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Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices

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Abstract: Bilberries (*Vaccinium myrtillus*) and blackcurrants (*Ribes nigrum*) were treated with extensive dosages of commercial cell wall degrading enzyme preparations, i.e. Econase CE, Pectinex Ultra SP-L, Pectinex Smash, Pectinex BE 3-L and Biopectinase CCM. The enzymes were dosed based on the polygalacturonase activity. The juice yield was improved in both berries as a result of the enzymatic treatment. The improvement was more pronounced with blackcurrants owing to their thicker cell walls. The impact of the enzymatic treatment on anthocyanins present in the juices was investigated using HPLC-DAD. The enzyme preparations affected the contents and composition of anthocyanins in the juices. Pectinex Ultra SP-L, Pectinex Smash, Pectinex BE 3-L and Biopectinase CCM increased the total content of anthocyanins by 13–41% in the bilberry juices and by 18–29% in the blackcurrant juices. Econase CE, however, produced a dramatic decrease in the total anthocyanin content in the bilberry juice due to its enzyme profile, whereas no such effect was observed with the blackcurrant juice. All the enzyme mixtures tested produced a total or extensive loss of anthocyanidin galactosides in bilberry juice. Commercial enzyme preparations used in the production of berry juices can improve extraction of anthocyanins into the juice. However, they may effectively hydrolyse certain glycosides and thus affect the profile of extracted anthocyanins.

Keywords: bilberry; blackcurrant; enzyme; juice; anthocyanins; phenolics

INTRODUCTION

Bilberries (wild European blueberries) (*Vaccinium myrtillus* L.) and blackcurrants (*Ribes nigrum* L. cv. Öjebyn) are used for juice manufacture, especially in Scandinavia. Bilberries and blackcurrants are rich in anthocyanins, which are a large and important group of flavonoid compounds. Anthocyanins are mostly responsible for the red and blue colours of these berries. Bilberry and blackcurrant anthocyanins are glycosides containing glucose, galactose, arabinose or rutinose as the sugar moiety linked to the aglycon (anthocyanidin). Blackcurrants contain mainly cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, delphinidin 3-*O*-glucoside and delphinidin 3-*O*-rutinoside.[1,2] In bilberries anthocyanins are composed of 3-*O*-galactosides, 3-*O*-glucosides and 3-*O*-arabinosides of delphinidin, cyanidin, petunidin, peonidin and malvidin.[3,4]

Anthocyanidins and their glycosidic forms are strong antioxidants *in vitro* mainly owing to their phenolic hydroxyl groups.[5–9] They may also have anticarcinogenic potential.[10] In mice, prevention of obesity and amelioration of hyperglycaemia was observed after feeding with an anthocyanin-rich diet.[11] Anthocyanins are absorbed to some extent both by humans and animals, and are found as intact glycosides in the blood and urine.[12–17] The chemical structure, ie the nature of the sugar conjugate and the phenolic aglycon, is known to affect bioavailability and to have an impact on the anthocyanin absorption and excretion.[15–17]

During juice manufacture the berries are crushed and pressed, whereafter the juice is separated for further use. Pectinolytic enzymes are currently used in industrial berry processing to facilitate juice extraction. With these enzymes, the cell wall network is disrupted and consequently the juice yield is enhanced.[18,19] Furthermore, enzymatic treatment is known to enhance the extractability of phenolic components from the cell wall matrix.[20] Enzyme manufacturers provide various types of enzyme mixtures for berry processing. Most of these enzyme preparations contain

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endo-acting pectinases, cellulases and hemicellulases in various ratios. In addition to these enzymes, exo-acting enzymes are also present in the enzyme mixtures, potentially affecting the chemistry of the phenolic glycosides extracted.[21] Wrolstad *et al*[22] and Wightman and Wrolstad[23] have screened various commercial pectinase preparations for their effect on cranberry juice anthocyanin hydrolysis. Arabinoside pigments were not degraded whereas galactosides were degraded by 2–100% depending on the enzyme used. The impact of β-glucosidase activity on boysenberry and raspberry anthocyanins has also been verified.[23,24]

In this work, the effect of extensive dosages of various commercial cell wall degrading enzyme preparations on the pressability of juice from bilberries and blackcurrants was investigated with special emphasis on improving the yield and maximizing the extraction of anthocyanins into the juice.

