JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization and Fate of Black Currant and Bilberry Flavonols in Enzyme-Aided Processing

JANI M. KOPONEN,*,† ANU M. HAPPONEN,† SEPPO AURIOLA,‡ HANNA KONTKANEN,§ JOHANNA BUCHERT,§ KAISA S. POUTANEN,†,§ AND A. RIITTA TÖRRÖNEN†

Food and Health Research Centre, Department of Clinical Nutrition, and Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland; and VTT, P.O. Box 1000, FIN-02044 VTT, Finland

The fate of black currant (*Ribes nigrum* L.) and bilberry (*Vaccinium myrtillus* L.) flavonols in enzyme-aided processing was studied. The flavonols were quantified and characterized by high-performance liquid chromatography equipped with a diode array detector and an electrospray ionization mass spectrometer. A tentative identification for 14 black currant and 19 bilberry flavonols is presented representing 11 previously unpublished conjugates. For the first time in any berry, the presence of laricitrin conjugates is reported. The enzyme-aided processing affected the flavonol extractability, elevating the yield in juices and decreasing that in press residues. Importantly, no significant loss of the berry flavonols was observed during the experiments, although some hydrolysis of flavonol conjugates was recorded. To maximize the effect on flavonol extractability, higher enzyme dosages were needed for black currants than for bilberries. The data show that the flavonol extractability and hydrolysis are dependent on the texture of raw material, the glycosylation pattern of the conjugates, and the activity profile of the enzyme preparation.

KEYWORDS: Flavonol; enzyme; pectinase; processing; juice; press residue; black currant; bilberry

INTRODUCTION

Black currants (*Ribes nigrum* L.) and bilberries (*Vaccinium myrtillus* L.) are usually consumed as fresh berries, jams, purees, nectars, and juices. Currently, also berry wines and dried berry products are available, and the interest in using berry-based ingredients in various foods and as nutraceuticals is increasing. Berries are rich in many health-promoting substances, such as vitamin C, soluble fiber, and flavonoids.

Flavonols are one of the main subclasses of flavonoids present in black currant and bilberry (*1*). These compounds possess a typical 2-phenyl-1,4-benzopyrone basic skeleton and a hydroxyl group in the 3-position, and approximately 90% of plant flavonols are hydroxylated in positions 5 and 7 (*2*). The most abundant flavonols found in nature are quercetin, kaempferol, myricetin, and isorhamnetin (**Figure 1**) (*3*), of which quercetin has received much attention for its potential health benefits, such as antiatherosclerotic, anti-inflammatory, anticarcinogenic, antiviral, and antithrombic effects as reviewed by Formica and Regelson (*4*). Quercetin and other flavonols are predominantly found in glycosylated form in foods. Mono-, di-, tri-, and tetrasaccharides consist of moieties of glucose (the most frequent), galactose, rhamnose, arabinose, xylose, and glucuronic acid and are predominately attached in the 3-position, less frequently in the 7-position, and rarely in the positions 5-, 3′-, and 4′ (*3*). The glycosides may also be substituted by an acyl group. The chemical structure, that is, the presence and structure of the sugar and acid moiety, may significantly influence the bioavailability and absorption of flavonols and their conjugates (*5, 6*).

Pectinolytic enzymes are commonly used in industrial berry processing to facilitate juice extraction. These enzymes cause disruption of the cell wall network, enhancing subsequently the juice yield (*7, 8*). Concomitantly with the juice yield the extractability of phenolic components is increased (*9–11*). We have previously shown that the activity profile of commercial pectinolytic enzyme preparations in berry juice processing also has an impact on the structure of extracted anthocyanins (*11*). Glycosidase activities present in the enzyme preparation can hydrolyze anthocyanidin glycosides to the corresponding aglycones, if dosages used are high enough.

Berry flavonols and their fate during the enzyme-aided processing are poorly understood. The objective of this study was to tentatively characterize the black currant and bilberry flavonols and to investigate the impact of enzyme-aided processing on them.

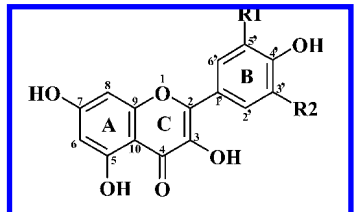
* Address correspondence to this author at the Department of Environmental Health, National Public Health Institute, P.O. Box 95, FIN-70701 Kuopio, Finland (telephone +358 17 201350; fax +358 17 201265; e-mail Jani.Koponen@ktl.fi).

† Department of Clinical Nutrition, University of Kuopio.

‡ Department of Pharmaceutical Chemistry, University of Kuopio.

§ VTT.

10.1021/jf703676m CCC: $40.75
Published on Web 04/22/2008



Name	R1	R2
Kaempferol	H	H
Quercetin	H	OH
Myricetin	OH	OH
Isorhamnetin	H	OCH_3
Laricitrin	OH	OCH_3

Figure 1. Chemical structures of flavonols.

MATERIALS AND METHODS

Samples. Black currants (*R. nigrum* L.) and bilberries (*V. myrtillus* L.) were obtained from Kiantama Ltd. (Suomussalmi, Finland). Berries were harvested in 2002 and kept frozen (–20 °C) until processing.

Chemicals and Standards. Acetonitrile (HPLC grade, >99.8%), methanol (HPLC grade, >99.8%), formic acid (pro analysi, 98–100%), and hydrochloric acid (HCl) were purchased from VWR International Ltd. (Espoo, Finland). Myricetin, quercetin, kaempferol, rutin (quercetin 3-*O*-rhamnosylglucoside), *p*-nitrophenyl β-D-glucuronide, sodium citrate, sodium carbonate, and *p*-nitrophenol were all obtained from Sigma (St. Louis, MO).

Enzyme Preparations and Their Activity Profiles. Four commercial enzyme preparations were used. Econase CE was obtained from AB Enzymes (Rajamäki, Finland). Biopectinase CCM from Quest International Ireland Ltd. (Carrigaline, Ireland), Pectinex Smash XXL from Novozymes (Bagsvaerd, Denmark), and Pectinex BE-3L from Novozymes (Dittinger, Switzerland). The activity profiles of these preparations and the activity assays have been reported elsewhere (*10, 11*), excluding the activity for glucuronidases. α-Glucuronidase activity of the enzyme preparations was measured in 0.05 M sodium citrate buffer, pH 3.5, at 50 °C using 0.25% 2-*O*-(4-*O*-methylglucuronic acid)xylobiose (MeGlcAX2) as substrate (*12*). β-Glucuronidase activity was determined using 1 mM *p*-nitrophenyl β-D-glucuronide in 0.05 M sodium citrate buffer, pH 3.5, as substrate. The activity assay was carried out by incubating 0.02 mL of enzyme preparation with 0.18 mL of substrate solution at 50 °C for 10 min, whereafter the reaction was terminated by the addition of 0.1 mL of 1 M Na_2CO_3. The amount of released *p*-nitrophenol was analyzed spectrophotometrically at 405 nm using external standard calibration.

