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Chemical factors contributing to orosensory profiles of bilberry (*Vaccinium myrtillus*) fractions

Oskar Laaksonen · Mari Sandell · Heikki Kallio

Abstract The effects of fractionation on the sensory profile of bilberry (*Vaccinium myrtillus*) were studied. The berries were fractionated by juice pressing, four ethanol extractions and ethanol evaporation. High-performance liquid chromatography and gas chromatography were used to analyze phenolic compounds, sugars and organic fruit acids of the fractions, and sensory properties of the fractions were studied using generic descriptive analysis. The majority of the phenolic compounds were anthocyanins, and they were mainly found to be located in the press residue. Anthocyanins and other phenolic compounds dissolved in ethanol, and the extracts were perceived as astringent and bitter as the sensory profile of the press residue was very mild. The juice was dominant in sweetness because it contained most of the sugars and fruit acids. Many flavonol glycosides and hydroxycinnamic acid conjugates were discovered to be the compounds contributing to astringency and bitterness, especially myricetin-3-*O*-arabinoside, myricetin-3-*O*-glucoside, myricetin-3-*O*-galactoside and an unknown caffeoyl quinic acid derivative. By supplementing the juice fraction with extracts, the content of phenolic compounds was significantly increased without altering the sensory profile of the juice.

Keywords Astringency · Bilberry · Bitterness · Ethanol extraction · Phenolic compounds

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Introduction

There is a growing interest in edible plants rich in phenolic compounds such as anthocyanins, flavonols, flavanols, flavones and phenolic acids and other oligomers among others. These phenolic compounds are potent antioxidants [1–5], and they have also been reported to have antimicrobial activity [1]. Some phenolic compounds have many potential beneficial effects on human health [6–8], and therefore, there is a growing demand to increase their contents in daily diets. Many phenolic compounds, on the other hand, have interesting sensory properties such as astringency and bitterness, which are often regarded as unwanted characteristics [9–11].

Berries have a high content of phenolic compounds, and their composition varies widely between species [1, 12, 13]. In addition to this, berries are a good source of dietary fiber [14]. Bilberry (*Vaccinium myrtillus* L.) belongs to the genus *Vaccinium* along with many wild and cultivated commercially available berries, such as blueberries (*V. corymbosum* L. and other blueberries), lingonberries (*V. vitis-idaea* L.) and cranberries (*Vaccinium oxycoccos* L. and other cranberries) among others. Bilberries are traditionally used in juices or jams and as colorants due to their high anthocyanin content. The anthocyanin composition of bilberry is well known [3, 5, 15, 16], and the

content of other phenolics has also been studied [17, 18]. The location and distribution of these compounds in bilberries have not yet been studied, and their relation to the sensory properties of the berry is unknown. Only a few studies have been conducted concerning the composition of non-volatile phenolic compounds in berries and their relation to sensory properties. In blackcurrants (*Ribes nigrum*), phenolic compounds are located in skin fractions of the berry and strongly correlate with astringency [19]. In redcurrants (*Ribes rubrum*), phenolic compounds correlate with astringency also [20].

The aim of this study was to isolate and identify compounds contributing the orosensory properties of bilberry fractions. Orosensory profiles of fractions consist of taste, flavor and tactile perceptions in mouth. Concentrations of phenolic compounds, sugars and acids in berries, especially in juice and press residue fractions of bilberry, 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 follow a minimal-processing strategy, to avoid all unnecessary unit operations and to keep the flavor-modifying process as simple, safe and natural as possible for later food industry applications.

Materials and methods

Berries

Wild bilberries were received from Riitan Herkku Oy (Mustasaari, Finland). Berries had been collected in 2008 from throughout Finland. Berries were stored frozen at −20 °C in polyethylene bags to maintain their quality for analyses.

Chemicals

Myricetin, quercetin, ferulic acid, caffeic acid and *p*-coumaric acid were obtained from Sigma (St. Louis, MO). Quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside and malvidin-3-*O*-galactoside were obtained from Extrasynthese (Genay, France). Sorbitol and tartaric acid were obtained from Merck (Darmstadt, Germany). Acetonitrile, ethyl acetate, methanol, formic acid and hydrochloric acid were of HPLC grade or of the highest purity available. Activated-carbon-filtered tap water was used in preparing the samples for sensory analysis. Ethanol used in sample preparation was 96% ETAX A (Altia, Helsinki, Finland).

Berry fractionation

Berries were fractionated according to Sandell et al. [19] using juice pressing, ethanol extraction and ethanol evaporation. The press residue (Residue I) was extracted four consecutive times with 90% ethanol to produce fractions (Extract-1 to Extract-4). Ethanol was evaporated from the extracts, and the solutes were diluted with water in concentrations 25 g/L for sensory analyses. The filtrated ethanol extracts were used as such for chemical analyses. The residues after ethanol extractions (Residue II and Residue III) were air-dried and stored in room temperature. For sensory analyses, Residue II was soaked in filtrated water to correspond to the moisture of Residue I. Fractions used in the sensory analyses are shown in Table 4. In addition to juice, four extracts and three residues, one additional sample was prepared by returning the two fractions (Extract-1 to Extract-2) extract to juice according to the amounts that were achieved from the berries (Table 1).

Dry matter

For the gravimetric dry matter measurement of berries, juice and Residue I, the samples were kept at +110 °C overnight before weighing. Weights of Extract-1 to Extract-4 were measured after evaporation of ethanol.

HPLC-DAD analysis of anthocyanins

Anthocyanins of Extract-1 to -4 were isolated in quadruplicate according to the method of Buchert et al. [15] with minor changes. Anthocyanins were analyzed with a modified method of Lätti et al. [16]. The HPLC-DAD system was a Shimadzu LC-10AVP (Shimadzu, Kyoto, Japan) with LC-10AT pump, SIL-10A auto-sampler and 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 Luna RP-18(2) 100A column with a 30 × 4.60 mm i.d., 5 μm Phenomenex Luna precolumn. The analysis of anthocyanins was performed using 5% formic acid and acetonitrile as solvents. Flow rate of the mobile phase was 1.0 ml/min. Anthocyanins were detected at 520 nm. Quantitative analysis was carried out using delphinidin-3-*O*-galactoside as an external standard. For the EtOH extracts, concentrations were expressed in mg per 100 ml of water-diluted dry extract and for other fractions in mg per 100 g of fresh weight. Concentrations were also expressed as fractions of 1 kg of berry.

Table 1 Distribution of the dry matter and phenolic compounds in bilberry and its fractions

Fractions[a]	Fresh weight (g)	Dry weight (g)	Total anthocyanins (mg)	Total flavonols (mg)	Hydroxy-cinnamic acids (mg)	Sugars (mg)	Fruit acids (mg)
Berry	1,000	105	2,500	160	230	47,000	10,000
Juice	850	45	440	65	120	45,000	9,500
Residue I	150	55	2,300	60	70	2,200	690
Extract-1		6.5	1,100	70	100	1,500	490
Extract-2		2.5	490	20	30	540	180
Residue II		50	710	7	6	170	50
Extract-3		1.0	190	7	8	140	50
Extract-4		0.5	120	2	3	30	*
Residue III		48	90	1	1	*	*

[a] Juice and Residue I are the fractions of the whole berry. Residue II is the residue after two ethanol extractions and Residue III after four extractions. Contents of phenolic compounds in Residue III were calculated according to the total contents in four ethanol extracts

* Not detected

HPLC-DAD analysis of other phenolic compounds

Flavonols, their aglycones and hydroxycinnamic acid derivatives were isolated in quadruplicate using the method of Määttä et al. [21] with minor changes. The analyses were carried out by the method of Koponen et al. [17] with minor changes. Analyses were executed with the same Shimadzu HPLC-DAD apparatus as described earlier using 1% formic acid and acetonitrile as solvents. Flavonols were detected at 360 nm and hydroxycinnamic acids and their conjugates at 320 nm. Quantitative analysis was carried out using quercetin-3-*O*-galactoside as an external standard for flavonol glycosides, quercetin for hydrolyzed flavonol aglycones and *p*-coumaric acid for hydroxycinnamic acid derivatives. For the EtOH extracts, concentrations were expressed as mg per 100 ml of water-diluted dry extract and for other fractions in mg per 100 g of fresh weight. Concentrations were also expressed as fractions of 1 kg of berry.