MATERIALS AND METHODS

Raw materials

Bilberries (*Vaccinium myrtillus* L.) were obtained from Kiantama Ltd, Suomussalmi, northern Finland. Blackcurrants (*Ribes nigrum* L. cv. Öjebyn) were obtained from Peltohermanni Ltd, Ilomantsi, eastern Finland. Both berries were of 2002 harvest. Prior to the experiments, they were stored frozen at −23 °C for 3 months.

Enzymes and enzyme activities

Various enzyme preparations obtained from commercial suppliers were used (Table 1). The enzymes used were Econase CE (supplied by AB Enzymes, Rajamäki, Finland), Pectinex Smash (supplied by Novozymes, Dittinger, Switzerland), Pectinex BE-3L (supplied by Novozymes, Dittinger, Switzerland), Pectinex Ultra SP-L (supplied by Novozymes, Bagsvaerd, Denmark) and Biopectinase CCM (supplied by Quest International Ireland Ltd, Carrigaline, Ireland). The activity profiles of the enzyme preparations were determined at pH 3.5 using standard assays. The overall cellulolytic activity was measured with filter paper as substrate. The activity was expressed as filter paper units (FPU).[25] Endoglucanase, mannanase, xylanase and β-glucanase activities of the enzyme preparations were analysed using hydroxyethylcellulose,[25] locust bean gum,[26] birchwood glucuronoxylan[27] and β-glucan[28] as substrate, respectively. Endopolygalacturonase, pectin lyase and β-glucosidase activities were measured using polygalacturonic acid,[29] citrus pectin[30] and 4-nitrophenyl-β-D-glucopyranoside[31] as substrate, respectively. Pectin methylesterase was assayed by titration of the liberated carboxyl groups of citrus pectin using an automatic titrator.[32] β-Galactosidase activity was measured using *o*-nitrophenyl-β-D-galactopyranoside as substrate.[31] α-Arabinosidase activity was analysed according to Poutanen *et al*[33] by using *p*-nitrophenyl-α-L-arabinofuranoside as substrate.

Juice pressing

Frozen berries (60 g) were separated from stalks and leaves, etc. Bilberries and blackcurrants were thawed for 20 and 35 min, respectively. After thawing, the berries were mashed for 8 s (16 s for blackcurrants) in a multifunctional chopper (Multitrio, Moulinex, Ireland). Fifty grams of the mashed berries were weighed into a 0.5 L plastic bag and kept in a 45 °C water bath until the temperature of the mash reached 45 °C. Enzymes were dosed based on endopolygalacturonase activity corresponding to 1000 nkat g^{-1} in the treatment. Each enzyme was diluted with distilled water to the constant volume of 10 mL before adding to the mashed berries, with the exception of the Econase CE dosage of 1000 nkatg^{-1}. Owing to the low endopolygalacturonase activity of the Econase CE preparation, a high enzyme volume had to be added to the berry mash (39 mL per 50 g berries). Separate reference treatment was therefore used in this treatment (39 mL of water instead of enzyme). The incubation was continued at 45 °C for 2 h. The treatments were carried out at the intrinsic pH of the berries (about pH 3) and the pH was checked from the mash and the juice after the treatment. Reference treatments were carried out correspondingly but omitting the enzyme. Reference treatments were also carried out using denatured enzymes. The enzymes were denatured by boiling in a water bath for 5 min.

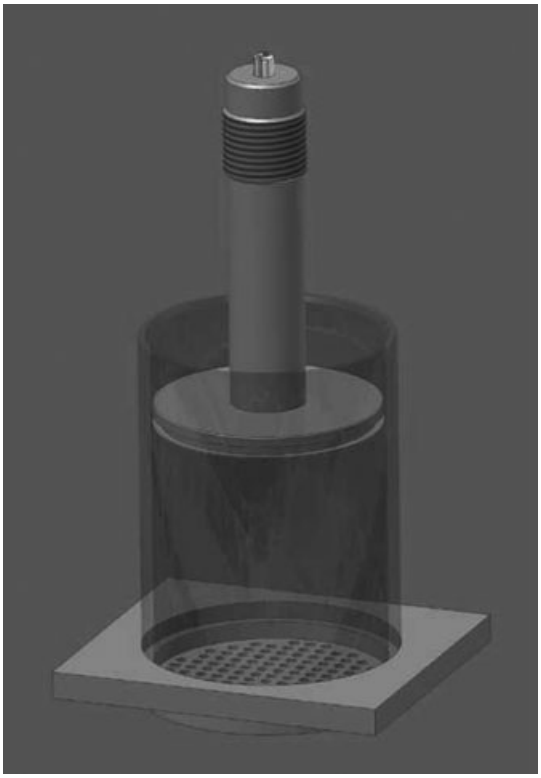
The juice was extracted by a juice pressing device attached to a TA-HDi texture analyser (Stable Micro Systems, Godalming, UK). The pressing device was specifically developed for this work and built by Protoshop Oy (Espoo, Finland) (Fig 1(a)). The inner