Enzyme-Aided Processing. Fifty grams of black currants and bilberries was processed in laboratory scale as described in detail by Buchert and co-workers (*10*). Enzyme preparations were dosed at 0, 1, 10, and 100 nkat/g of berries (Econase CE, Biopectinase CCM and Pectinex BE 3-L) or 0, 1, 10, and 20 nkat/g of berries (Pectinex Smash XXL) on the basis of their polygalacturonase (PG) activity. To evaluate the effect of pectin depolymerizing activity on berry processing, we note that Pectinex Smash XXL contains pectin lyase (PL) as the main pectin depolymerizing activity in addition to PG activity. Thus, the dosage used in treatments with Pectinex Smash XXL actually contained a 10-fold higher pectin depolymerizing activity (PG + PL) as compared to the other preparations used at the same PG dosage. After the processing, the juice yield was determined by weighing the pressed juice, which was compared to the initial sample weight. The residual berry mash was considered as the press residue yield. The treatments were carried out in four replicates. Control treatment was carried out correspondingly but omitting the enzymes. The samples were frozen and stored at –20 °C until flavonol analyses.

Quantitative Analysis of Flavonols. Frozen berry, juice, and press residue samples were thawed and weighed for the analysis. For berry (4.8–5.2 g) and press residue (1.2–5.2 g) samples, the extraction of flavonols was performed at room temperature according to a previously published three-step procedure (*13*). The extracts obtained from the berry and press residue was concentrated in a 30 °C water bath using a Laborota 4000 efficient rotary evaporator (Heidolph, Schwabach, Germany) under vacuum (Vacuubrand PC 2001 Vario, Wertheim, Germany). The semidried sample was reconstituted with purified water to give a final volume of 5 mL. For juices, a 4.7 mL sample was weighed for the analysis. After the addition of concentrated HCl (50 μL) to adjust the pH below 1, the sample volume was adjusted to 5 mL with methanol.

Prior to HPLC analysis, the samples were filtered through a 0.45 μm syringe filter (Pall Life Sciences, Ann Arbor, MI). A 25 μL injection of the filtrates was separated on a 250 × 4.6 mm i.d., 5 μm LiChroCart Purospher Star RP-18e column (Merck, Darmstadt, Germany) with a 4 × 4 mm i.d. guard column using a HP 1100 series HPLC (Waldbronn Analytical Division, Waldbronn, Germany) equipped with a quaternary pump, an autosampler, and a diode array detector linked to an HP ChemStation data handling system. The analysis of flavonols was performed using 0.25% formic acid in water as eluent A and HPLC grade acetonitrile/methanol (85:15, v/v) as eluent B with a gradient program as follows: 0–6 min, 16−18% B; 6–9 min, 18% B; 9–14 min, 18−20% B; 14–30 min, 20% B; 30–42 min, 20−26% B; 42–50 min, 26−29% B; 50–52 min, 29−42% B; 52–62 min, 42% B; 62–66 min, 42−54% B; 66–68 min, 54−80% B followed by an isocratic elution for 3 min and then returning to the initial conditions for 5 min before the next injection. The flow rate of the mobile phase was 0.4 mL/min for 0–9 min, followed by a gradual increase to 0.6 mL/min for 9–14 min and 0.6 mL/min for 14–71 min. Flavonols were detected at 360 nm. Quantification was carried out using quercetin 3-*O*-rhamnosylglucoside (rutin) as external standard for all of the detected compounds. The concentrations were expressed in milligrams per kilogram of sample fresh weight, for the weight of the aglycone.

Identification of Flavonols. Flavonols were separated using the same HPLC conditions as described above. For HPLC-DAD-ESI-MS^n analysis the HPLC apparatus was interfaced to a Finnigan LTQ linear ion trap mass spectrometer (Thermo, San Jose, CA). The UV−vis (240–550 nm) and MS spectra were simultaneously detected by splitting the solvent flow after the chromatographic separation onto a DAD and a electrospray ion source (split ratio 5:1). ESI-MS was performed in positive ion mode using a source voltage of 3.8 kV and a temperature of 275 °C, a heated capillary voltage of 33 V, a tube lens voltage of 70 V, and a collision energy of 35% in the MS^2 and MS^3 analyses. Nitrogen was used as both sheath and nebulizer gas. In the MS analysis (full scan), data were collected over a mass range of *m/z* 250–700. The MS^2 and MS^3 spectra were recorded by isolation and degradation of the fragment ions of interest. The UV−vis spectra, retention times, and MS spectra of compounds, as well as literature data (*1, 14–18*) were used for the identification. The identity of aglycone moieties was confirmed by comparing the fragmentation pattern to that of standard compounds when available.

Statistics. Differences between nonenzymatic and enzyme treatments were tested using one-way analysis of variance (ANOVA) with Bonferroni post hoc test. All data were processed by SPSS 14.0 for Windows (SPSS Inc., Chicago, IL). Differences at $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Characterization of Flavonols in Black Currant and Bilberry. Flavonol profiles of black currant and bilberry detected at 360 nm with HPLC-DAD are shown in **Figure 2**. UV−vis spectra of all the numbered peaks showed two absorbance maxima in the ranges of 340–370 and 250–270 nm as illustrated in **Figure 3**. These absorbance maxima are characteristic for flavonols (*19*), and thus these compounds were analyzed in more detail with ESI-MS in the positive ion mode. On the basis of the MS data together with spectrometric characteristics, the black currant and bilberry flavonols were tentatively identified, and the results are summarized in **Table 1**. Although the level of some flavonols was close to the detection limit with the HPLC-DAD, it was clearly high enough for reliable identification with the HPLC-DAD-ESI-MS^n. HPLC coupled with mass spectrometry offers a powerful method for more precise identification than retention times and UV−vis spectra by providing exact information about the molecular weight and typical fragment ion pattern for each aglycone moiety. Furthermore, the coeluting

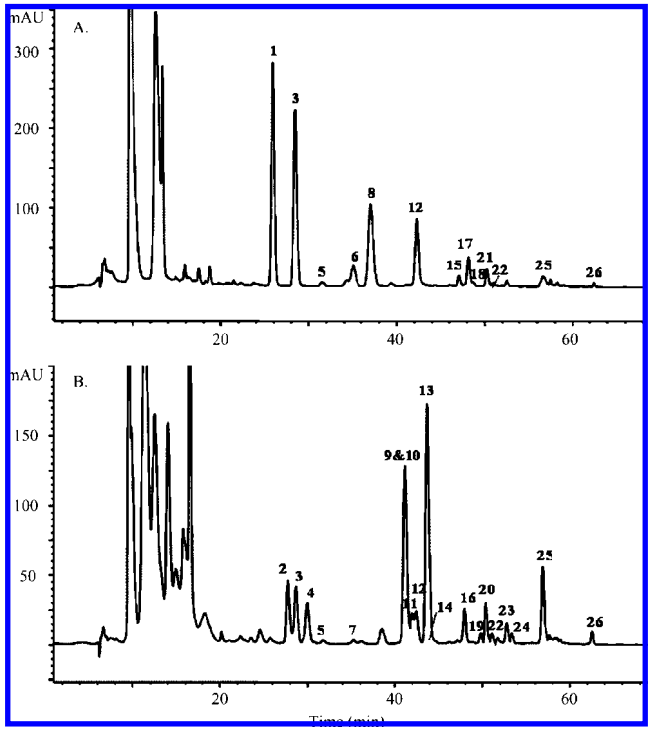


Figure 2. HPLC-DAD chromatograms of flavonol profiles in black currant (**A**) and bilberry (**B**) detected at 360 nm. For identification of the numbered peaks, see **Table 1**.