Total content of phenolic compounds

Total content of each anthocyanin, flavonol glycoside, flavonol aglycone and hydroxycinnamic acid derivative was calculated according to four consecutive extracts forming a descending curve using Origin 8 software (Originlab Corporation, Northampton, MA) using the method of Sandell et al. [19]. The equation of the descending curve ($y = A \times e^{(-x/B)} + C$) was chosen according to simplicity and adjusted R^2 values. A (amplitude) and B (decay constant) were constants calculated by the software, and C (offset) was set to zero. In the case of anthocyanins, the total contents were calculated on the basis of four consecutive MeOH–HCl extractions of

Residue I (corresponding calculated value shown as CRI in Table 3) and Residue II (CRII) analyzed separately and the other phenolic compounds were calculated according to ethanol extractions (Extract-1 to -4). Contents in Residue III (CRIII) were calculated using the aforementioned equation.

Identification of phenolic compounds by uHPLC-MS

HPLC conditions were as described previously, and the apparatus was an Acquity™ Ultra Performance LC (Waters, Milford, MA) interfaced to a Waters Quattro Premier quadruple mass spectrometer. Electrospray ionization-mass spectrometry (ESI-MS) analysis for anthocyanins was performed in positive ion mode using a capillary voltage 3.5 kV, cone voltage 40 V and extractor voltage 3 V, and for flavonols: capillary 5 kV, cone 20 V and extractor 3 V. In both cases, source temperature was 120 °C and desolvation temperature 300 °C. In the MS-analysis (full scan), data were acquired over a mass range of *m/z* 250–1,000. The UV–vis spectra, retention times, reference compounds and mass spectra, as well as literature data [3–5, 13, 15–19], were used for identification.

Analyses of sugars and acids

Sugars and acids of juice and extracts were analyzed in triplicate by gas chromatography as trimethylsilyl (TMS) derivatives [22]. Sorbitol and tartaric acid were used as internal standards. Sugar and acid content of Residue I was calculated as a sum of contents in extracts, and the contents in berries were calculated as sum of sugars and acids in juice and Residue I.

Sensory evaluation

The general guidelines for the selection, training and monitoring of assessors (ISO 8586-1, 1988) were used. Sensory descriptive profiling was applied with 15 voluntary panelists, of whom 8 were women and 7 were men (ages 21–62 years). The assessors were selected according to their willingness, good health (self-reported) and availability. The majority of the assessors were trained panelists, and a few assessors without previous experience were pre-trained to recognize different taste samples to rank the taste solutions and to differentiate samples in triangle tests [19]. The descriptors were generated following DIS 11035 standards (ISO/DIS 1992) during three independent sessions. During the training sessions, the descriptors were created and the assessors were familiarized to the usage of the attributes, the intensity scale and the Compusense-*five* data collection software (version 4.6, Compusense, Guelph, ON, Canada). Sample set consisted of fractions as such and they were thawed to room temperature before analyses. Samples were evaluated in triplicate sessions in randomized order. Intensities of the attributes were rated on the line scale anchored from 0 (none) to 10 (very strong) with help of the references (Table 6) in each session. All attributes of a given sample were evaluated at a session in non-randomized order. All the sensory analyses were performed at the sensory laboratory in accordance with the ISO 8589-1988 standard.

Statistical analyses

Differences between samples were analyzed by a one-way analysis of variance (ANOVA) together with post-hoc tests: Tukey's *t*-test and Tamhane test $p < 0.05$. For interpreting the results for eight sensory attributes, principal component analysis (PCA) was applied. To find the relationships between the two data matrices, partial least squares regression (PLS2) 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 a number of principal components for statistically reliable models. Statistical analyses were performed using SPSS 16.0 (SPSS Inc. H, Chicago, IL) and Unscrambler 9.8 (Camo Process AS, Oslo, Norway).

Results and discussion

Fractionation of the berries

The unit operations in fractionation of bilberries were juice pressing, ethanol extraction of the press residue and

evaporation of ethanol under reduced pressure. For Residue I, 100 g was extracted with a total of 1,200 ml 90% ethanol. The 90% EtOH was chosen as a solvent due to suitability for the process in our previous study [19]. Recoveries of each fraction in the process are presented in Table 1. In the extraction process, the yield of juice was $83 \pm 2\%$ and of the press residue in the juice extraction (Residue I) $14 \pm 1\%$ ($n = 35$). The loss (approximately 3% of juice) in the process was mainly due to the small-scale, effective hydraulic press. Recoveries of the fractions were rounded to 850 and 150 g as indicated in Table 1. Four consecutive extractions of Residue I were carried out to investigate the extractability of different phenolic compounds. Dry matter from the first extract, representing 1 kg of berries, was 6 g, whereas the corresponding value from Extract-4 was only 0.5 g (Table 1). Due to low yields from Extract-3 and Extract-4, these fractions were not taken to sensory analyses at all. Nevertheless, chemical compositions of all four extracts were analyzed.

Identification of phenolic compounds

Figure 1 shows HPLC-DAD chromatograms of the anthocyanins, flavonol compounds and phenolic acids in bilberry. Identification of the compounds was based on chromatographic and mass spectral information, reference compounds and the literature [3, 5, 13, 15–19]. A total of 58 phenolic compounds identified are listed in Table 2. All the compounds listed were found in Extract-1, while in other fractions, the contents of some compounds were less than the detection threshold (signal to noise ratio 3).

Identification of 15 different anthocyanins was based on the reference compounds, UV–vis spectra, mass spectra and the literature [3, 5, 15, 16], while one trace compound remained unidentified (peak 9, Fig. 1a). The anthocyanins included galactosides, glucosides and arabinosides of delphinidin, cyanidin, peonidin, petunidin and malvidin. Similar anthocyanins have also been detected in crowberry and other dark blue berries [4, 5, 13, 19], and data from these findings were also used to identify the mass fragments. In addition to anthocyanins, small amounts of anthocyanidins were also detected in the chromatograms, the most dominant being cyanidin (17) with traces of four other anthocyanidins. Anthocyanidins were more abundant in more processed samples, e.g., Residue II and Extract-2 to -4, indicating anthocyanins having been hydrolyzed in the fractionation process.

A total of 22 flavonol glycosides and 5 flavonol aglycons were found (Fig. 1b). These glycosides included galactosides, glucosides, glucuronides, arabinosides and xylosides of myricetin, laricitrin, quercetin, syringetin and isorhamnetin. Quercetin-3-*O*-galactoside (peak 27) eluted just before quercetin-3-*O*-glucoside (peak 29), as

