 ± 0.0 c	4.4 ± 0.0 b	7.3	3.0 ± 0.5 ab	2.0 ± 0.1 c	1.0 ± 0.0 c	0.4 ± 0.0 c	*
fer8	5.5 ± 0.1 c	2.2 ± 0.0 d	4.0 ± 0.0 c		2.3 ± 0.1 b	1.7 ± 0.1 cd	0.7	*	*
fer13	4.1 ± 0.0 d	1.6 ± 0.0 e	3.2 ± 0.0 d	6.3	2.6 ± 0.1 ab	1.8 ± 0.1 cd	0.8 ± 0.0 d	0.3 ± 0.0 d	*
coum20	8.5 ± 0.2 e	3.3 ± 0.0 f	5.6 ± 0.2 e	13	4.9 ± 0.1 c	3.6 ± 0.3 a	1.7 ± 0.0 a	0.8 ± 0.0 a	*
fer21	7.0 ± 0.0 b	2.8 ± 0.0 c	6.7 ± 0.1 a		4.5 ± 0.2 c	3.0 ± 0.2 ab	1.2 ± 0.0 e	0.5 ± 0.0 e	*
caff22	2.2 ± 0.1 f	0.4	2.2 ± 0.1 f		1.3 ± 0.0 d	1.1 ± 0.1 d	*	*	*

[a] Compounds refer to **Table 2**; *, below the detection limit of S/N > 3. [b] Extracts are sample set A (diluted in water, 25 g/L). Significant differences between samples in each compound (top section) based on Tukey's test ($p < 0.05$) are marked with letters a−e. [c] Contents in each original fraction (Table**1**). Significant differences between compounds (bottom section; flavonol glycosides and hydroxycinnamic acid conjugates compared separately) in each fraction based on Tukey's test ($p < 0.05$) are marked with letters a−h. [d] Calculated theoretical values of residue I (Figure**3**).

Table 6. Flavonol Aglycons[a] and Hydroxycinnamic Acids[a] in Sensory Samples[b] and in Fractions[c]

	berry	juice	residue I	extract 1	extract 2	extract 3	extract 4	residue II
			Sensory Samples (Milligrams per 100 g)					
my	1.4 ± 0.0 ac	0.4	5.1 ± 0.0 b	1.7 ± 0.3 a	1.8	1.5 ± 0.0 ac	1.0 ± 0.1 c	*
qu	3.3 ± 0.1 a	1.4	10.5 ± 0.1 b	3.5 ± 0.7 a	3.9	2.9 ± 0.1 a	2.2 ± 0.1 a	*
ka	1.1 ± 0.0 ab	0.6	0.9 ± 0.0 ab	1.3 ± 0.3 b	1.4	1.0 ± 0.1 ab	0.7 ± 0.0 ab	*
fe	1.1 ± 0.0 a	0.9	5.8 ± 0.1 b	1.3 ± 0.3 a	1.5	1.3 ± 0.0 a	1 ± 0.1 a	*
co	2.6 ± 0.0 a	1.4	7.2 ± 0.3 b	2.1 ± 0.5 ac	2.3	2.0 ± 0.1 ac	1.5 ± 0.1 c	*
ca	1.9 ± 0.1 a	0.6	5.7 ± 0.2 b	0.9 ± 0.1 c	0.9	0.8 ± 0.0 c	0.6 ± 0.1 c	*
			Fractions (Milligrams per Kilogram of Berry)					
my	14 ± 0.4 a	3.2	12 ± 0.0 a	12 ± 2.4 a	7.4	3.0 ± 0.1 a	1.3 ± 0.1 a	*
qu	33 ± 0.9 b	11	25 ± 0.1 b	25 ± 5.2 a	16	5.9 ± 0.2 b	2.7 ± 0.1 b	*
ka	11 ± 0.4 c	4.4	2.1 ± 0.1 c	9.3 ± 1.8 a	5.8	1.9 ± 0.1 c	0.9 ± 0.0 c	*
fe	11 ± 0.1 a	7.0	14 ± 0.3 a	9.6 ± 1.9 a	6.1	2.6 ± 0.0 a	1.2 ± 0.1 a	*
co	26 ± 0.4 b	11	17 ± 0.8 b	15 ± 3.3 a	9.2	4.1 ± 0.1 b	1.8 ± 0.1 b	*
ca	19 ± 0.6 c	4.6	14 ± 0.5 a	6.6 ± 0.8 a	3.7	1.7 ± 0.1 c	0.8 ± 0.1 c	*