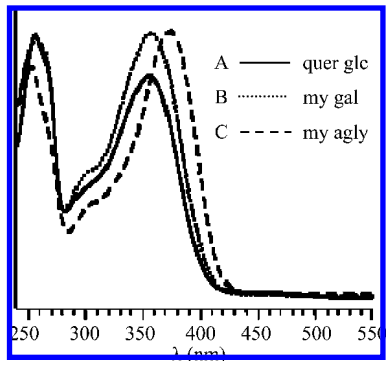


Figure 3. UV−vis spectra of flavonols detected in black currant (A, quercetin glucoside) and bilberry (B, myricetin galactoside; C, myricetin aglycone).

peaks, which may occur in samples with complex flavonol compositions, can be distinguished by means of MS data, if the fragmentation pattern of the molecules differs. However, MS data will not necessarily provide all the information about the conjugates; for example, the structures of sugar and acyl moieties may not be completely identified. Thus, the retention times obtained from standard compounds and literature data (*1, 14–18*) were also needed to confirm the flavonol conjugate identities.

Black Currant. Previously published identification (*1, 14, 15*) for the conjugates of *m/z* 317 myricetin (compounds **1**, **3**, and **6**), *m/z* 303 quercetin (compounds **8**, **12**, and **17**), *m/z* 287 kaempferol (compounds **15** and **21**), and *m/z* 317 isorhamnetin (compound **18**) was confirmed by the present MS data. As a result, to the best of our knowledge, this is the first report of the presence of myricetin pentoside (compound **5**) and isorhamnetin hexoside (compound **22**) in black currant. The mass differences of 132, 162, and 248 amu between the aglycone and the molecular ions were indicative for pentoside, hexoside, and malonylhexoside moieties, respectively, for which the more detailed identity was based on the literature data (*1, 14, 15*). For flavonol rutinosides, a cleavage of glycosidic bonds resulted in the fragment ions of $[M - 146]^+$ for the loss of rhamnose and $[M - 146 - 162]^+$ for the loss of rhamnose and glucose. There is a strong possibility that each aglycone moiety is linked to the same sugar substitute pattern. Thus, compound **22** was tentatively identified as isorhamnetin glucoside. We may also hypothesize on the basis of the presented assumption that malonylglucoside of kaempferol and isorhamnetin are found in black currant, but in extremely low levels. Compound **5** was tentatively identified as myricetin arabinoside due to the same retention time and fragment ion pattern as observed in bilberry for this compound (see below). A bathochromic shift of about 20 nm in higher λ_{max} value (340–370 nm) was observed for compounds **25** and **26** as compared to the other compounds present in the samples (**Figure 3**). This shift can be used as a marker for a nonconjugated aglycone moiety, which was also confirmed by the MS data. In addition to flavonol conjugates, a derivative of aurone (*m/z* 287) was detected and identified with the support of previously reported data (*14–16*). This aureusidin glucoside eluted before myricetin malonylglucoside (retention time of 34.3 min).

Bilberry. In agreement with the previously published identification of bilberry and blueberry flavonols (*1, 17, 18*), conjugates of myricetin (compounds **2**, **3**, and **5**) and quercetin (compounds **9**, **12**, **13**, and **20**) were detected in this study. Interestingly, we found nine unreported bilberry flavonols, which were tentatively identified as conjugates of myricetin (compounds **4** and **7**), quercetin (compound **23**), isorhamnetin (compounds **19**, **22**, and **24**), and laricitrin (compounds **10**, **11**, and **14**). The laricitrin conjugates have been previously found in grapes (*20*), but to the best of our knowledge never in berries. The identification of these compounds was strongly based on the MS^3 fragmentation pattern, which was quite similar to that of isorhamnetin with the exception of a mass difference of 16 amu. Although the molecular ion (*m/z* 495) is the same as for quercetin 3-methoxyhexoside, a compound found in blueberry (*Vaccinium corymbosum* L.) by Cho and co-workers (*17*), the fragment pattern suggests that compounds **10** and **11** are not quercetin conjugates. As noted above, the flavonol hexosides showed a loss of 162 amu from molecular ion resulting in a fragment ion for the corresponding aglycone moiety. On the basis of the elution order and literature data (*17*), the identity of flavonol galactosides and glucosides found in bilberry were assigned. The mass difference of 176 amu between the aglycone and the molecular ions for compounds **4**, **13**, **14**, and **24** were indicative of a glucuronic acid moiety. The flavonol pentosides, compounds **5** and **16**, were tentatively identified as myricetin arabinosides and quercetin xyloside, respectively, on the basis of the previously published data (*17, 18*). From the elution order of pentosides, compound **7** was identified as myricetin xyloside. The MS^3 fragmentation pattern showed that compound **23** was a quercetin conjugate, but the identity of the sugar moiety remained to be studied. Compounds **25** and **26** were identified as myricetin and quercetin aglycones, respectively, on the basis of the UV−vis and MS data.

Flavonol Levels in Berries, Juices, and Press Residues. Black currants and bilberries were processed with the aid of four commercial enzyme preparations (Econase CE, Biopectinase CCM, Pectinex Smash XXL, and Pectinex BE 3-L). For each preparation, four dosage levels based on polygalacturonase activity were used in the treatments. The flavonol content in unprocessed berry and the processed juice and press residue were analyzed with HPLC.

Black Currant. The unprocessed berry contained flavonols at the level of 156 mg/kg, which agreed with the previous

Table 1. Characterization of Black Currant and Bilberry Flavonols by HPLC-DAD-ESI-MS^n Detection[a]