Fig. 1 HPLC-DAD chromatograms of anthocyanins (**a**, 520 nm), flavonol glycosides and hydroxycinnamic acid conjugates (**b**, 360 nm), hydrolyzed flavonol aglycons and hydroxycinnamic acids (**c**, 360 nm) in the first ethanol extract (Extract-1). *Numbers* of peaks refer to Table 2
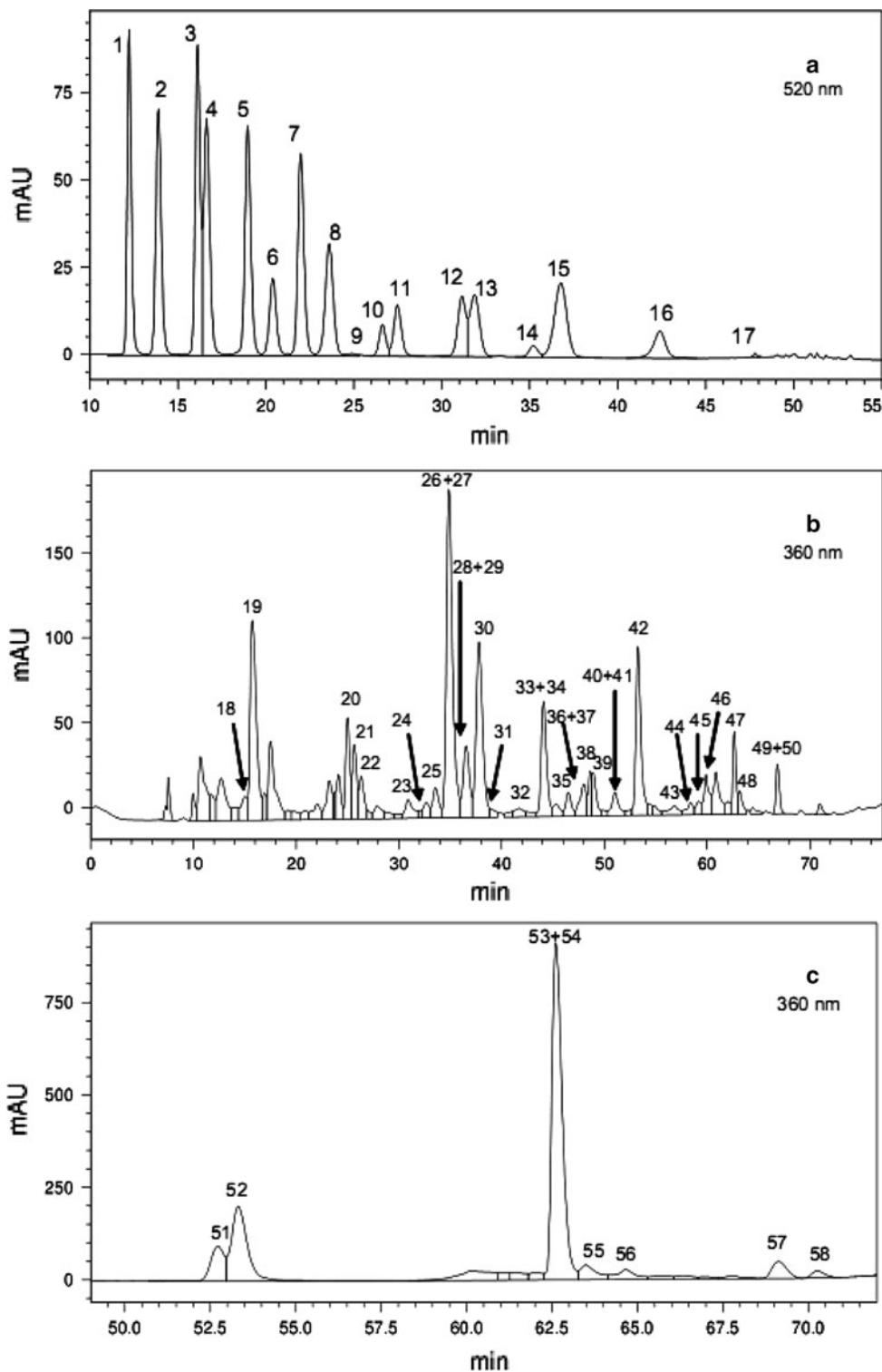
recognized with the reference compounds and literature [17, 19]. Galactosides and glucosides have the same molar masses and mass spectra, but they differ according to their retention. Similarly, galactosides of other flavonols were assumed to elute before glucosides. Peak 34 was identified as quercetin xyloside according to the mother ion *m/z* 435

and fragment *m/z* 303 as the mass difference *m/z* 132 indicates the existence of xylose. The same fragments were detected in peak 32 as in quercetin-3-*O*-arbinoside in the literature [17], and arabinosides of the flavonols detected were also assumed to elute before xylosides. According to the retention pattern of the reference compounds and of

Table 2 Identification of phenolic compounds in bilberry

No	Code	Compounds[a] Tentative identifications	UPLC-MS[b] [M+H]+ (m/z)	Fragment ions (m/z)	HPLC-DAD Ref[c]	HPLC[d] (nm)	Lit[e]
1	dp-gal	Delphinidin-3-O-galactoside	465	303	x	524	3,5,15,16
2	dp-glc	Delphinidin-3-O-glucoside	465	303	x	524	3,5,15,16
3	cy-gal	Cyanidin-3-O-galactoside	449	287	x	516	3,5,15,16
4	dp-ara	Delphinidin-3-O-arabinoside	435	303		525	3,5,15,16
5	cy-glc	Cyanidin-3-O-glucoside	449	287	x	516	3,5,15,16
6	pt-gal	Petunidin-3-O-galactoside	479	317		527	3,5,15,16
7	cy-ara	Cyanidin-3-O-arabinoside	419	287		516	3,5,15,16
8	pt-glc	Petunidin-3-O-glucoside	479	317		526	3,5,15,16
9	anthoc	Unknown anthocyanin	–	–		526	–
10	pn-gal	Peonidin-3-O-galactoside	463	301		516	3,5,15,16
11	pt-ara	Petunidin-3-O-arabinoside	449	317		527	3,5,15,16
12	pn-glc	Peonidin-3-O-glucoside	493	331		516	3,5,15,16
13	ma-gal	Malvidin-3-O-galactoside	463	301	x	528	3,5,15,16
14	pn-ara	Peonidin-3-O-arabinoside	493	331		512	3,5,15,16
15	ma-glc	Malvidin-3-O-glucoside	433	301		527	3,5,15,16
16	ma-ara	Malvidin-3-O-arabinoside	463	331		527	3,5,15,16
17	cy	Cyanidin	287	–	x		–
18	HA1	Caffeoyl quinic acid derivative	685	355,579,339		317	18
19	chlor	Chlorogenic acid derivative	731	355,579,291,372		325	13,18
20	my-gal	Myricetin-3-O-galactoside	481	319		357	13,17–19
21	my-glc	Myricetin-3-O-glucoside	481	319		355	13,17–19
22	my-gla	Myricetin-3-O-glucuronide	495	319		352	17,18
23	my-ara	Myricetin-3-O-arabinoside	451	319		357	17,19
24	my-xyl	Myricetin-3-O-xyloside	451	319		354	17
25	HA2	Coumaric acid derivative + hexose	519	357		313	–
26	la-gal	Laricitrin-3-O-galactoside	495	333		–	17
27	qu-gal	Quercetin-3-O-galactoside	465	303	x	355	13,17–19
28	la-glc	Laricitrin-3-O-glucoside	495	333		–	17
29	qu-glc	Quercetin-3-O-glucoside	465	303	x	356	13,17–19
30	qu-gla	Quercetin-3-O-glucuronide	479	303		355	17
31	la-gla	Laricitrin-3-O-glucuronide	509	333		x	17
32	qu-ara	Quercetin-3-O-arabinoside	435	303		x	18,19
33	la-xyl	Laricitrin-3-O-xyloside	465	333		–	17
34	qu-xyl	Quercetin-3-O-xyloside	435	303		356	17,18
35	is-gal	Isorhamnetin-3-O-galactoside	479	317		350	17
36	sy-gal	Syringetin-3-O-galactoside	509	347		–	–
37	qu-rha	Quercetin-3-O-rhamnoside	449	303		354	18
38	sy-glc	Syringetin-3-O-glucoside	509	347		358	–
39	is-glc	Isorhamnetin-3-O-glucoside	479	317		355	17
40	is-gla	Isorhamnetin-3-O-glucuronide	449	317		347	17
41	sy-gla	Syringetin-3-O-glucuronide	479	347		–	–
42	my	Free myricetin	319	-		373	13,17
43	HA3	Coumaric acid derivative + hexose	435	251,413		312	–
44	is-xyl	Isorhamnetin-3-O-xyloside	449	317		363	–
45	sy-xyl	Syringetin-3-O-xyloside	479	347		364	–
46	HA4	Caffeic acid derivative + hexose	593	429,267,447,461		318	–
47	la	Free laricitrin	333	–		–	–

Table 2 continued

No	Code	Compounds[a]	UPLC-MS[b]		HPLC-DAD		Lit[e]
		Tentative identifications	[M+H]$^+$ (m/z)	Fragment ions (m/z)	Ref[c]	HPLC[d] (nm)	
48	qu	Free quercetin	303	–		371	13,18
49	sy	Free syringetin	347	–		–	–
50	is	Free isorhamnetin	317	–		367	–
51	Caff1	Caffeic acid derivative	–	–		327	–
52	Myr	Myricetin aglycon	319	–	x	372	19
53	Quer	Quercetin aglycon	303	-	x	372	19
54	Lar	Laricitrin aglycon	333	–		–	–
55	p-Coum	p-Coumaric acid	–	–	x	310	19
56	Caff2	Caffeic acid	–	–	x	327	19
57	Syr	Syringetin aglycon	347	–		372	–
58	Isor	Isorhamnetin aglycon	317	–		373	–

[a] Numbering and codes of the peaks are used throughout in figures and other tables

[b] Mass spectral comparison, positive ion mode. In the cases of peaks 18, 19, 43 and 46, the first mass fragments had highest intensities (fragments are in decreasing order according to the intensities)

[c] Retention time and UV–vis spectrum compared to reference compound

[d] Identification according to UV–vis spectra. Spectrum maxima of the analytes shown or x indicates identification as anthocyanin, flavonol glycoside or as conjugative of hydroxycinnamic acid derivative, "–" not detected in UV–vis spectra

[e] Literature cited [3–5, 13, 15–19]

literature reference [20], the compounds were all evidently 3-O-glycosides. Quercetin and myricetin glycosides were the most dominant flavonols as the others were found in smaller amounts. Laricitrin glycosides as well as the free aglycone co-eluted with corresponding quercetin compounds but were detected in much lower quantities. Syringetin and its derivatives were identified according to mass fragment m/z 247 present in the peaks. To our knowledge, syringetin glycosides have not previously been identified in bilberry.