Table 1. Activity profiles of enzymes measured at pH 3.5

Enzyme	CE (FPU mL^{-1})	EG (nkat mL^{-1})	XYL (nkat mL^{-1})	MAN (nkat mL^{-1})	PG (nkat mL^{-1})	β-GLU (nkat mL^{-1})	β-GAL (nkat mL^{-1})	α-ARA (nkat mL^{-1})	PME (nkat mL^{-1})
Econase CE	47	16 780	30 040	2 110	1 280	63	64	640	0
Pectinex Smash	0	1 990	590	30 915	34 885	42	1 910	774	7 807
Pectinex BE-3L	0	986	21 630	1 287	4 900	338	2 804	2 988	2 090
Biopectinase CCM	0	1 467	1 762	3 139	22 540	136	691	1 688	5136
Pectinex Ultra SP-L	0	1 653	900	16 160	29 300	8	1 464	715	2 537

CE = overall cellulolytic activity; EG = endoglucanase; XYL = xylanase; MAN = mannanase; PG = endopolygalacturonase; β-GLU = β-glucosidase; β-GAL = β-galactosidase; α-ARA = α-arabinosidase; PME = pectin methylesterase.

(a)



(b)

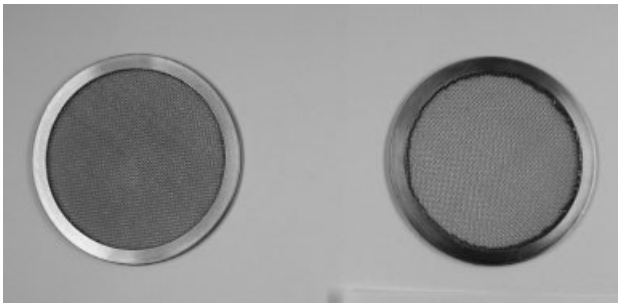


Figure 1. (a) Juice pressing device developed for the texture analyser; (b) net discs for bilberries (left) and blackcurrants (right).

diameter of the pressing cell was 70 mm and the height was 105 mm. It had a fixed metallic base that was perforated, the holes having a diameter of 3 mm. On the fixed base a thin net disc (edge 0.5 cm) (Spinea Ltd, Helsinki, Finland) with the desired mesh size could be placed. For the bilberries a net with 0.5 mm × 0.5 mm holes (thread thickness 0.29 mm) was used. For the blackcurrants a net with somewhat larger holes was needed (0.75 mm × 0.75 mm, thread thickness 0.3 mm) (Fig 1(b)). The berry mash was poured into the pressing cell and compressed with a speed of 0.5 mm s^{-1} until a force of 245 kg was reached. This force, which equates to a pressure of 0.62 MPa (6.2 bar), was maintained for 5 s and thereafter the piston was moved up to its starting position. After pressing, the weights of the resulting juice and press cake were determined and the corresponding yields were calculated. The treatments were carried out in duplicate. The samples were frozen and stored at −25 °C before the analyses.

Analysis of total phenolics and sugars

Total phenolics were determined from the raw materials and juices using the Folin–Ciocalteau method according to Singleton and Rossi.[34] The phenolic compounds were extracted from the berries with methanol. The sugars present in the juice were analysed by the HPLC assay procedure using a modified gradient.[35] Initial eluent conditions consisted of 85% water and 15% 100 mmol L^{-1} NaOH. The NaOH concentration was decreased with a concave curve from 15% to 0% between 0 and 4 minutes. From 4 to 35 minutes the gradient was held at 100% water. The column was washed with 300 mmol L^{-1} sodium acetate in 100 mmol L^{-1} NaOH for 3 min and after that with 300 mmol L^{-1} NaOH for 4 min. After these steps the gradient was returned to 85% of water and 15% of 100 mmol L^{-1} NaOH and held at these initial conditions for 15 min.