peak	tentative identification (*detected in*)	RT (min)	MS^n analysis (*m*/*z*)		
			$[M + H]^+$	MS^2	MS^3
1	myricetin rutinoside (*black currant*)	25.9	627	609 (64), 591 (33), 523 (67), 481 (100), 463 (32), 319 (28)	301 (39), 273 (100), 245 (31), 165 (35), 153 (28)
2	myricetin galactoside (*bilberry*)	27.6	481	463 (45), 385 (44), 319 (100)	301 (40), 273 (100), 245 (33), 165 (29), 153 (32)
3	myricetin glucoside (*black currant, bilberry*)	28.5	481	463 (25), 319 (100)	301 (43), 273 (100), 245 (29), 165 (36), 153 (31)
4	myricetin glucuronide (*bilberry*)	29.8	495	319 (100)	301 (39), 273 (100), 245 (31), 165 (33), 153 (28)
5	myricetin arabinoside (*black currant, bilberry*)	31.7	451	433 (10), 319 (100)	301 (33), 273 (100), 245 (36), 165 (42), 153 (29)
6	myricetin malonylglucoside (*black currant*)	35.0	567	549 (8), 319 (100)	301 (40), 273 (100), 245 (31), 165 (33), 153 (27)
7	myricetin xyloside (*bilberry*)	35.4	451	433 (17), 319 (100)	301 (52), 273 (100), 245 (36), 165 (33), 153 (26)
8	quercetin rutinoside (*black currant*)	37.1	611	465 (21), 303 (100)	285 (58), 257 (100), 247 (29), 229 (69), 165 (51)
9	quercetin galactoside (*bilberry*)	41.0	465	447 (55), 399 (26), 369 (31), 303 (100)	285 (44), 257 (100), 247 (30), 229 (62), 165 (40)
10	laricitrin galactoside (*bilberry*)	41.3	495	477 (5), 333 (100)	318 (100), 301 (48), 287 (29), 273 (37), 165 (30)
11	laricitrin glucoside (*bilberry*)	41.8	495	333 (100)	318 (100), 301 (37), 287 (22), 273 (25), 165 (30)
12	quercetin glucoside (*black currant, bilberry*)	42.3	465	303 (100)	285 (55), 257 (100), 247 (31), 229 (74), 165 (48)
13	quercetin glucuronide (*bilberry*)	43.5	479	303 (100)	285 (57), 257 (100), 247 (30), 229 (73), 165 (50)
14	laricitrin glucuronide (*bilberry*)	44.1	509	333 (100)	318 (100), 301 (51), 287 (32), 273 (37), 165 (27)
15	kaempferol rutinoside (*black currant*)	47.1	595	449 (28), 287 (100)	287 (47), 241 (100), 231 (39), 213 (77), 165 (91)
16	quercetin xyloside (*bilberry*)	47.8	435	417 (13), 399 (22), 303 (100)	285 (62), 257 (100), 247 (28), 229 (68), 165 (43)
17	quercetin malonylglucoside (*black currant*)	48.2	551	533 (6), 303 (100)	285 (58), 257 (100), 247 (32), 229 (75), 165 (52)
18	isorhamnetin rutinoside (*black currant*)	48.7	625	479 (82), 461 (20), 317 (100)	302 (100), 285 (38), 271 (6), 257 (9), 165 (6)
19	isorhamnetin galactoside (*bilberry*)	49.6	479	317 (100)	302 (100), 285 (35), 271 (7), 257 (9),165 (6)
20	quercetin rhamnoside (*bilberry*)	50.1	449	431 (5), 303 (100)	285 (63), 257 (100), 247 (21), 229 (77), 165 (57)
21	kaempferol glucoside (*black currant*)	50.2	449	287 (100)	287 (46), 241 (100), 231 (26), 213 (63), 165 (72)
22	isorhamnetin glucoside (*black currant, bilberry*)	51.0	479	317 (100)	302 (100), 285 (39), 271 (6), 257 (10), 165 (5)
23	quercetin derivative (*bilberry*)	52.6	493	303 (100)	285 (59), 257 (100), 247 (30), 229 (69), 165 (55)
24	isorhamnetin glucuronide (*bilberry*)	53.2	493	317 (100)	302 (100), 285 (39), 271 (6), 257 (9),165 (6)
25	myricetin (aglycone) (*black currant, bilberry*)	56.7	319	301 (39), 273 (100), 245 (32), 165 (34), 153 (28)	
26	quercetin (aglycone) (*black currant, bilberry*)	62.5	303	285 (57), 257 (100), 247 (33), 229 (72), 165 (52)	

[a] The UV−vis spectrum was monitored at 240–550 nm, and the ESI-MS^n was done using positive ionization mode. The table shows detected ions (*m*/*z*) with their relative intensities in parentheses. The molecular ion in the MS analysis was fragmented to produce a MS/MS spectrum of which the most intense ion (base peak) was further fragmented to produce a MS^3 spectrum.

studies, where a variation from 101 to 158 mg/kg was reported (*1, 14, 15, 21*). The main flavonols were conjugates of myricetin (61% of total) and quercetin (36% of total). In addition, minor levels of kaempferol derivates were detected, whereas the level of isorhamnetin derivative was below the quantification limit (1.5 μg/mL). These findings are in agreement with the previously reported data (*1*).

After processing, the total flavonol concentration (TFC) of juices, including both glycosidic and aglyconic forms of flavonols, varied from 151 to 168 mg/kg (**Table 2**). The TFC in all of the enzyme-treated black currant juices was not significantly changed as compared to the control treatment without enzymes. The TFC in press residues was 150–175 mg/kg, with no significant differences between control and enzyme-aided treatments (with the exception of treatment with the highest dosage of Econase CE in which the TFC was decreased). This indicates that the changes in the juice/press residue yield does not have a diluting/concentrating effect on the TFC; that is, the release of flavonols from unprocessed berry matrix increases with an increased juice yield, supporting the previously published study (*22*).

Bilberry. The TFC in unprocessed bilberry was 164 mg/kg, which was very similar to that in black currant. The previous findings have shown that the level of TFC varies widely from 41 to 111 mg/kg for bilberry (*V. myrtillus* L) (*1, 21*) and from 192 to 401 mg/kg for blueberry (*V. corymbosum* L) (*17, 23*). The majority of flavonols were quercetin derivatives (76% of

Table 2. Total Flavonol Concentration (TFC) in Enzyme-Treated Juices and Press Residues of Black Currant and Bilberry[a]

			TFC			
	enzyme dosage		black currant		bilberry	
treatment	nkat/g[b]	mL/kg[c]	juice	press residue	juice	press residue
control (no enzyme)			151 ± 20	174 ± 10	117 ± 9	239 ± 20
Econase CE	1	0.78	157 ± 3	169 ± 2	112 ± 10	239 ± 8
	10	7.8	161 ± 5	163 ± 8	120 ± 8	226 ± 5
	100	78	164 ± 7	150 ± 7*	143 ± 6**	239 ± 10
Biopectinase CCM	1	0.03	161 ± 5	175 ± 10	116 ± 5	248 ± 6
	10	0.27	161 ± 5	166 ± 10	135 ± 10	243 ± 20
	100	2.7	165 ± 5	168 ± 3	133 ± 9	235 ± 10
Pectinex Smash XXL	1	4.6	163 ± 5	170 ± 10	126 ± 7	240 ± 10
	10	46	168 ± 4	159 ± 10	120 ± 6	212 ± 7
	20	92	166 ± 10	155 ± 10	131 ± 10	214 ± 10
Pectinex BE 3-L	1	0.08	159 ± 2	171 ± 6	124 ± 4	255 ± 9
	10	0.84	163 ± 7	170 ± 8	132 ± 5	256 ± 8
	100	8.4	163 ± 5	162 ± 7	128 ± 9	309 ± 3***

[a] Values are mean ± standard deviation of four experiments and expressed as quercetin aglycone equivalent mg/kg of juice or press residue. Significant differences between control and enzymatic treatments are indicated as *, $p < 0.05$; **, $p < 0.01$; or ***, $p < 0.001$. [b] Polygalacturonase activity in enzyme preparation nkat/g of fresh berries. [c] Enzyme preparation mL/kg of fresh berries.

total), followed by myricetin (18%) and a minor level of isorhamnetin derivatives. No detectable levels of kaempferol conjugates were found in bilberry.