Hydroxycinnamic acid conjugates (Fig. 1b) were identified by comparing their UV–vis and mass spectra with data published earlier [13, 18] and quantified at 320 nm. Five relatively large compounds in the fractions were identified as hydroxycinnamic acid derivatives as three of them were derivatives of caffeic acid (peaks 18, 19 and 46) and two were derivatives of p-coumaric acid (peaks 25 and 43), according to their UV–vis spectra. Any smaller peaks of hydroxycinnamic acids were co-eluted with flavonol glycosides or anthocyanins and therefore not detected. Peak 19 was identified as a derivative of chlorogenic acid (m/z 355) as this phenolic acid has been reported in bilberries [13]. Hokkanen et al. [18] reported different isomers of chlorogenic acid present in bilberry leaves. Compound 18 could be one of these as mass m/z 355 was the most abundant ion detected in these compounds. Analysis of the mass spectral data of compounds 18 and 19 was difficult due to overlapping anthocyanins. The loss of mass m/z 162 from the mother ion in compounds 25, 43 and 46 could indicate a presence of glucose as these compounds might be esters of caffeic or p-coumaric acid.

In addition to flavonol glycosides and hydroxycinnamic acid derivatives, flavonol aglycons in bilberry were also analyzed to confirm the identifications of glycosides (Fig. 1c). Myricetin, quercetin, laricitrin, syringetin and isorhamnetin were the aglycones detected as in the case of the glycosides. Also p-coumaric acid derivative and two different caffeic acid derivatives were found according to their UV–vis spectra. The latter (56) could be actual caffeic acid as in the literature [19], and the former remained unidentified. Other phenolic compounds such as flavan-3-ols and their polymers were tentatively identified with support of data previously reported [13, 18], but the contents of these compounds were not further defined in this study.

Profiles of phenolic compounds

Contents of phenolic compounds may vary largely in foods according to the regional [16] or altitudinal [23] differences or storage conditions [24, 25]. Type and degree of variation depends on plant species and the compound classes. In bilberries, the main phenolics of each fraction collected were anthocyanins. Distributions of each anthocyanin in various fractions are shown in Table 3, in sensory samples in Table 4 and the corresponding sum of the anthocyanins in Table 1. Over 80% of anthocyanins remained in the press residue (Residue I) after fractionating, and less than

Table 3 Phenolic compounds in fractions of berry

Compounds[a]	Berry	Juice	Residue I	CRI [c]	Extract-1	Extract-2	Residue II	CRII[c]	Extract-3	Extract-4	CRIII[c]
Anthocyanins (mg/1 kg of berry)[b]											
dp-gal	360 ± 30	59 ± 6	340 ± 40	350	120 ± 4	58 ± 6	150 ± 12	150	27 ± 1	21 ± 4	15
dp-glc	300 ± 20	48 ± 4	270 ± 30	280	120 ± 4	56 ± 6	100 ± 9	96	23 ± 0.2	16 ± 0.3	11
cy-gal	300 ± 30	54 ± 5	270 ± 30	270	140 ± 5	60 ± 7	72 ± 6	71	23 ± 0.1	14 ± 0.2	7.7
dp-ara	340 ± 20	55 ± 5	320 ± 40	330	120 ± 5	61 ± 7	120 ± 10	110	26 ± 0.1	19 ± 0.4	14
cy-glc	220 ± 20	39 ± 3	200 ± 30	210	120 ± 5	48 ± 5	44 ± 3	45	16 ± 0.1	9.0 ± 0.2	4.6
pt-gal	120 ± 9	22 ± 2	100 ± 15	110	49 ± 3	22 ± 3	36 ± 3	35	9.4 ± 0.1	6.4 ± 0.1	4.1
cy-ara	220 ± 20	36 ± 3	200 ± 30	210	110 ± 4	46 ± 5	47 ± 4	45	16 ± 0.1	9.2 ± 0.2	11
pt-glc	170 ± 10	30 ± 3	150 ± 20	150	80 ± 4	35 ± 4	41 ± 3	40	13 ± 0.1	8.1 ± 0.2	4.8
anthoc	6.5 ± 0.7	1.1 ± 0.1	5.6 ± 3	7.3	3.2 ± 0.9	1.7 ± 0.2	1.3 ± 0.4	1.9	0.6 ± 0.0	0.3 ± 0.0	0.3
pn-gal	36 ± 4	7.9 ± 0.7	33 ± 6	33	19 ± 2	7.9 ± 0.8	6.1 ± 0.6	6,2	2.6 ± 0.1	1.4 ± 0.0	0.8
pt-ara	84 ± 7	13 ± 2	76 ± 13	69	36 ± 4	18 ± 2	24 ± 2	28	9.0 ± 0.1	6.0 ± 0.2	5.3
pn-glc	75 ± 6	13 ± 1	65 ± 9	71	45 ± 2	16 ± 2	10 ± 0.8	9.8	4.9 ± 0.0	2.2 ± 0.0	1.1
ma-gal	100 ± 10	23 ± 2	89 ± 13	88	52 ± 3	20 ± 2	17 ± 1	18	6.9 ± 0.0	3.8 ± 0.2	1.7
pn-ara	18 ± 3	3.7 ± 0.3	16 ± 4	16	10 ± 1	3.7 ± 0.4	3.1 ± 0.5	2.5	1.2 ± 0.0	0.6 ± 0.0	0.3
ma-glc	150 ± 8	30 ± 3	120 ± 20	130	82 ± 4	27 ± 2	19 ± 1	18	8.7 ± 0.0	4.7 ± 0.1	1.5
ma-ara	53 ± 5	9.8 ± 0.9	50 ± 8	47	30 ± 3	12 ± 1	9.3 ± 0.8	12	5.0 ± 0.1	2.8 ± 0.1	1.3
cy	3.0 ± 0.6	*	3.6 ± 2	–	1.4 ± 0.6	1.1 ± 0.2	1.2 ± 0.4	2.4	0.8 ± 0.0	0.3 ± 0.0	1.0
Hydroxycinnamic acids (mg/1 kg of berry)[b]											
HA1	24	13 ± 0.4	6.1 ± 0.7		7.8 ± 0.4	2.9 ± 0.9	*		1.0	*	
chlor	100	50 ± 5	21 ± 0.4	52	38 ± 2	10 ± 1	2.2 ± 0.2	3.7	3.7 ± 0.4	1.4 ± 0.4	0.3
HA2	91	38 ± 3	30 ± 2	53	39 ± 2	11 ± 1	2.6 ± 0.1	3.7	3.4 ± 0.3	1.1 ± 0.5	0.3
HA3	22	12 ± 1	5.4 ± 0.6		7.2 ± 0.3	1.6 ± 0.2	0.4 ± 0.0		0.4 ± 0.3	*	
HA4	16	2.5 ± 0.3	8.0 ± 0.7	14	11 ± 0.4	2.4 ± 0.2	1.0 ± 0.0	0.7	0.8 ± 0.0	0.3 ± 0.1	0.03
Flavonols and their glycosides (mg/1 kg of berry)[b]											
my-gal	9.2	3.4 ± 0.3	2.1 ± 0.1	5.8	3.5 ± 0.2	1.5 ± 0.2	0.3 ± 0.0	0.9	0.6 ± 0.1	0.3 ± 0.1	0.1
my-glc	7.2	2.7 ± 0.2	1.4 ± 0.1	4.5	2.7 ± 0.1	1.0 ± 0.1	0.2 ± 0.0	0.5	0.4 ± 0.0	0.1 ± 0.0	0.1
my-gla	3.4	0.8 ± 0.3	0.8 ± 0.2	2.6	1.5 ± 0.2	0.7 ± 0.1	0.3 ± 0.0	0.4	0.3 ± 0.1	0.3	0.1
my-ara	1.4	*	*		1.2 ± 0.0	0.2 ± 0.0	*		*	*	
my-xyl	1.4	0.6	1.0 ± 0.3		0.8 ± 0.1	*	*		*	*	
la-gal + qu-gal	41	17 ± 2	12 ± 0.7	24	17 ± 08	4.9 ± 0.5	1.3 ± 0.0	1.9	1.7 ± 0.1	0.6 ± 0.2	0.1
la-glc + qu-glc	10	5.0 ± 0.4	2.7 ± 0.2	5.4	3.9 ± 0.2	1.1 ± 0.1	0.3 ± 0.0	0.4	0.4 ± 0.0	0.1 ± 0.0	0.03
qu-gla	35	16 ± 1	14 ± 1	18	9.8 ± 0.5	4.8 ± 0.5	1.8 ± 0.0	4.0	2.2 ± 0.4	1.0 ± 0.4	0.9
la-gla	0.5	0.5	0.5 ± 0.2		0.4 ± 0.0	*	*		*	*	
qu-ara	0.6	0.6	0.5 ± 0.2		0.6 ± 0.1	*	*		*	*	
la-xyl + qu-xyl	10	3.4 ± 0.3	4.2 ± 0.5	6.8	5.3 ± 0.2	1.2 ± 0.1	0.4 ± 0.0	0.3	0.4 ± 0.0	0.1 ± 0.0	0.02
is-gal	1.9	0.7 ± 0.1	0.7 ± 0.1		1.2 ± 0.1	*	*		*	*	
sy-gal + qurha	3.5	1.3 ± 0.1	1.5 ± 0.3		1.7 ± 0.1	0.4 ± 0.0	*		0.1 ± 0.0	*	
sy-glc	4.7	2.9 ± 0.2	1.2 ± 0.1		1.3 ± 0.1	0.4 ± 0.0	0.1 ± 0.0		0.1 ± 0.0	*	
is-glc	2.6	0.7 ± 0.1	1.2 ± 0.1		1.5 ± 0.0	0.3 ± 0.0	0.1 ± 0.0		0.1 ± 0.0	*	
is-gla + sy-gla	3.1	1.2 ± 0.1	1.2 ± 0.1		1.4 ± 0.1	0.3 ± 0.0	0.1		0.1 ± 0.0	*	
my	14	4.8 ± 1	6.3 ± 0.6	9.6	7.6 ± 0.5	1.6 ± 0.1	1.0 ± 0.1	0.4	0.6 ± 0.1	0.3 ± 0.0	0.02
is-xyl	0.7	*	0.6		0.7 ± 0.0	*	*		*	*	
sy-xyl	0.6	*	0.5		0.6 ± 0.0	*	*		*	*	
qu	6.8	3.7 ± 0.6	5.8 ± 0.9		2.4 ± 0.2	0.5 ± 0.0	0.7 ± 0.0		0.2 ± 0.0	*	
la	0.8	*	0.5 ± 0.1		0.6 ± 0.0	*	*		*	*	
sy + is	2.4	0.7	1.1 ± 0.2		1.5 ± 0.1	0.3 ± 0.0	*		*	*	