[a] Names refer to Table**2**; *, below the detection limit of S/N > 3. [b] Extracts are sample set A (diluted in water, 25 g/L). Significant differences between samples in each compound (top section) based on Tukey's test ($p < 0.05$) are marked with letters a−e. [c] Contents in each original fraction (Table**1**). Significant differences between compounds (bottom section) in each fraction based on Tukey's test ($p < 0.05$) are marked with letters a−h.

the phenolic compounds. This results in a sound hypothesis that the four ethanol fractions produced may have different sensory and even nutritional properties.

Quercetin-3-*O*-glucoside (qu-glc) was the most abundant compound followed by myricetin-3-*O*-glucoside (my-glc) and quercetin-3-*O*-rutinoside (qu-rut). They together comprised approximately two-thirds of all the flavonol glycosides. Many less abundant compounds appeared in some of the extracts below the quantitation limit or even below the detection limit. This does not, however, lead to the automatic contention of a complete absence of biological significance of the compounds, even at these very low concentrations. When compared to anthocyanins, the total content of glucosides (14.8 mg/100 g) was higher than that of rutinosides (8.8 mg/100 g) in residue I. Most of the flavonol glycosides remained in residue I after berry pressing, as in the case of anthocyanins.

Aglycons of flavonol glycosides and hydroxycinnamic acid derivatives are shown in **Table 6**. This table shows the total content of each hydrolyzed aglycon in each fraction. Quercetin dominated in residue I and in all of the extracts and fractions. Myricetin and kaempferol were the second most abundant class of flavonols. In earlier studies, quercetin has been reported to be the main flavonol in the variety Öjebyn (*5*) followed by myricetin and kaempferol, whereas myricetin was found to be the major aglycon in the variety Mortti (*11*). Both myricetin and kaempferol are known to be sensitive to long-term storage of the frozen berries (*32*), and this may have had some effect on the actual contents in our study. *p*-Coumaric acid was the main hydroxycinnamic

Table 7. Sugars and Fruit Acids in Black Currant Sensory Samples[a] and in Fractions[b]

	berry	juice	residue I	extract 1
	Sensory Samples (Grams per 100 g)			
fructose	4.1 ± 0.1 a	4.2 ± 0.3 a	1.0 ± 0.0 b	2.7 ± 0.2 c
glucose	5.6 ± 0.1 a	6.0 ± 0.3 a	1.2 ± 0.0 b	3.1 ± 0.2 c
sucrose	0.9 ± 0.0 a	3.3 ± 0.2 b	0.2 ± 0.0 c	0.2 ± 0.0 c
malic acid	0.2 ± 0.0 a	0.1 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c
citric acid	2.8 ± 0.1 a	1.9 ± 0.1 b	0.5 ± 0.0 c	0.2 ± 0.0 d
	Fractions (Grams per Kilogram of Berry)			
fructose	41 ± 0.9 a	32 ± 2.2 a	2.5 ± 0.1 a	20 ± 1.4 a
glucose	56 ± 0.7 b	46 ± 2.6 b	3.0 ± 0.1 b	22 ± 1.6 a
sucrose	8.8 ± 0.1 c	25 ± 1.7 a	0.5 ± 0.0 c	1.2 ± 0.1 b
malic acid	1.5 ± 0.0 a	0.9 ± 0.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a
citric acid	28 ± 1.3 b	15 ± 0.9 b	1.3 ± 0.1 b	1.3 ± 0.2 a

[a] Extract 1 is sample set A (diluted in water, 25 g/L). Significant differences between samples in each compound (top section) based on Tukey's test ($p < 0.05$) are marked with letters a−e. [b] Contents in each original fraction (Table**1**). Significant differences between compounds (bottom section; sugars and acids compared separately) in each fraction based on Tukey's test ($p < 0.05$) are marked with letters a−h.

acid in all of the fractions, whereas the content of ferulic acid and caffeic acid was lower.