After the bilberry processing, the TFC in bilberry juices varied from 112 to 143 mg/kg and in press residues from 212 to 309 mg/kg (**Table 2**). The difference in TFCs between bilberry and black currant juices and press residues can mostly be explained by the differences in juice/press residue yields. The juice yields, which are reported in detail elsewhere (*11*), varied from 47 to 65% and from 60 to 75% in black currant and bilberry treatments, respectively. Furthermore, the flavonol recoveries from unprocessed berries into the juice and press residue, as shown in detail below, were the same for both berries. This indicates that the same level of flavonols was extracted into a higher volume of bilberry juice as compared to black currant juice, resulting in the different TFC levels in juices and press residues. Some differences in the TFCs may also result in the differences in the flavonol structures. This effect of flavonol structure on the extractability is discussed later this paper.

It is worth noting that the flavonol level in unprocessed bilberry and in processed press residue is actually slightly higher than that we report above. The quantity of myricetin 3-glucoside (peak 2 in **Figure 2**) could not be determined due to an overlapping peak with the present HPLC-DAD method. This overlapping peak was not present in processed juices, resulting in a better quantification for TFC in juices (in which myricetin 3-glucoside comprised 6–8% of total flavonols) than for that in berry and press residues. In most of the experiments the TFC found in juices and press residues was not significantly changed by enzymatic processing as compared to control treatment.

Effect of Enzyme-Aided Processing on Total Flavonol Yield and Profile. The TFC alone is insufficient to evaluate the effectiveness of enzymatic processing. Thus, the total flavonol yield (TFY) was used to describe the effectiveness of enzymatic processing on berry flavonols. The TFYs for juice and press residue, that is, flavonol recovery from berry into the juice and press residue, were calculated according to the following equation:

$$\text{TFY (mg/kg)} = \text{TFC (mg/kg)} \times \text{juice or press residue yield (\%)}/100$$

To make the following results comparable between berry, press residue, and juice, the concentration and yield of bilberry flavonols are presented by omitting the quantity of myricetin 3-glucoside (see above). The black currant and bilberry juice yields are reported in detail elsewhere (*11*), and the press residue yields are calculated on the basis of those results.

As illustrated in **Figure 4**, all of the flavonols present in black currant or bilberry were recovered in juice or press residue, and no significant loss of the berry flavonols was observed during the processing with the exception of treatment of bilberries with the two highest dosages of Pectinex Smash XXL. The reason for this disappearance of flavonols in the Pectinex Smash XXL treatment is not known. Pectinex Smash XXL is known to contain pectin lyase as key activity, and by dosing 10 nkat/g of PG, also 89 nkat/g of pectin lyase is simultaneously dosed (*11*). Pectin lyase depolymerizes pectin chains by β-elimination, resulting in a double bond to the reaction product (*8*). The minor flavonol losses in bilberry treatments may be explained by the fact that the enzyme-aided processing affects the chemical nature of berry matrix and favors an interaction between flavonols and other substances, such as proteins (*24*), other phenolics, or pectin lyase catalyzed reaction products. This results in the formation of new flavonol complexes, which decreases the quantity of complex-free flavonol compounds.

A ratio of the TFY in juice versus the TFY in press residue describes the flavonol extractability between juice and press residue. This ratio was 0.8 for black currant and 0.7 for bilberry in control treatments, indicating that more of the flavonols remained in press residue than were extracted into the juice. However, this ratio was clearly affected by enzyme treatments, which resulted in a ratio of up to 1.8 for both berries (corresponding to TFY 64% in juice and 36% in press residue). Overall, this indicates that the berry cell wall structure is disrupted by depolymerizing enzymes facilitating the flavonol extraction into the juice. However, a notable level of flavonols remains in press residues, indicating that the residues are concentrated with rather resistant polymer structures, into which the flavonols and other phenolics are imbedded.

Previous studies have shown that the yield of total phenol and total anthocyanin tends to increase in berry juices in a dosage-dependent manner in enzyme-aided juice production (*9, 11, 22*). Moreover, we have reported (*11*) that the extractability and the hydrolysis of different anthocyanidin glycosides

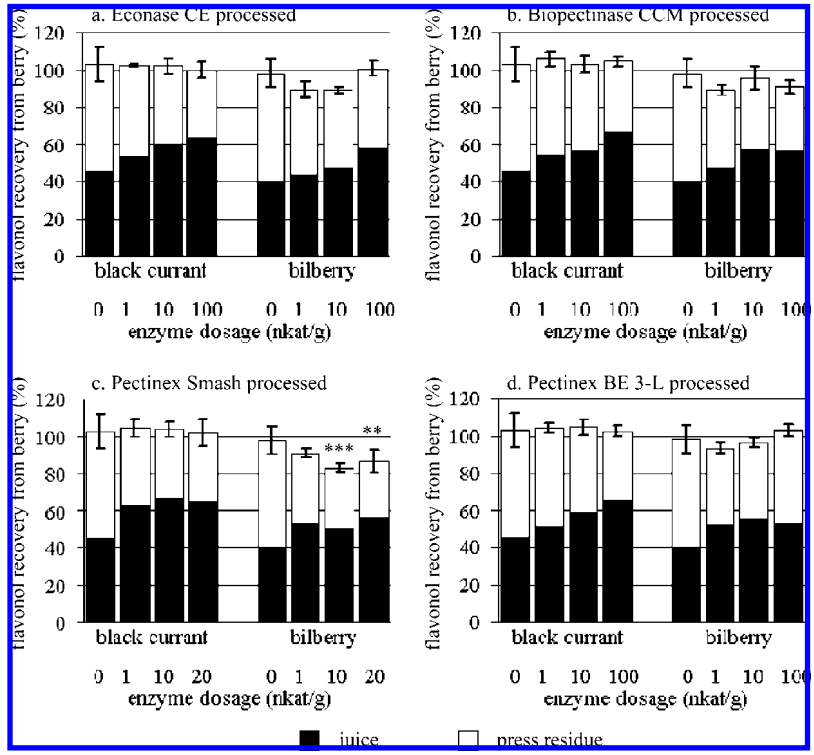


Figure 4. Effect of enzyme-aided processing on black currant and bilberry flavonols. The significant differences between unprocessed berry (100%) and enzymatic treatments are indicated as **, $p < 0.01$, and ***, $p < 0.001$.