[a] Compounds refer to Table 2, * below the detection limit

[b] Contents in each original fraction (Table 1). In the case of hydroxycinnamic acids and flavonols, the contents in berry are the sum of contents in juice and calculated Residue I (CRI or Extract-1 to -4)

[c] Calculated theoretical values of Residue I (CRI), Residue II (CRII) and Residue III (CRIII)

Table 4 Phenolic compounds in sensory samples

	Juice	Juice + extract	Residue I	Combined extract	Extract-1	Extract-2	Residue II
Anthocyanins (mg/100 g)							
dp-gal	7.0 ± 0.6 [e]	130 ± 6[c]	220 ± 30[b]	42 ± 10[d]	46 ± 2[d]	57 ± 6[d]	300 ± 20[a]
dp-glc	5.7 ± 0.5[d]	110 ± 5[b]	180 ± 20[a]	40 ± 9[c]	46 ± 2[c]	56 ± 6[c]	200 ± 20[a]
cy-gal	6.3 ± 0.6[e]	110 ± 3[c]	180 ± 20[a]	50 ± 12[d]	54 ± 2[d]	60 ± 7[d]	140 ± 10[b]
dp-ara	6.5 ± 0.6[e]	120 ± 8[c]	210 ± 30[a]	41 ± 10[d]	47 ± 2[d]	61 ± 7[d]	250 ± 20[b]
cy-glc	4.5 ± 0.4[d]	83 ± 4[b]	130 ± 20[a]	40 ± 10[c]	46 ± 2[c]	48 ± 5[c]	88 ± 6[b]
pt-gal	2.6 ± 0.2[d]	41 ± 0.6[b]	69 ± 10[a]	17 ± 4[c]	19 ± 1[c]	22 ± 3[c]	72 ± 6[a]
cy-ara	4.3 ± 0.4[d]	82 ± 3[b]	130 ± 20[a]	36 ± 8[c]	42 ± 1[c]	46 ± 5[c]	93 ± 7[b]
pt-glc	3.5 ± 0.3[e]	60 ± 2[c]	98 ± 13[a]	26 ± 6[d]	31 ± 1[d]	35 ± 4[d]	82 ± 6[b]
anthoc	0.1 ± 0.0[c]	2.3 ± 0.8[abc]	3.8 ± 2[a]	1.2 ± 0.3[bc]	1.2 ± 0.3[bc]	1.7 ± 0.2[abc]	2.5 ± 0.9[ab]
pn-gal	0.9 ± 0.1[d]	14 ± 0.6[b]	22 ± 4[a]	7.2 ± 2[c]	7.5 ± 0.9[c]	7.9 ± 0.8[c]	12 ± 1[b]
pt-ara	1.5 ± 0.2[d]	30 ± 1[b]	51 ± 9[a]	16 ± 4[c]	14 ± 1[c]	18 ± 0.2[c]	47 ± 4[a]
pn-glc	1.6 ± 0.1[d]	28 ± 1[b]	43 ± 6[a]	14 ± 3[c]	17 ± 0.9[c]	16 ± 2[c]	20 ± 2[c]
ma-gal	2.7 ± 0.3[d]	37 ± 0.5[b]	59 ± 9[a]	18 ± 5[c]	20 ± 1[c]	20 ± 2[c]	35 ± 2[b]
pn-ara	0.4 ± 0.0[e]	6.7 ± 0.5[b]	11 ± 3[a]	3.4 ± 0.7[d]	3.8 ± 0.6 [cd]	3.7 ± 0.4 [cd]	6.1 ± 1[bc]
ma-glc	3.6 ± 0.3[d]	52 ± 3[b]	82 ± 12[a]	26 ± 6[c]	31 ± 2[c]	27 ± 2[c]	37 ± 3[c]
ma-ara	1.1 ± 0.1[e]	21 ± 0.8[b]	34 ± 6[a]	13 ± 3 [cd]	12 ± 1[d]	12 ± 1[d]	18 ± 2[bc]
cy	*	1.3 ± 0.5	2.4 ± 1[a]	1.2 ± 0.3	0.5 ± 0.2	1.1 ± 0.2	2.3 ± 0.9[a]
Hydroxycinnamic acids (mg/100 g)							
HA1	1.5 ± 0.0[d]	5.0 ± 0.5[a]	4.0 ± 0.5[ab]	2.9 ± 0.3[bc]	3.0 ± 0.2[bc]	2.9 ± 0.9[c]	*
chlor	4.5 ± 0.3[d]	21 ± 2[a]	20 ± 1[a]	14 ± 0.2[b]	15 ± 0.8[b]	11 ± 1[c]	5.3 ± 0.1[d]
HA2	6.0 ± 0.6[d]	22 ± 2[a]	14 ± 0.3[b]	13 ± 0.7[b]	15 ± 0.6[b]	11 ± 1[c]	4.5 ± 0.4[d]
HA3	1.5 ± 0.1[d]	4.4 ± 0.4[a]	3.6 ± 0.4[b]	2.4 ± 0.1[c]	2.8 ± 0.1[c]	1.6 ± 0.2[d]	0.8 ± 0.0[e]
HA4	0.3 ± 0.0[e]	4.6 ± 0.4[b]	5.3 ± 0.4[a]	3.6 ± 0.1[c]	4.1 ± 0.2[bc]	2.4 ± 0.2[d]	2.0 ± 0.0[d]
Flavonols and their glycosides (mg/100 g)							
my-gal	0.4 ± 0.0[c]	2.1 ± 0.2[a]	1.4 ± 0.0[b]	1.4 ± 0.0[b]	1.4 ± 0.1[b]	1.5 ± 0.2[b]	0.6 ± 0.1[c]
my-glc	0.3 ± 0.0[c]	1.5 ± 0.1[a]	1.0 ± 0.1[b]	1.0 ± 0.0[b]	1.0 ± 0.0[b]	1.0 ± 0.1[b]	0.4 ± 0.1[c]
my-gla	0.1 ± 0.0[b]	0.8 ± 0.2[a]	0.6 ± 0.1[a]	0.6 ± 0.1[a]	0.6 ± 0.1[a]	0.7 ± 0.1[a]	0.6 ± 0.1[a]
my-ara	*	0.4 ± 0.1[ab]	*	0.4 ± 0.0[b]	0.5 ± 0.0[a]	*	*
my-xyl	0.1[c]	0.3 ± 0.0[ab]	0.4 ± 0.1[a]	0.2 ± 0.0[b]	0.3 ± 0.0[ab]	*	*
la-gal + qu-gal	2.0 ± 0.2[e]	9.3 ± 0.8[a]	8.0 ± 0.5[b]	6.1 ± 0.1[c]	6.6 ± 0.3[c]	4.9 ± 0.5[d]	2.5 ± 0.1[e]