Sugars and Acids. The contents of sugars and acids of various samples are given in **Table 7**. The main sugars of black currant were glucose and fructose with minor amounts of sucrose. The total sugar content of the berry was 105 g/kg, and >90% ended up in the juice after pressing. The sugar level of the sensory sample extract 1 was still quite high (6 g/L) due to the residual juice in press cake and good extractability of sugars in ethanol. Citric acid was the main organic acid (95−100%) in every fraction, whereas malic acid was found in significantly lower amounts. The majority of the organic fruit acids were in the juice. Practically all of the sugars were dissolved during the first ethanol extraction.

Orosensory Profiles of Fractions. The orosensory profile of black currant fractions was chosen for eight different attributes (total intensity of flavor, roundness, sweetness, fruitiness, sourness, sharpness, bitterness, astringency; **Table 8**). The sensory profile was the most multidimensional in juice (**Table 9**). It was strongest in all of the other properties except in bitterness and astringency. In general, the expected bitterness was very weak in every fraction. Residue I was the second strongest in roundness, fruitiness, and sharpness. There was no difference between residue I and residue III. This finding proved the suitability of ethanol in our extraction process. Due to this result, the chemical composition of residue III was not analyzed. Sensory properties became weaker with consecutive extractions. Residue II had the weakest sensory profile and almost no perceived sensation. Extract 1 was significantly stronger than extract 4 in all of the properties excluding bitterness. Interestingly, the first extract was the most astringent sample of all the fractions. The combined sample of extractions was similar to extract 1. Only in total intensity was it closer to extract 2 than extract 1.

The total intensities of the extracts were compared with a ranking test using the ordinary scale (sample set B). When dried extracts were diluted in a constant volume of water, the total flavors of the samples were significantly different from each other ($\chi^2 = 126.7 > \chi^2_{(0.001)}(3) = 16.27$). Concentrations varied from 150 g/L of the first extract to 25 g/L of the fourth extract. The total intensity of flavor was strongest in the first extract. The mass of the fourth extract was smallest and its total flavor was weakest. When residues II and IV were analyzed with the triangle test, no significant difference was detected between samples at the level of significance 0.01, showing that the small amount of compounds extracted with SFE had no effect on the sensory properties.

Compounds Contributing to Orosensory Properties. Black currant juice had a strong sensory profile, and it was rich in sugars and acids. Sweetness, roundness, fruitiness, and sharpness of black currant juice were interacting with sugars and acids. Residue I was milder than juice when sensory profiles were compared. Pressed juice was rich in sugars and acids, whereas the majority of the phenolic compounds remained in berry skin-rich press residue. In general, the phenolic compounds are thought to interact with the astringency and bitter taste. Our findings showed that residue I was neither astringent nor bitter and that its total flavor was milder than that of juice. However, in the case of astringency, extract 1 and combined extract were significantly stronger than all of the other samples. Astringency declined significantly with the first three consecutive extractions.

The PLS method was applied to relate the sensory and chemical data matrices to identify the compounds contributing to the orosensory characteristics. The model was applied to the sensory properties and nonvolatile chemical variables (PLS2) with black currant fractions except berry, residue III, combined extract, and juice. Our sensory panel did not evaluate whole berries. In addition, residues I and III did not differ in their sensory profiles, and only residue I was selected for the chemical composition analyses. We also excluded the juice from the model, because it was clearly the strongest sample in most of the sensory attributes (**Table 9**), and also sugars and acids, because these were predominantly in the Jjuice (**Table 7**). The predicted *Y*-values (sensory properties, $n = 6$) were computed by applying the model equation to the observed *X*-variables (phenolic compounds, $n = 26$). Sweetness and sourness were excluded because of their relationship to the content of sugars and acids. When the two principal components were taken into account, 95% of the chemical variables explained 93% of the sensory data (**Figure 4**). The model showed again a strong correlation between residue I and many phenolic compounds on the right side of the plot using PC1. However, both residue I and most of the phenolic compounds did not correlate with astringency. Chemical variables ka-gly (unknown kaempferol glycoside), ka-mal (kaempferol-3-*O*-(6″-malonyl)glucoside), and my-gal (myricetin-3-*O*-galactoside) were close to extract 1 and astringency. We suggest that those three flavonol glycosides contribute significantly to the astringency of extract 1 of black currant even at minor quantities. Two kaempferol glycosides indicate a relationship between kaempferol compounds and astringency. However, only a part of the compounds in the extracts were identified in this study.