HPLC analysis of anthocyanins

The frozen juices were thawed and a 4.7 mL sample was weighed for the analysis. After adding concentrated HCl (50 μL), the sample volume was adjusted to 5 mL with methanol. Frozen berry samples were homogenised with a household blender, and the homogenised sample (5 g) was extracted with 3 × 15 mL of acidified methanol (MeOH:HCl, 99:1, v/v). After vigorous mixing for 1 min, the suspension was centrifuged at 3400 × *g* for 10 min. The supernatants from three extractions were combined and made to 50 mL. The pH of the final samples was below 1.

Prior to HPLC analysis, the samples were filtered through a syringe filter (0.45 μm TITAN, Gloucester, UK). A 10 μL injection of the filtrates was separated on a LiChroCart Purospher Star RP-18e column (250 mm × 4.6 mm, i.d. 5 μm; 4 mm × 4 mm guard column; Merck, Darmstadt, Germany) using a HP 1100 series HPLC (Waldbronn Analytical Division, Waldbronn, Germany) equipped with a quaternary pump, an autosampler and a diode-array detector (DAD) linked to an HP-ChemStation data handling system. The analysis of anthocyanins was performed using 10% formic acid in water as solvent A and acetonitrile:methanol (85:15 v/v, HPLC grade) as solvent B. The flow rate of the mobile phase was 1.0 mL min^{-1} for 0–10 min and 0.8 mL min^{-1} for 10–70 min. The gradient used was as follows: 0–2 min, 6% B; 2–4 min, 6 to 7% B; 4–11 min, 7% B; 11–20 min, 7 to 9% B; 20–30 min, 9 to 10% B; 30–50 min, 10 to 12% B; 50–55 min, 12 to 16% B; 55–64 min 16% B; 64–67 min, 16 to 90% B, followed by an isocratic gradient for 3 min and then returning to the initial conditions for 5 min before the next injection. Anthocyanins were detected at 520 nm.

Identification of anthocyanins was based on reference compounds (UV-visible spectra and retention times), the literature[1,3,4] and our previous studies.[36–38] Quantification of anthocyanins was carried out using representative anthocyanidin 3-*O*-glucosides (Polyphenols AS, Sandnes, Norway) as

external standards, and the contents were expressed in mg kg^{-1} of fresh weight, for the weight of the aglycon.

RESULTS AND DISCUSSION

Activity profiles of the enzyme preparations

Enzyme activity profiles of the enzyme preparations measured at pH 3.5 and the calculated enzyme activities in the enzyme mixtures with constant endopolygalacturonase activity of 1000 nkat g^{-1} used for the treatments are seen in Tables 1 and 2, respectively. The enzyme preparations were clearly different with respect to the activity profiles. The highest β-glucosidase activity as related to endopolygalacturonase activity was in the Pectinex BE-3L and Econase CE preparations, whereas Pectinex Ultra SP-L contained practically no β-glucosidase (Table 2). The β-glucosidase activities of Pectinex Smash and Biopectinase CCM were also relatively low compared with those of Econase CE and Pectinex BE-L. All enzyme mixtures contained significant amounts of β-galactosidase, with the highest relative activity in Pectinex BE-3L. Clear differences in the α-arabinosidase activities were found, and Econase CE and Pectinex BE-3L contained higher relative activities than the other enzymes used. Of the enzyme preparations tested, Pectinex BE-3L was relatively the richest with respect to β-glucosidase, β-galactosidase and α-arabinosidase activities. Econase CE was devoid of any pectin methylesterase activity, which was present at practically similar levels in the other preparations used (Table 1).

Effect of enzyme treatment on juice yield

Both berries, ie bilberry and blackcurrant, were treated for 2 h at 45 °C with a relative high enzyme dosage (1000 nkat g^{-1}) based on endopolygalacturonase activity. The dosage was selected to be able to compare different enzyme preparations and to see a clear impact of the role of minor activities in the preparations. The reference treatments were carried out at similar conditions but by adding water instead of enzyme preparation. After the treatments the berries were pressed and the juice yields were measured (Tables 3 and 4). With the pressing device used, the bilberry juice yields obtained after the enzyme treatments were 116–118% of the corresponding references (Table 3). As mentioned, the enzyme dosages used were, however, very high and maximal bilberry juice yield was obtained already at much lower enzyme dosages (below 50 nkat g^{-1}; results not shown). The pH of the bilberry mash after enzyme addition was found to be 2.6–2.7, with the exception of Econase CE treatment which (owing to the high volume of the enzyme preparation used) raised the pH to 3.3.