Table 3. Flavonol Yield in Enzyme-Treated Black Currant Juices[a]

		flavonol yield		
treatment	enzyme dosage[b]	rutinosides	glucosides	other[c]
control (no enzyme)		37 ± 3.2 a	27 ± 3.0 a	6.6 ± 1.0 a
Econase CE	1	43 ± 1.4 ab	32 ± 1.4 ab	8.8 ± 0.4 b
	10	47 ± 2.3 bce	36 ± 2.8 bcef	11 ± 0.6 cef
	100	49 ± 2.2 be	37 ± 2.2 bg	9.3 ± 0.6 f
Biopectinase CCM	1	43 ± 0.9 ab	32 ± 0.9 abd	9.3 ± 0.5 bd
	10	45 ± 4.4 bcd	33 ± 3.4 bcd	11 ± 0.6 deg
	100	53 ± 2.0 e	41 ± 1.5 g	11 ± 0.4 ef
Pectinex Smash XXL	1	51 ± 1.6 de	38 ± 1.2 deg	10 ± 0.4 de
	10	53 ± 2.3 e	40 ± 1.1 fg	11 ± 0.4 ef
	20	51 ± 3.3 de	40 ± 2.1 eg	12 ± 0.9 fg
Pectinex BE 3-L	1	41 ± 2.2 ac	30 ± 1.6 ac	9.5 ± 0.2 bcd
	10	47 ± 4.0 bce	35 ± 3.4 bce	11 ± 0.2 cef
	100	51 ± 2.2 de	41 ± 1.3 g	10 ± 0.4 bde

[a] Values are mean ± standard deviation of four experiments and expressed as quercetin aglycone equivalent mg/kg of fresh berry. Values sharing the same letter in columns were not significantly different ($p > 0.05$). [b] Polygalacturonase activity in enzyme preparation nkat/g of fresh berry. [c] This group contains all the other flavonols detected in the samples, excluding those presented by name in this table.

are different and dependent on the dosage and activity profile of enzyme preparation. As a result, the glycosidic profile of anthocyanidins in produced juices is dependent on the type of enzyme used. Therefore, the dosage dependence of flavonol extraction and the profile of processed products were investigated in detail in the current study.

Black Currant. The yields and profiles of flavonols present in juices and press residues are summarized in **Tables 3** and **4**. Only the yields of the main glycoside groups (flavonol rutinosides and glucosides) are shown in detail, because the relative level of other glycoside groups was <5%. The flavonol yield significantly increased in juices and subsequently decreased in press residues treated with a pectin depolymerizing enzyme dosage of ≥10 nkat/g as compared to nonenzymatic treatment. Furthermore, the yield obtained from the treatments with the moderate (10 nkat/g) and the highest enzyme dosage was at the same level. This indicates that an optimal depolymerizing dosage for improving the flavonol extraction in juices and subsequently decreasing in press residues is >10 nkat/g, but not necessarily as high as 100 nkat/g. The optimal pectin depolymerizing dosage for anthocyanin extraction from black currant has been reported to be near 100 nkat/g (*11*). This may indicate that the flavonols are bound differently from the anthocyanins, that is, interaction with other substances or location in berry cell wall matrix are different. Moreover, we note that the action of pectin lyase is as effective as that of polygalacturonase in facilitating the extraction of berry flavonols. The treatments

Table 4. Flavonol Yield in Enzyme-Treated Black Currant Press Residues[a]

treatment	enzyme dosage	flavonol yield		
		rutinosides	glucosides	other
control (no enzyme)		40 ± 4.7 a	39 ± 4.9 a	11 ± 1.0 ab
Econase CE	1	33 ± 1.4 bcd	33 ± 1.3 bc	11 ± 1.7 b
	10	29 ± 1.1 cf	28 ± 1.6 cd	8.5 ± 0.4 bd
	100	26 ± 1.5 ef	24 ± 0.9 d	7.7 ± 0.9 ad
Biopectinase CCM	1	36 ± 1.3 ac	35 ± 1.9 ab	9.7 ± 2.2 bd
	10	32 ± 5.6 bce	30 ± 4.3 bd	10 ± 3.1 ab
	100	25 ± 0.6 f	25 ± 0.2 d	7.7 ± 0.2 ad
Pectinex Smash XXL	1	28 ± 2.0 def	29 ± 2.1 cd	8.0 ± 1.0 bd
	10	25 ± 1.2 f	25 ± 1.5 d	6.9 ± 0.6 cd
	20	25 ± 2.5 f	24 ± 2.3 d	6.7 ± 1.2 d
Pectinex BE 3-L	1	36 ± 2.2 ab	35 ± 2.8 ab	11 ± 0.4 b
	10	31 ± 2.4 bcf	30 ± 1.4 bd	9.8 ± 0.6 abc
	100	25 ± 1.5 f	25 ± 2.0 d	7.2 ± 0.3 cd

[a] See **Table 3** footnotes.

Table 5. Yield of Flavonol Conjugates and Aglycones in Enzyme-Treated Bilberry Juices[a]

treatment	enzyme dosage	flavonol yield			
		galactosides	glucuronides	aglycones	other
control (no enzyme)		21 ± 1.8 a	26 ± 2.4 a	6.3 ± 1.0 a	13 ± 1.1 a
Econase CE	1	23 ± 1.4 ab	27 ± 3.6 ab	6.1 ± 1.1 a	15 ± 1.2 ab
	10	25 ± 1.9 ac	29 ± 2.4 ac	7.6 ± 0.9 a	16 ± 1.6 acd
	100	28 ± 1.7 bcd	36 ± 2.4 e	13 ± 0.4 bd	18 ± 0.7 cd
Biopectinase CCM	1	25 ± 0.9 ac	30 ± 1.9 ac	7.6 ± 0.9 a	16 ± 0.4 ac
	10	29 ± 1.8 ce	34 ± 3.0 cde	12 ± 1.0 bc	19 ± 1.0 cd
	100	23 ± 2.1 ab	29 ± 1.3 abd	22 ± 1.4 e	19 ± 1.5 d
Pectinex Smash XXL	1	27 ± 1.8 bcd	34 ± 1.6 bce	8.4 ± 0.5 a	18 ± 0.9 bcd
	10	27 ± 0.6 bcd	31 ± 2.2 ae	7.6 ± 0.5 a	17 ± 0.8 bcd
	20	30 ± 3.7 de	35 ± 3.0 ce	8.7 ± 0.6 ac	18 ± 1.6 cd
Pectinex BE 3-L	1	26 ± 0.9 bcd	32 ± 1.5 bce	9.0 ± 0.6 ac	18 ± 0.8 bcd
	10	26 ± 1.1 bcd	31 ± 2.3 ae	15 ± 0.6 d	19 ± 1.2 cd
	100	16 ± 0.7 f	13 ± 1.3 f	40 ± 3.3 f	18 ± 2.0 cd

[a] See **Table 3** footnotes.

with the PL dosage of 9 nkat/g and PG dosage of 1 nkat/g (the lowest dosage of Pectinex Smash XXL) resulted in the same flavonol yield as the treatments with the PG dosages of 10 nkat/g.

The individual flavonols were not similarly extracted, resulting in changes in flavonol profiles of juices and press residues as compared to unprocessed berry. The relative extractability of rutinosides was higher than that of glucosides, which changed the relative level of these glycosides as compared to unprocessed berry. This supports our previously reported theory (*11*) that the sugar moiety influences the extractability of flavonoids, suggesting that some glycosides are more tightly bound to the berry matrix than others or that they have differences in solubility. If the glycosides are tightly bound to the berry matrix, higher enzyme dosages or completely different preparations with different profile may be needed to obtain the maximal flavonol recovery into the juice.