la-glc + qu-glc	0.6 ± 0.0[e]	2.2 ± 0.2[a]	1.8 ± 0.1[b]	1.4 ± 0.0[c]	1.5 ± 0.1[c]	1.1 ± 0.1[d]	0.5 ± 0.0[e]
qu-gla	2.0 ± 0.2[e]	6.8 ± 0.5[b]	9.4 ± 0.7[a]	4.1 ± 0.1 [cd]	3.8 ± 0.2[d]	4.8 ± 0.5[c]	3.7 ± 0.1[d]
la-gla	*	0.1 ± 0.0[b]	0.4 ± 0.1[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	*	*
qu-ara	*	0.2 ± 0.0[ab]	0.3 ± 0.1[a]	0.2 ± 0.0[b]	0.2 ± 0.0[ab]	*	*
la-xyl + qu-xyl	0.4 ± 0.0[d]	2.6 ± 0.2[a]	2.8 ± 0.3[a]	1.8 ± 0.1[b]	2.1 ± 0.1[b]	1.2 ± 0.1[c]	0.8 ± 0.0[d]
is-gal	0.1 ± 0.0[c]	0.5 ± 0.0[a]	0.5 ± 0.1[a]	0.3 ± 0.0[b]	0.4 ± 0.0[a]	*	*
sy-gal + qurha	0.2 ± 0.0[e]	0.8 ± 0.1[ab]	1.0 ± 0.2[a]	0.6 ± 0.0[c]	0.7 ± 0.0[bc]	0.4 ± 0.0[d]	*
sy-glc	0.3 ± 0.0 [cd]	0.9 ± 0.1[a]	0.8 ± 0.1[a]	0.5 ± 0.0[b]	0.5 ± 0.0[b]	0.4 ± 0.0[bc]	0.3 ± 0.0[d]
is-glc	0.1 ± 0.0[e]	0.7 ± 0.1[b]	0.8 ± 0.1[a]	0.5 ± 0.0[c]	0.6 ± 0.0[c]	0.3 ± 0.0[d]	0.3 ± 0.0[d]
is-gla + sy-gla	0.1 ± 0.0[d]	0.7 ± 0.1[a]	0.8 ± 0.1[a]	0.5 ± 0.0[bc]	0.5 ± 0.0[b]	0.3 ± 0.0[c]	0.1[d]
my	0.6 ± 0.1[e]	3.6 ± 0.3[b]	4.2 ± 0.4[a]	2.5 ± 0.1[c]	2.9 ± 0.2[c]	1.6 ± 0.1[d]	2.0 ± 0.1[d]
is-xyl	*	0.2 ± 0.0[b]	0.4[a]	0.2 ± 0.0[c]	0.3 ± 0.0[a]	*	*
sy-xyl	*	0.2 ± 0.0[b]	0.3[a]	0.2 ± 0.0[c]	0.2 ± 0.0[a]	*	*
qu	0.4 ± 0.1[c]	1.4 ± 0.1[b]	3.9 ± 0.6[a]	0.8 ± 0.0[c]	0.9 ± 0.1[bc]	0.5 ± 0.0[c]	1.4 ± 0.1[b]
la	*	0.3 ± 0.0[bc]	0.4 ± 0.1[a]	0.2 ± 0.0[c]	0.3 ± 0.0[ab]	*	*
sy + is	0.1[d]	0.6 ± 0.1[ab]	0.7 ± 0.1[a]	0.5 ± 0.0[b]	0.6 ± 0.0[b]	0.3 ± 0.0[c]	*

Phenolic compounds: names refer to Table 2, * below the detection limit

Sensory samples: extracts are diluted in water, 25 g/L. Significant differences between samples in each compound based on one-way ANOVA and Tukey's test ($p < 0.05$) are marked with superscript a–e

Table 5 Sugars and fruit acids in fractions of berry and in sensory samples

	Berry	Juice	Juice + extract	Residue I	Combined extract	Extract-1	Extract-2	Residue II	Extract-3	Extract-4	Residue III
Fractions of berry (mg/1 kg of berry)											
Sugars											
Xylose	380 ± 80	380 ± 80	–	*	–	*	*	*	*	*	*
Fructose	20,000 ± 700	19,000 ± 700	–	920 ± 30	–	640 ± 30	270 ± 10		50 ± 2	0.6 ± 0.0	*
Glucose	26,000 ± 700	25,000 ± 700	–	1,200 ± 20	–	900 ± 19	270 ± 10		85 ± 0.5	0.6 ± 0.0	*
Myo-inositol	410 ± 9	410 ± 9	–	*	–	*	*	*	*	*	*
Acids											
Malic acid	2,000 ± 30	1,900 ± 40	–	120 ± 6	–	98 ± 5	27 ± 0.4	*	*	*	*
Citric acid	4,000 ± 300	3,700 ± 200	–	320 ± 7	–	220 ± 7	81 ± 1	24 ± 0.2	24 ± 0.2	*	*
Quinic acid	4,200 ± 300	3,900 ± 300	–	250 ± 3	–	170 ± 9	61 ± 0.9	22 ± 0.2	22 ± 0.2	*	*
Sensory samples (mg/100 g)											
Sugars											
Xylose	–	44 ± 9	44 ± 9	*	*	*	*	*	–	–	–
Fructose	–	2,200 ± 80	2,300 ± 80	610 ± 20	260 ± 10	250 ± 10	270 ± 10	130 ± 0.0	–	–	–
Glucose	–	2,900 ± 90	3,000 ± 80	830 ± 10	330 ± 5	350 ± 7	270 ± 10	360 ± 5	–	–	–
Myo-inositol	–	48 ± 1	48 ± 1	*	*	*	*	*	–	–	–
Acids											
Malic acid	–	220 ± 6	230 ± 6	80 ± 4	33 ± 3	38 ± 2	27 ± 0.4	*	–	–	–
Citric acid	–	430 ± 30	450 ± 30	210 ± 4	86 ± 2	86 ± 3	81 ± 1	60 ± 0.5	–	–	–
Quinic acid	–	460 ± 30	470 ± 30	170 ± 2	62 ± 8	67 ± 3	61 ± 0.9	54 ± 4	–	–	–

* Not detected

Table 6 Sensory attributes, descriptions with references and their intensities used in sensory profiling of bilberry fractions