Blackcurrant cell walls are known to be thicker and to contain more pectins than those of bilberry.[39] Thus the effect of the enzymatic treatment on the pressability was more pronounced compared with bilberry pressing (Table 4). The highest juice yields compared with reference treatment (133–135%) were obtained by Pectinex BE-3L and Biopectinase CCM, whereas the effect obtained by Econase CE was clearly lower (114%). The lower effect of the Econase CE preparation is partially expected to be due to lack of pectin methylesterase activity in the mixture (Tables 1 and 2), as polygalacturonase is unable to attack cell wall pectins efficiently without concomitant demethylation of the pectins. Significant variation in juice yields have usually been obtained depending on pressing device. The juice yields reported by Landbo

Table 2. Calculated enzyme activities with PG activity of 1000 nkat g^{-1} berry sample

Enzyme	PG (nkat g^{-1})	β-GLU (nkat g^{-1})	β-GAL (nkat g^{-1})	α-ARA (nkat g^{-1})	Wt%
Econase CE	1000	49	50	500	78
Pectinex Smash	1000	1.2	56	22	3.4
Pectinex BE-3L	1000	69	572	610	2.9
Biopectinase CCM	1000	6	31	75	20
Pectinex Ultra SP-L	1000	<0.5	50	24	4.4

PG = endopolygalacturonase; β-GLU = β-glucosidase; β-GAL = β-galactosidase; α-ARA = α-arabinosidase.

Table 3. Enzyme-aided pressing of bilberry

Enzyme	pH after enzyme addition	pH of juice	Juice (g)	Juice yield[a] (%)	Juice yield (% of reference)
Reference (10 mL H_2O)	2.74	2.97	40.1	66.8	100
Pectinex Ultra SP-L	2.59	2.84	47.6	79.3	119
Pectinex Smash	2.61	2.85	47.2	78.7	118
Pectinex BE-3L	2.60	2.84	47.9	79.8	119
Biopectinase CCM	2.66	2.94	47.6	79.3	119
Reference (39 mL H_2O)[b]	2.82	3.07	67.7	76.1	100
Econase CE	3.33	3.48	78.6	88.3	116

[a] Juice yield = [(mass of juice)/(mass of mash into the pressing system)] × 100.
[b] Reference to Econase CE treatment.

Table 4. Enzyme-aided pressing of blackcurrant

Enzyme	pH after enzyme addition	pH of juice	Juice (g)	Juice yield[a] (%)	Juice yield (% of reference)
Reference (10 mL H_2O)	2.57	2.84	31.0	51.6	100
Pectinex Ultra SP-L	2.49	2.76	39.3	65.5	127
Pectinex Smash	2.48	2.75	40.0	66.7	129
Pectinex BE-3L	2.45	2.72	42.0	70.0	135
Biopectinase CCM	2.48	2.76	41.2	68.7	133
Reference (39 mL H_2O)[b]	2.61	2.85	60.7	68.2	100
Econase CE	2.89	3.14	68.9	77.4	114

[a] Juice yield = [(mass of juice)/(mass of mash into the pressing system)] × 100.
[b] Reference to Econase CE treatment.

Table 5. Phenolic and carbohydrate composition of bilberry juices

Enzyme	Total phenolics[a] ($g\,L^{-1}$)	Increase (%)	Fru ($g\,L^{-1}$)	Glc ($g\,L^{-1}$)	Gal ($g\,L^{-1}$)	Man ($g\,L^{-1}$)	Ara ($g\,L^{-1}$)	Xyl ($g\,L^{-1}$)	GalA ($g\,L^{-1}$)	Total sugars ($g\,L^{-1}$)
Reference (10 mL H_2O)	3.33	—	31.0	23.0	0	0	0	0	0	54.0
Pectinex Ultra SP-L	4.22 (3.05)	27	31.0	23.0	1.1	0	0.4	0	3.9	59.4
Pectinex Smash	4.21 (2.42)	26	30.0	22.0	0.9	0	0.4	0	3.5	56.8
Pectinex BE-3L	4.50 (2.89)	35	30.0	23.0	1.3	0	0.6	0	3.8	58.7
Biopectinase CCM	4.38 (3.13)	32	32.0	25.0	1.4	0	0.7	0	3.8	62.9
Reference (39 mL H_2O)[b]	2.33	—	22.0	15.5	0	0	0	0	0	37.5
Econase CE	2.67 (1.87)	15	20.0	16.5	1.5	2.9	0.4	0	0	41.3