Bilberry. Compared to the control treatment, the TFY in enzymatically processed juices increased significantly when a pectin depolymerizing dosage of >1 nkat/g was used, with a concomitant decrease in press residue already with the lowest enzyme dosage. Surprisingly, no dosage dependence was observed within the same preparation, and only with few exceptions between the preparations. This implies that an optimal dosage is >1 nkat/g, but not >10 nkat/g. Thus, the dosage response for bilberries differed from that for black currants. Black currant cell walls are known to be thicker and to contain more pectins than those of bilberry (*25*), which may explain the lower need for the pectin depolymerizing activity. As for the black currants (above), the optimal dosage for maximal flavonol yield in bilberry juices was shown to be lower than for maximal anthocyanin yield, for which the optimal depolymerizing activity dosage was suggested to be between 10 and 100 nkat/g (*11*).

Although the TFY in most of the enzyme-aided treatments was not affected by the dosage or the origin of preparation, the flavonol profile detected in juices and press residues was dramatically changed in some of the treatments as compared to unprocessed berry or control. **Tables 5** and **6** illustrate clearly a hydrolysis of flavonol conjugates in treatments with moderate or the highest dosages of Biopectinase CCM and Pectinex BE 3-L. This results in a decreased yield of flavonol galactosides and glucuronides and an increased yield of aglycones as compared to the presumed levels. These observations are highly linked to the glycosidase side activities present in enzyme preparations. The galactosidase and glucuronidase dosages (nanokatals per gram) in the treatments with the highest pectin

Table 6. Yield of Flavonol Conjugates and Aglycones in Enzyme-Treated Bilberry Press Residues[a]

treatment	enzyme dosage	flavonol yield			
		galactosides	glucuronides	aglycones	other
control (no enzyme)		21 ± 1.5 a	19 ± 3.1 a	22 ± 5.5 ab	34 ± 5.3 a
Econase CE	1	16 ± 1.1 b	12 ± 1.7 bd	18 ± 1.2 ac	30 ± 1.2 ab
	10	15 ± 1.0 bc	11 ± 1.2 be	16 ± 2.0 cd	26 ± 2.7 bc
	100	16 ± 1.0 b	18 ± 1.6 ac	16 ± 0.9 cd	20 ± 0.3 de
Biopectinase CCM	1	15 ± 0.5 bc	15 ± 1.2 ab	16 ± 0.3 ac	23 ± 1.0 cd
	10	15 ± 1.0 b	15 ± 1.4 ab	14 ± 1.9 ce	19 ± 0.8 df
	100	11 ± 0.5 de	9.4 ± 0.9 de	20 ± 1.9 abd	15 ± 2.0 ef
Pectinex Smash XXL	1	15 ± 1.1 bc	13 ± 0.5 bcd	14 ± 1.4 ce	20 ± 1.8 cde
	10	14 ± 0.8 bc	12 ± 1.9 bd	10 ± 0.7 ef	18 ± 1.7 df
	20	15 ± 1.3 bc	14 ± 0.9 bc	7.8 ± 1.0 f	14 ± 1.7 f
Pectinex BE 3-L	1	15 ± 0.7 b	15 ± 2.2 ab	17 ± 1.0 ac	21 ± 2.5 cd
	10	13 ± 1.0 cd	11 ± 2.5 be	24 ± 1.7 b	20 ± 2.8 de
	100	10 ± 0.3 e	6.8 ± 0.9 e	53 ± 2.4 g	13 ± 0.6 f

[a] See **Table 3** footnotes.

Table 7. β-Galactosidase, β-Glucuronidase, and α-Glucuronidase Dosages in Treatments with the Highest Pectin Depolymerizing Dosage[a]

enzyme preparation	dosage (nkat/g)		
	β-galactosidase[b]	β-glucuronidase	α-glucuronidase
Econase CE	5	–	–
Biopectinase CCM	2	0.05	0.004
Pectinex Smash XXL	–	–	–
Pectinex BE 3-L	23	0.2	0.3

[a] The PG dosage for Econase CE, Biopectinase CCM, and Pectinex BE 3-L was 100 nkat/g, and that for Pectinex Smash XXL was 20 nkat/g. –, activity not detected. [b] Data adopted from our previous study (*10*).

depolymerizing dosages are shown in **Table 7**. The present results support the previously reported fact (*11, 26*) that the β-galactosidase dosage of 2 nkat/g is high enough to significantly hydrolyze the galactose-substituted flavonoids to their aglycone and sugar moieties. However, it is interesting to note that the glucuronides are effectively hydrolyzed by an extremely low level of glucuronidase. Similar levels of both α- and β-glucuronidase activities are present in Biopectinase CCM and Pectinex BE 3-L treatments, which implies that the bilberry glucuronides can be either α or β anomers. The exact stereoisomerism remains to be studied. Moreover, the results show that in Econase CE treatments the galactosidase dosage of 5 nkat/g is insufficient to hydrolyze flavonol galactosides. This indicates that the glycoside moieties are not equally accessible to the action of the glycosidases, and the mode of action depends on the microbial origin of the enzyme preparation, which is supported by previous findings (*11, 27*).

In addition, the bilberry flavonol profile was changed by the fact that the flavonol glucuronides were more easily extracted into the juice than the other glycosides, and the aglycones favored the press residue over the juice. Moreover, the association of flavonols with proteins (*24*) and presumably with other reactive compounds is better for aglycones than for glycosides, which can affect both the flavonol level and profile in processed samples.

In conclusion, we note that the enzyme-aided black currant and bilberry processing facilitates significantly the extraction of flavonols from berry matrix, resulting in the increased TFY in juices and decreased TFY in press residues. No significant loss of the berry flavonols was observed during the experiments, although some hydrolysis of flavonol glycosides was observed. The maximal flavonol recoveries in pressed juice were 64 and 65% for enzymatically processed black currant and bilberry, respectively, which were clearly higher than those obtained for enzyme-aided processing of blueberry (53%) and bayberry (19%) (*23, 28*). To optimize the pectin depolymerizing dosage used in berry processing, we suggest the dosage of 10 nkat/g of berry mash as a proper starting point. However, the required dosage is highly dependent on the texture of the raw material and the functional properties of extracted compounds. In the present treatments, the optimal dosage for black currant flavonols was between 10 and 100 nkat/g, whereas the dosage of <10 nkat/g was sufficient for bilberry flavonols. For an industrial point of view it is presumed that the following processing steps of fining, filtering, and thermal treatments will cause a decrease in flavonol recovery, but evidently not during the enzyme treatment step. However, the hydrolysis of flavonols may occur during the enzyme treatment, but this is dependent on both the flavonol structures present in the berry and the activity profile of the enzyme preparation. The aspects related to the storage stability of the flavonol glycosides and aglycones in the enzymatically processed juices remain to be studied.