Sensory attribute	Description	Reference	Intensity[a]
Total intensity	Perceived first expression of all flavors	–	–
Roundness	Compact, rich and multidimensional, opposite to weak, watery and simple	–	–
Berryness	Berried flavor	Unsweetened bilberry juice (Kaskein Marja Ky, Taavetti, Finland) + 0,7% fructose (Sigma, St. Louis, MO)	5
Astringency	Puckering, drying mouthfeel	0.10% AlSO$_4$ (Linnan Apteekki, Turku, Finland)	5
Sourness	Sour taste	0.1% Citric acid (J.T. Baker, Deventer, Netherlands)	4
Sweetness	Sweet taste	0.7% Fructose (Sigma, St. Louis, MO)	3
Bitterness	Bitter taste	0.04% Caffeine (Yliopiston Apteekki, Helsinki, Finland) + 0.001% quinine-HCl	4

[a] Scale is from 0 (no sensation) to 10 (very strong sensation)

one fifth was obtained in the juice. The first two ethanol extractions removed nearly 70% of anthocyanins from Residue I, and after four ethanol extractions, less than 5% of the anthocyanins of Residue I remained in Residue III. Glycosides of delphinidin and cyanidin were the major anthocyanins throughout the fractions. According to Lätti et al. [16], there is a high variation in the content of anthocyanins in bilberries collected throughout Finland as the northern berries have higher contents than the southern berries. Theoretical values of residue fractions are congruent with the measured values (Table 3), and more accurate contents in berries could be calculated as a sum of contents in juice and Residue I. Different extraction methods used in analyses and sample preparations could have resulted in diverse extractability of individual anthocyanins as some compounds dissolved more effectively in ethanol than in acidified methanol. Also, by adding two extracts back to the juice fraction, anthocyanin content of the sample increased by 17-fold on average. Riihinen et al. [26] reported that anthocyanins locate mainly in the peel of berries as a fraction was found in the pulp of the berry and in the red leaves of the plant.

Flavonol glycosides and hydroxycinnamic acid derivatives in the fractions and in the sensory samples are shown in Tables 3 and 4. A total of five derivatives of hydroxycinnamic acids were included in the phenolic profiles despite their tentative identification. The total content of these phenolic acids was higher than the content of flavonols in each fraction. The systematically decreasing trend of concentrations in the ethanol fractions is unambiguous without any exception. Among the phenolic compounds in

the isocratic samples prepared for sensory analysis, the concentrations were typically highest in Extract-2. Quercetin-3-O-glucuronide and quercetin-3-O-galactoside were the most abundant compounds followed by other quercetin glycosides. Altogether, these comprised approximately two-thirds of all the flavonol glycosides. Laricitrin glycosides eluted with corresponding quercetin compounds but were found in trace amounts only. Many less abundant compounds appeared in some of the extracts below the quantification limit or even below the detection limit. These compounds may still have some biological significance, even at these very low concentrations. Contents of phenolic compounds in bilberries are very different to blueberries [26], as the latter berries have higher contents of phenolic acids and flavonols. Contents of flavonols are also higher in bog bilberries (*V. uliginosum*) [13], which are also known as northern bilberries.

Most of the flavonol glycosides and hydroxycinnamic acids remained in the press residue after berry pressing, as has been previously demonstrated [19, 26]. According to Riihinen et al. [26], most of the phenolic acids and flavonols can be found in the leaves rather than in the berries of bilberry. In red leaves especially, contents of these compound classes could be dozens of times higher than in the berries. Juice supplemented with extracts had the highest contents of flavonols and hydroxycinnamic acids in some cases among sensory samples. Contents of these compounds were on average five times higher than those found in pure juice. Theoretical values of flavonol glycosides and hydroxycinnamic acid conjugates are shown in Table 3. Values were calculated for compounds that were

Table 7 Mean intensities ($n = 45$) for sensory attributes in bilberry fractions

	Juice	Juice + extract	Residue I	Extract-1	Extract-2	Combined extract	Residue II
Total int.	6.5 ± 1.3^a	6.6 ± 1.3^a	2.8 ± 1.1^c	4.7 ± 2.3^b	4.2 ± 1.9^b	5.0 ± 2.2^b	1.7 ± 1.1^d
Roundness	6.4 ± 1.4^a	6.0 ± 1.8^a	3.6 ± 1.6^b	2.0 ± 1.5^c	1.8 ± 1.2^c	1.8 ± 1.4^c	1.7 ± 1.3^c
Berryness	6.8 ± 1.3^a	6.5 ± 1.6^a	3.2 ± 1.3^b	1.6 ± 1.2^c	1.8 ± 1.4^c	1.5 ± 1.2^c	1.5 ± 1.0^c
Astringency	2.9 ± 2.2^b	3.9 ± 2.3^b	1.3 ± 1.3^b	7.1 ± 1.9^a	5.9 ± 2.2^a	7.0 ± 1.7^a	1.0 ± 1.1^c
Sourness	4.5 ± 1.8^a	4.8 ± 1.7^a	2.4 ± 1.3^b	4.3 ± 2.1^a	4.3 ± 2.0^a	4.2 ± 2.5^a	1.3 ± 1.0^c
Sweetness	4.6 ± 2.1^a	4.2 ± 1.8^a	1.9 ± 1.1^b	0.9 ± 0.7^c	0.9 ± 0.7^c	0.8 ± 0.6^c	1.0 ± 0.7^c
Bitterness	2.1 ± 2.0^{bc}	2.4 ± 2.0^b	1.4 ± 1.5^{bc}	5.0 ± 2.1^a	5.0 ± 2.3^a	5.5 ± 2.3^a	1.2 ± 1.3^c

Mean intensities: scale is from 0 (no sensation) to 10 (very strong sensation). Significant differences between samples in each attribute based on one-way ANOVA and Tamhane test ($p < 0.05$) are marked with a–d

measurable in all four ethanol extracts. On average, 90% of these compounds were removed by two ethanol extractions when compared with calculated values of Residue I (CRI in Table 3) and 98% by four ethanol extractions. Calculated values show more accurately the real contents of these compounds than other values.

Sugars and acids

The sugar and acid contents of various samples are given in Table 5. Identification of the compounds is based on the literature [22, 27] and reference compounds. The main sugars of bilberry were glucose and fructose with minor amounts of xylose and *myo*-inositol which were only found in the juice fraction. Sucrose has previously been found in bilberry [28], but our samples did not appear to contain any. The total content of sugars in the berries was 47 g/kg, and more than 95% ended up in the juice after pressing. Most of the sugars in Residue I were dissolved to Extract-1, and over 96% were removed from the press residue by two extractions. Traces were still detected in the third and fourth extracts.

Quinic and citric acids were the main organic fruit acids in every fraction, while malic acid was found only at low levels. The majority of the organic acids were found to be in the juice (over 93%). Practically, all the acids were dissolved during the first ethanol extraction.

Orosensory profiles of fractions

Seven different attributes (total intensity of flavor, roundness, berryness, astringency, sourness, sweetness and bitterness) were chosen for the orosensory profile of the fractions (Tables 6, 7). Fractions used in the sensory analyses were chosen according to earlier findings in blackcurrant where similar attributes decreased during the ethanol extraction process [19]. The sensory profile of the bilberry juice was sweet, round and berried, while the

extracts of Residue I were bitter and astringent. Juice supplemented with the extract did not differ significantly from pure juice in any attribute although it was perceived to be slightly more astringent than the juice. By adding more than two extract fractions to the juice, the astringency may increase significantly. The three extract fractions (Extract-1, -2 and the combination) were all similar in each attribute. Two consecutive extractions significantly decreased the total intensity, sweetness, sourness and berried flavor from Residue I when compared with Residue II. To decrease the bitterness and astringency of Residue I, more than two extractions would be needed. Nevertheless, Residue II was found to be lacking in flavor and taste properties, and it gave very few perceived sensations. Interestingly, the first extract was the most astringent sample of all the fractions though the difference was not statistically significant.

Compounds contributing to orosensory properties

Bilberry juice had a sweet, round and berried sensory profile, and it was rich in sugars and acids. The profile of Residue I was very mild, but it was richer in phenolic compounds than the juice. In general, the phenolic compounds are thought to be interacting with astringency and bitterness [9, 10, 20]. Our findings showed that while Residue I was neither astringent nor bitter, its extracts were significantly stronger in these attributes than all the other samples, and these extracts were also rich in phenolic compounds. On the other hand, juice supplemented with extracts was similar to pure juice even though it contained significantly more phenolic compounds.