Fru = fructose; Glc = glucose; Gal = galactose; Man = mannose; Ara = arabinose; Xyl = xylose; GalA = galacturonic acid.
[a] The value obtained with denatured enzymes is in parentheses.
[b] Reference to Econase CE treatment.

and Meyer[40] ranged from 66.4% to 78.9% by wet weight of mash in experimental blackcurrant berry juice production with various pectolytic enzymes.

Effect of enzyme treatment on bilberry juice quality

The bilberry juices produced were analysed with respect to the phenolics and monosaccharides present (Table 5). The total phenolic content of bilberry juice as measured by the Folin–Ciocalteau method was increased by 35%, from 3.3 to 4.5 $g\,L^{-1}$ with the most effective enzyme used, Pectinex BE-3L. Pectinex Ultra SP-L, Pectinex Smash and Biopectinase CCM improved the extractability of phenolics by 26–32%. The added enzyme amount was not found to affect the Folin measurement. The galacturonic acid content of bilberry juice was 3.5–3.9 $g\,L^{-1}$ after all other enzyme treatments but Econase CE, indicating that Econase CE does not contain exo-pectinase activity.

The content of individual anthocyanins of the initial berries and the juices were analysed by HPLC (Fig 2, Table 6). The most abundant anthocyanidin glycosides in whole bilberry were delphinidin 3-*O*-arabinoside and delphinidin 3-*O*-galactoside (each 14% of total anthocyanins), followed by cyanidin 3-*O*-galactoside (12%) (Table 6). The total anthocyanin yields in bilberry juices were enhanced in all other treatments except with Econase CE (Fig 3). The most efficient enzyme preparation to increase the anthocyanin extraction was Pectinex BE-3L, increasing the yield by 41% (when identified and

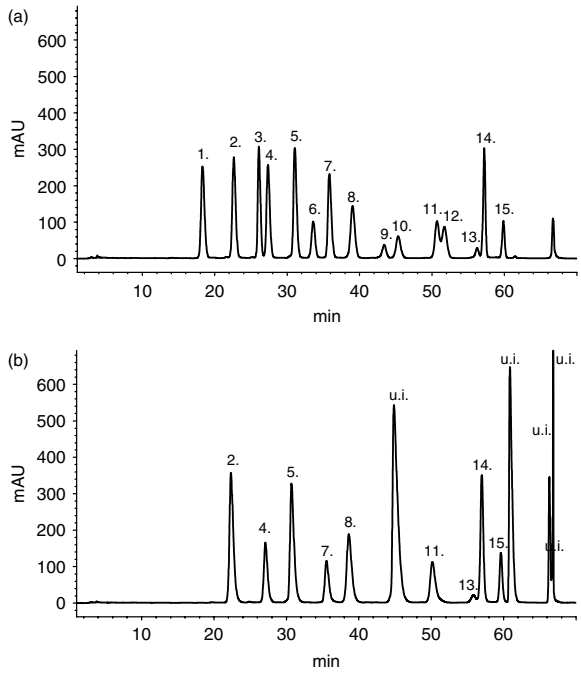


Figure 2. HPLC chromatograms (520 nm) of anthocyanins in bilberry juices: (A) reference juice (no enzyme treatment); (B) enzyme-treated (Pectinex BE-3L) juice. Peak identification: 1. delphinidin 3-*O*-galactoside, 2. delphinidin 3-*O*-glucoside, 3. cyanidin 3-*O*-galactoside, 4. delphinidin 3-*O*-arabinoside, 5. cyanidin 3-*O*-glucoside, 6. petunidin 3-*O*-galactoside, 7. cyanidin 3-*O*-arabinoside, 8. petunidin 3-*O*-gluco-side, 9. peonidin 3-*O*-galactoside, 10. petunidin 3-*O*-arabinoside, 11. peonidin 3-*O*-glucoside, 12. malvidin 3-*O*-galactoside, 13. peonidin 3-*O*-arabinoside, 14. malvidin 3-*O*-glucoside, 15. malvidin 3-*O*-arabinoside, u.i. unidentified.