In the previous studies of Schwarz and Hofmann (*15*), quercetin rutinoside, kaempherol rutinoside, kaempherol glucoside, quercetin galactoside, and quercetin glucoside among other flavonol glycosides were reported to be the compounds contributing to astringency in red currant. They also reported some indolyl glycosides to be very astringent in red currant (*33*). Myricetin-3-*O*-galactoside is one of the astringent compounds in black tea with a taste threshold of

Table 8. Sensory Attributes, Descriptions with References, and Their Intensities Used in Sensory Profiling of Black Currant Fractions

sensory attribute	description	reference	intensity[a]
total intensity	perceived first impression of sample in mouth		
sourness	sour taste	0.4% citric acid (J. T. Baker, Deventer, Netherlands)	5
sharpness	sharp, acidic, and tangy mouthfeel	1:6 diluted lemon juice (Sicilia, Segmuller GmbH, Singen, Germany) + 0.07% malic acid (Fluka, Buchs SG, Switzerland)	5
astringency	puckering mouthfeel	0.14% $NH_4Al(SO_4)_2$ (Riedel-de Haen, Seelze, Germany)	7
sweetness	sweet taste	0.5% fructose (Sigma, St. Louis, MO)	3
fruitiness	berry rich flavor	1:6 diluted black currant juice (Aten, Marja-aitta, Puumala, Finland)	5
bitterness	bitter taste	0.08% caffeine (Yliopiston Apteekki, Helsinki, Finland)	7
roundness	compact and multidimensional, opposite to weak, watery, and simple		

[a] Scale 0−10.

Table 9. Mean Intensities[a] (n = 45) for Sensory Attributes in Black Currant Fractions

sample	juice	residue I	residue III	combined extract	extract 1	extract 2	extract 3	extract 4	residue II
total	7.9 ± 0.4 a	4.3 ± 0.3 bcd	4.8 ± 0.3 b	3.5 ± 0.2 def	4.4 ± 0.3 bc	3.7 ± 0.3 cde	2.8 ± 0.2 ef	2.6 ± 0.4 f	0.6 ± 0.0 g
roundness	7.3 ± 0.4 a	4.2 ± 0.4 b	3.9 ± 0.2 b	2.2 ± 0.1 cd	2.9 ± 0.1 c	2.6 ± 0.6 cd	1.9 ± 0.5 d	2.0 ± 0.6 d	0.9 ± 0.1 e
sweetness	3.9 ± 0.5 a	2.0 ± 0.3 b	2.3 ± 0.2 b	1.7 ± 0.2 b	1.7 ± 0.2 b	1.6 ± 0.3 b	1.6 ± 0.3 b	1.7 ± 0.4 b	0.7 ± 0.1 c
fruitiness	6.5 ± 0.2 a	4.8 ± 0.7 b	4.3 ± 0.2 bc	3.4 ± 0.0 cd	3.4 ± 0.3 cd	3.0 ± 0.5 de	2.6 ± 0.4 de	2.3 ± 0.5 e	0.6 ± 0.0 f
sourness	5.5 ± 0.5 a	3.7 ± 0.4 b	3.7 ± 0.3 b	3.5 ± 0.1 b	3.9 ± 0.2 b	3.1 ± 0.4 bc	3.0 ± 0.3 bc	2.5 ± 0.4 bc	0.6 ± 0.2 d
bitterness	2.0 ± 1.0 a	1.3 ± 0.2 a	1.1 ± 0.3 a	1.4 ± 0.4 a	1.5 ± 0.3 a	1.4 ± 0.2 a	1.2 ± 0.3 a	1.3 ± 0.3 a	0.7 ± 0.0 a
sharpness	6.9 ± 0.6 a	2.6 ± 0.5 b	3.3 ± 0.2 b	1.5 ± 0.4 c	1.4 ± 0.4 cd	1.2 ± 0.4 cd	0.8 ± 0.1 d	0.9 ± 0.3 cd	0.2 ± 0.0 e
astringency	2.3 ± 0.6 cd	1.4 ± 0.5 d	1.2 ± 0.2 de	4.6 ± 0.4 ab	5.2 ± 0.4 a	3.4 ± 0.6 bc	2.3 ± 0.3 cd	2.3 ± 0.7 cd	0.6 ± 0.1 e