The PLS2 method was applied to relate the sensory and chemical data matrices to see the compounds contributing to the orosensory characteristics. The model was applied on all the sensory properties and non-volatile chemical variables (PLS2) with bilberry fractions except the combined extract and berries. Our sensory panel did not evaluate whole berries at all and we excluded the combined extract
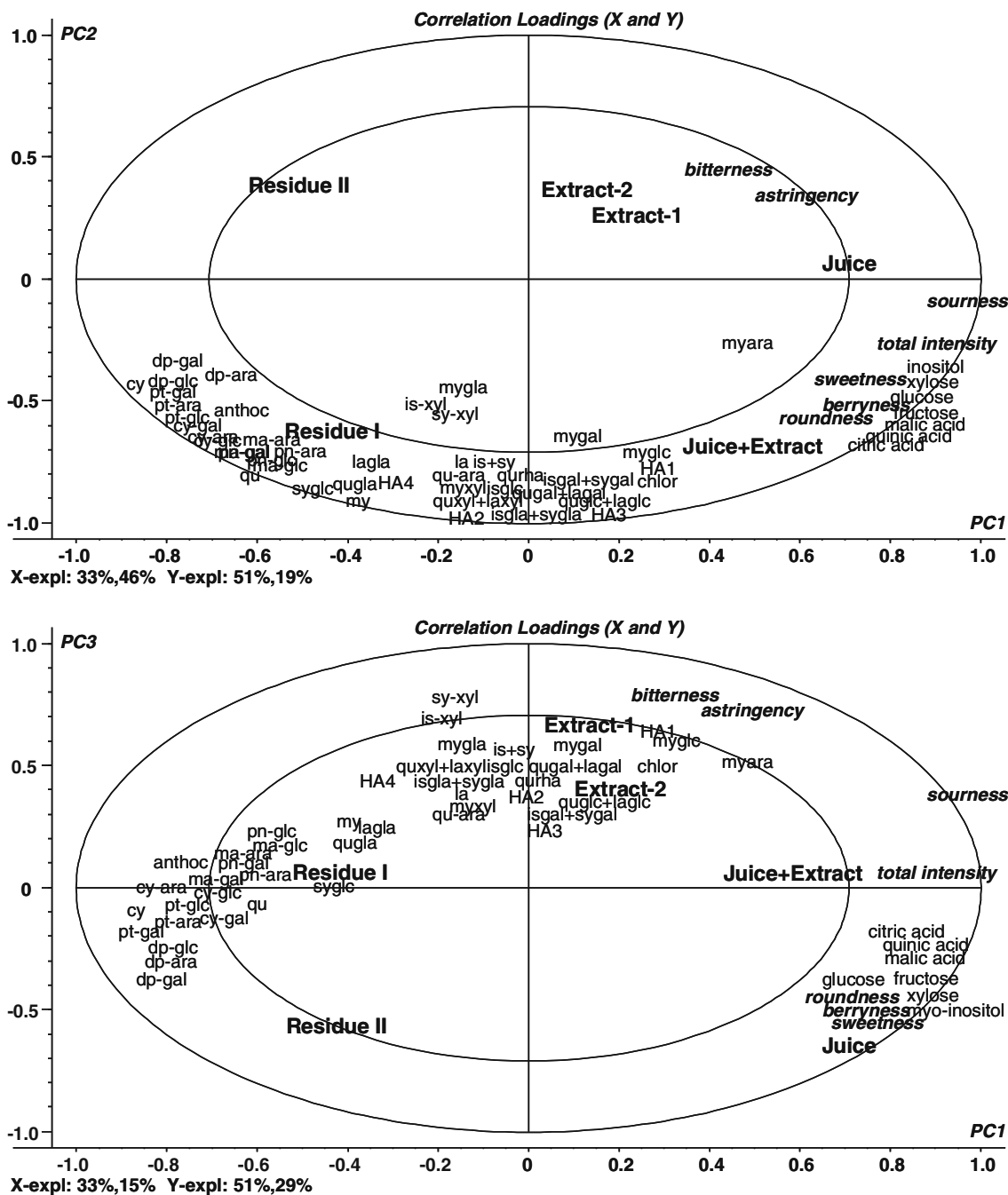
Fig. 2 Partial least squares regression (PLS2) plots of the interaction between sensory profiles (*n* = 7) and chemical variables (*n* = 51) in bilberry samples (*n* = 6) with 3 principal components (in the first plot PC1 and PC2: in the second PC1 and PC3). Samples are in *bold* and *bigger letter size*, and attributes are in *bold* and *italics*. Abbreviations of phenolic compounds refer to Table 2

from the model, because it was very similar to Extract-1 and -2 in every attribute. The predicted *Y*-values (sensory properties, *n* = 7) were computed by applying the model equation to the observed *X*-variables (phenolic compounds, sugars and fruit acids, *n* = 51). When three principal components were taken into account, 94% of chemical variables explained 99% of the sensory data (Fig. 2). The model showed, again, a strong correlation between Residue

I and many phenolic compounds on the left side of both plots using PC1. However, most of these compounds were anthocyanins and other phenolics correlated with supplemented juice (juice + extract), which was significantly neither astringent nor bitter (PC2). In addition to mild profiles of the residues, PC1 also showed a strong relationship between sugars and sweetness. PC3 showed a correlation between phenolics and astringency and

bitterness in Extract-1 and -2. Anthocyanins do not notably correlate with any of the sensory attributes. In the lower plot, chemical variables, especially my-ara (myricetin-3-*O*-arabinoside), my-glc (myricetin-3-*O*-glucoside), my-gal (myricetin-3-*O*-galactoside) and HA1 (caffeoyl quinic acid derivative), were close to Extract-1 and its astringency. In addition to these compounds, some other phenolic compounds in the lower plot close to Extract-1 could contribute significantly to the astringency of bilberry Extract-1. However, not all of the compounds in the extracts were identified in this study.

In our previous study on blackcurrants [19], we found myricetin-3-*O*-galactoside to be one of the compounds correlating with astringency in similar extracts. Schwarz and Hofmann [20] reported quercetin galactoside and quercetin glucoside, among other flavonol glycosides, and hydroxycinnamic acid derivatives to contribute to astringency in redcurrants. Also, some indolyl glycosides have been reported to be very astringent in redcurrants [29]. Myricetin-3-*O*-galactoside has been found to be one of the astringent compounds in black tea [30]. Quercetin glycosides were generally more astringent than myricetin or kaempferol glycosides [20, 30]. Hydroxycinnamic acids were reported as puckering astringent compounds in red wine, present at very low concentrations, and their ethyl esters were both bitter and astringent [31]. Flavonol glycosides, for example, syringetin-3-*O*-glucoside and quercetin-3-*O*-galactoside, were reported as more velvety astringent in red wine and were perceived at even lower concentrations than phenolic acids. Many phenolic compounds, such as flavonol glycosides, have been found to be astringent at low concentrations but bitter at higher levels [30, 31]. In our study, the content of these compounds was significantly higher in Residue I and supplemented juice than in Extract-1 or -2, but neither of Residue I nor supplemented juice was astringent or bitter. As we previously suggested, these compounds may be released from the berry skin structures more effectively with ethanol than with saliva [19]. In addition to this, sugars and acids in the juice weaken the astringency and bitterness of the added extract. Although there were no significant differences between juice and supplemented juice, astringency was slightly higher in the latter sample. Adding more than two extracts to the juice fraction may significantly effect astringency and therefore result in a decreased liking of the taste.

Finally, the location of orosensory contributing compounds in bilberry fractions was investigated. Pressed juice was rich in sugars and acids while the majority of the phenolic compounds remained in press residue rich in berry skin fragments. Phenolic compounds were effectively concentrated in the first two ethanol extracts. These extracts were more astringent and bitter than the other

fractions, and some flavonol glycosides, as well as hydroxycinnamic acid derivatives, contributed to these sensory attributes. Future studies would be needed to investigate the effects of other phytochemicals in the extracts that may contribute to astringency and bitterness in bilberries. It was also found that by adding phenolic compounds from the press residue to the juice, the content of these nutritionally significant compounds was increased without altering the overall sensory profile.

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