Table 6. Contents[a] of anthocyanidin glycosides in whole berries and juices of bilberry

Treatment	Delphinidin glycosides			Cyanidin glycosides			Petunidin glycosides			Peonidin glycosides			Malvidin glycosides			Unidentified anthocyanins	Total anthocyanins[b]		
	Gal	Glc	Ara	Gal	Glc	Ara	Gal	Glc	Ara	Gal	Glc	Ara	Gal	Glc	Ara		Gal	Glc	Ara
Bilberry (whole berry)	859	691	857	766	574	585	223	389	206	60	203	32	167	460	143		2074	2316	1823
Reference (10 mL H_2O)	230	239	221	194	237	191	82	140	62	29	88	19	110	191	69	57[c]	645	895	562
Pectinex Ultra SP-L	0	354	236	0	306	175	0	195	ND[d]	0	114	20	0	251	94	705	0	1220	525[e]
Pectinex Smash	0	334	229	0	302	176	0	187	ND[d]	0	116	20	0	242	95	796	0	1181	520[e]
Pectinex BE-3L	0	364	158	0	307	110	0	204	ND[d]	0	117	19	0	268	105	1401	0	1260	392[e]
Biopectinase CCM	0	337	307	0	295	227	0	186	ND[d]	0	108	21	35	234	84	628	35	1160	639[e]
Reference (39 mL H_2O)[f]	156	177	150	140	183	136	57	105	42	22	70	13	82	148	48	42[c]	457	683	389
Econase CE	0	13	7	0	0	5	0	8	ND[d]	0	0	0	0	13	30	98	0	34	42[e]

Gal = galactoside; Glc = glucoside; Ara = arabinoside.
[a] Expressed as means of duplicate samples in mg kg^{-1} for the weight of the aglycon.
[b] The total content of anthocyanins was calculated as the sum of compounds representing anthocyanins.
[c] The unknown peaks in the reference samples are predominantly not the same as found after the enzymatic treatments.
[d] ND = not determined owing to overlapping peaks.
[e] Petunidin arabinoside has been excluded due to overlapping peaks.
[f] Reference to Econase CE treatment.

Figure 3. Distribution of anthocyanidin glycosides in bilberry juices.

unidentified compounds were calculated). With the others the increase was 13–16%. Econase CE, on the other hand, decreased the total amount of detectable anthocyanins from about 1570 mg kg^{-1} to below 180 mg kg^{-1}, ie by 89%. In all enzyme treatments, with the exception of Econase CE, a significant amount of unknown anthocyanin type of components was formed (Figs 2 and 3, Table 6). These components were not detected in the berries, and their chemical structure will be characterized in our further studies. We tentatively assume that these new components represent aglycons liberated by enzymatic hydrolysis of anthocyanidin glycosides as reported by Wightman and Wrolstad.[23,41] In addition to the formation of unknown components, the enzymatic treatments also caused clear changes in the anthocyanin profiles of the juices (Fig 3). As a result of all enzymatic treatments, practically no anthocyanidin galactosides were detected (Table 6), which is in line with the high galactosidase activities found in the preparations (Table 2). Hydrolysis of anthocyanidin galactosides has also been reported for enzymatically processed cranberries.[22,41] The hydrolysis could also be visualised in the increased galactose content of the juice after especially Econase CE, Biopectinase CCM and Pectinex BE-3L treatment (Table 5).

Pectinex BE-3L and Econase CE contained the highest relative α-arabinosidase activity (Table 2), which can be visualised in efficient arabinoside hydrolysis with hydrolysis degrees of 31% and 90%, respectively (Table 6). The amount of petunidin 3-*O*-arabinoside could not be analysed owing to overlapping with an unknown peak formed during the treatment. Glucosides were only hydrolysed by Econase CE (Table 6, Fig 3), although Pectinex BE-3L contained even higher glucosidase activity compared with Econase CE (Table 2). Thus the glucosidase(s) present in Econase CE and Pectinex BE-3L clearly have different modes of action.