[a] Scale is from 0 (no sensation) to 10 (very strong sensation). Significant differences between fractions based on Tamhane test (p < 0.05) are marked with letters a−g.

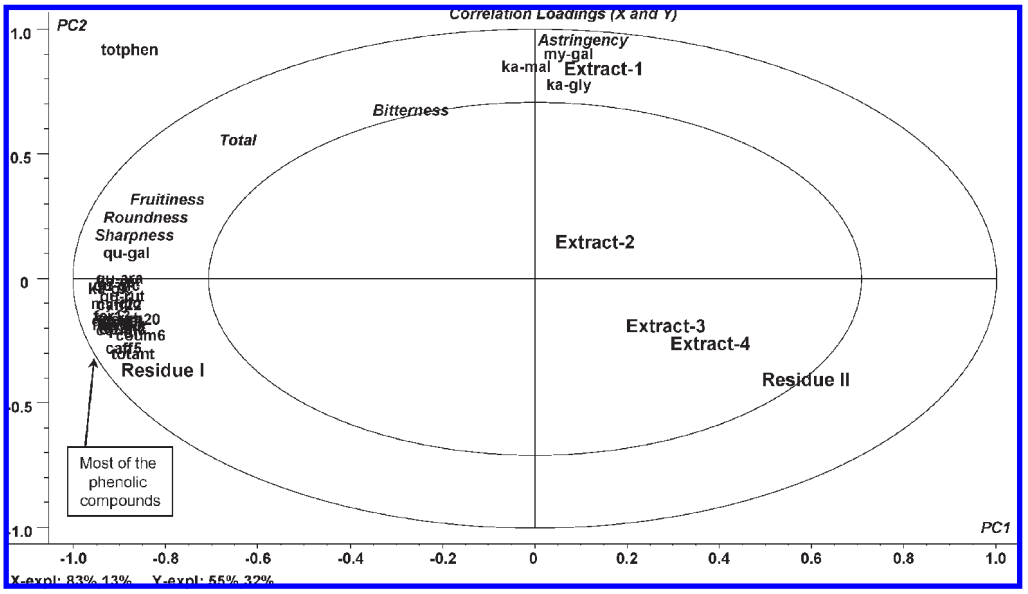

Figure 4. PLS2 plot of the interaction between sensory profiles (n = 6) and phenolic variables (n = 26) in black currant samples (n = 6) with two principal components. Different fonts are used for different data (sensory attributes = *italic*; samples = **bold**; chemical compounds = normal).

2.7 μmol/L (*34*). In tea, quercetin glycosides were more astringent than myricetin or kaempherol glycosides (*34*). Caffeic acid, ferulic acid, and *p*-coumaric acid are puckering astringent compounds in red wine at very low concentrations; on the other hand, their ethyl esters were both bitter and astringent (*35*). Flavonol glycosides were reported as more velvety astringent compounds in red wine and perceived at even lower concentrations. Many phenolic compounds, such as flavonol glycosides, have been perceived as astringent at lower concentrations and bitter at higher levels (*34*, *35*). In our study, the content of these compounds was significantly higher in residue I than in extract 1 or 2, but residue I was perceived as only slightly astringent. The reason may be that the compounds could be released from the berry skin structure better with ethanol than with saliva during the tasting session. Solid residues may also be more difficult to perceive as astringent compared to liquid samples. However, by understanding the location of different compounds in berry, the different fractions could be exploited by the food industry to develop innovative berry products.

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