ABBREVIATIONS USED

HCl, hydrochloric acid; PG, polygalacturonase; PL, pectin lyase; TFC, total flavonol concentration; TFY, total flavonol yield; TPC, total polyphenol concentration.

LITERATURE CITED

(1) Määttä-Riihinen, K. R.; Kamal-Eldin, A.; Mattila, P. H.; González-Paramás, A. M.; Törrönen, A. R. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *J. Agric. Food Chem.* **2004**, *52*, 4477–4485.

(2) Macheix, J.-J.; Fleuriet, A.; Billot, J. Flavonoids. In *Fruit Phenolics*; Macheix, J.-J., Fleuriet, A., Billot, J., Eds.; CRC Press: Boca Raton, FL, 1999; pp 39–80.

(3) Hollman, P. C. H.; Arts, I. C. W. Flavonols, flavones and flavanols—nature, occurrence and dietary burden. *J. Sci. Food Agric.* **2000**, *80*, 1081–1093.

(4) Formica, J. V.; Regelson, W. Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.* **1995**, *33*, 1061–1080.

(5) Hollman, P. C.; de Vries, J. H.; van Leeuwen, S. D.; Mengelers, M. J.; Katan, M. B. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *Am. J. Clin. Nutr.* **1995**, *62*, 1276–1282.
(6) Hollman, P. C.; Buijsman, M. N.; van Gameren, Y.; Cnossen, E. P.; de Vries, J. H.; Katan, M. B. The sugar moeity is a major determinant of the absorption of dietary flavonoid glycosides in man. *Free Radical Res.* **1999**, *31*, 569–573.
(7) Pilnik, W.; Voragen, A. G. The significance of endogenous and exogenous pectic enzymes in fruit and vegetable processing. In *Food Enzymology*; Fox, P. F., Ed.; Elsevier Applied Science: London, U.K., 1991; Vol. 1, pp 303–336.
(8) Voragen, A. G.; Pilnik, W.; Thibault, J.-F.; Axelos, M. A.; Renard, C. M. Pectins. In *Food Polysaccharides and Their Applications*; Stehen, A. M., Ed.; Dekker; New York, 1995; pp 287–339.
(9) Landbo, A.-K.; Meyer, A. Effects of different enzymatic treatments on enhancement of anthocyanins and other phenolics in black currant juice. *Innovative Food Sci. Emerging Technol.* **2004**, *5*, 303–313.
(10) Buchert, J.; Koponen, J. M.; Suutarinen, M.; Mustranta, A.; Lille, M.; Törrönen, R.; Poutanen, K. Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices. *J. Sci. Food Agric.* **2005**, *85*, 2548–2556.
(11) Koponen, J. M.; Buchert, J.; Poutanen, K. S.; Törrönen, A. R. Effect of pectinolytic juice production on the extractability and fate of bilberry and black currant anthocyanins. *Eur. Food Res. Technol.* **2008**, in press.
(12) Siika-aho, M.; Tenkanen, M.; Buchert, J.; Puls, J.; Viikari, L. An α-glucuronidase from *Trichoderma reesei* RUT C-30. *Enzyme Microb. Technol.* **1994**, *16*, 813–819.
(13) Koponen, J. M.; Happonen, A. M.; Mattila, P. H.; Törrönen, A. R. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. *J. Agric. Food Chem.* **2007**, *55*, 1612–1619.
(14) Anttonen, M. J.; Karjalainen, R. O. High-performance liquid chromatography analysis of black currant (*Ribes nigrum* L.) fruit phenolics grown either conventionally or organically. *J. Agric. Food Chem.* **2006**, *54*, 7530–7538.
(15) Määttä-Riihinen, K. R.; Kamal-Eldin, A.; Törrönen, A. R. High-performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: *Ribes* species. *J. Agric. Food Chem.* **2003**, *51*, 6736–6744.
(16) Lu, Y.; Foo, L. Y. Polyphenolic constituents of blackcurrant seed residue. *Food Chem.* **2003**, *80*, 71–76.
(17) Cho, M. J.; Howard, L. R.; Prior, R. L.; Clark, J. R. Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry. *J. Sci. Food Agric.* **2005**, *85*, 2149–2158.
(18) Zheng, W.; Wang, S. Y. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J. Agric. Food Chem.* **2003**, *51*, 502–509.
(19) Schieber, A.; Berardini, N.; Carle, R. Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. cv. 'Tommy Atkins') peels by high performance liquid chromatography-electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 5006–5011.
(20) Mattivi, F.; Guzzon, R.; Vrhovsek, U.; Stefanini, M.; Velasco, R. Metabolite profiling of grape: flavonols and anthocyanins. *J. Agric. Food Chem.* **2006**, *54*, 7692–7702.
(21) Häkkinen, S. H.; Kärenlampi, S. O.; Mykkänen, H. M.; Törrönen, A. R. Influence of domestic processing and storage on flavonol contents in berries. *J. Agric. Food Chem.* **2000**, *48*, 2960–2965.
(22) Bagger-Jørgensen, R.; Meyer, A. S. Effects of different enzymatic pre-press maceration treatments on the release of phenols into blackcurrant juice. *Eur. Food Res. Technol.* **2004**, *219*, 620–629.
(23) Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *J. Food Sci.* **2000**, *65*, 357–364.
(24) Laborde, B.; Moine-Ledoux, V.; Richard, T.; Saucier, C.; Dubourdieu, D.; Monti, J. P. PVPP−polyphenol complexes: a molecular approach. *J. Agric. Food Chem.* **2006**, *54*, 4383–4389.
(25) Hilz, H.; Bakx, E.; Schols, H.; Voragen, A. Cell wall polysaccharides in black currants and bilberries—characterisation in berries, juice, and press cake. *Carbohydr. Polym.* **2005**, *59*, 477–488.
(26) Wightman, J. D.; Wrolstad, R. E. Anthocyanin analysis as a measure of glycosidase activity in enzymes for juice processing. *J. Food Sci.* **1995**, *60*, 862–867.
(27) Mandalari, G.; Bennett, R. N.; Kirby, A. R.; Lo Curto, R. B.; Bisignano, G.; Waldron, K. W.; Faulds, C. B. Enzymatic hydrolysis of flavonoids and pectic oligosaccharides from bergamot (*Citrus bergamia* Risso) peel. *J. Agric. Food Chem.* **2006**, *54*, 8307–8313.
(28) Fang, Z.; Zhang, M.; Sun, Y.; Sun, J. How to improve bayberry (*Myrica rubra* Sieb. et Zucc.) juice color quality: effect of juice processing on bayberry anthocyanins and polyphenolics. *J. Agric. Food Chem.* **2006**, *54*, 99–106.

Received for review December 17, 2007. Revised manuscript received February 5, 2008. Accepted February 11, 2008. This study has been carried out with financial support from the Commission of the European Communities, specific RTD program "Quality of Life and Management of Living Resources", contract QLRT-CT-2002-02364 "Novel enzyme-aided extraction technologies for maximized yield and functionality of bioactive components in consumer products and ingredients from by-products", acronym MAXFUN. It does not reflect the Commission's views and in no way anticipates its future policy in this area.

JF703676M