Effect of enzyme treatment on blackcurrant juice quality

Blackcurrant juice (reference juice without enzymes) contained a lower concentration of total phenolics than the corresponding bilberry juice (Tables 5 and 7). The enzymatic improvement of phenolic extraction

Table 7. Phenolic and carbohydrate composition of blackcurrant juices

Enzyme	Phenolics[a] ($g\,L^{-1}$)	Increase (%)	Fru ($g\,L^{-1}$)	Glc ($g\,L^{-1}$)	Gal ($g\,L^{-1}$)	Man ($g\,L^{-1}$)	Ara ($g\,L^{-1}$)	Xyl ($g\,L^{-1}$)	GalA ($g\,L^{-1}$)	Total sugars ($g\,L^{-1}$)
Reference (10 mL H_2O)	2.88	—	36.0	32.0	0	0	0	0	0.8	68.8
Pectinex Ultra SP-L	4.07 (3.21)	41	42.0	35.0	1.1	0	0.9	0	11.1	90.1
Pectinex Smash	3.77 (3.09)	31	43.0	34.0	0.9	0	0.9	0	10.9	89.7
Pectinex BE-3L	4.20	46	40.0	33.0	1.3	0	1.8	0	10.3	86.4
Biopectinase CCM	4.60 (2.68)	60	41.0	34.0	1.4	0	1.7	0	10.5	88.6
Reference (39 mL H_2O)[b]	2.04	—	26.0	20.0	0	0	0	0	0.5	46.5
Econase CE	2.34 (2.21)	15	26.0	23.0	1.5	2.9	0.9	0.5	2.3	57.1

Fru = fructose; Glc = glucose; Gal = galactose; Man = mannose; Ara = arabinose; Xyl = xylase; GalA = galacturonic acid.
[a] The value obtained with denatured enzymes is in parentheses.
[b] Reference to Econase CE treatment.

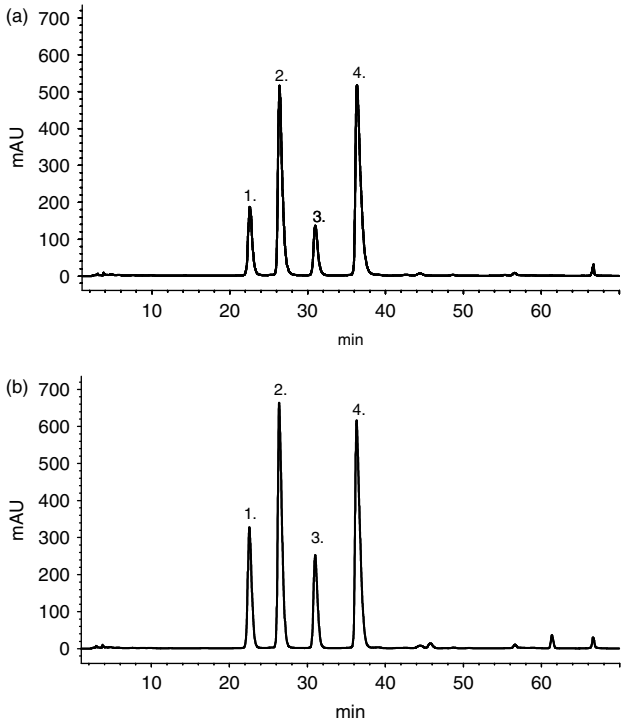


Figure 4. HPLC chromatograms (520 nm) of anthocyanins in blackcurrant juices: (A) reference juice (no enzyme treatment); (B) enzyme-treated (Pectinex BE-3L) juice. Peak identification: 1. delphinidin 3-*O*-glucoside, 2. delphinidin 3-*O*-rutinoside, 3. cyanidin 3-*O*-glucoside, 4. cyanidin 3-*O*-rutinoside.

was, however, higher than with bilberry treatments. Biopectinase CCM increased the phenol content by 60%, followed by Pectinex BE-3L (46%). Econase CE treatment resulted in only a 15% increase. After the enzymatic treatments with Pectinex Ultra SP-L, Pectinex Smash, Pectinex BE-3L or Biopectinase CCM the blackcurrant juices contained significant amounts of galacturonic acid, indicating extensive hydrolysis of cell wall pectins. This could be expected owing to the high concentration of pectins in these berries.[40] Econase CE treatment also resulted in slight liberation of galacturonic acid to the juice.

The individual anthocyanins were analysed by HPLC (Fig 4) from the berries and juices, and the results are presented in Table 8. Enzyme-aided pressing resulted in improved liberation of anthocyanins

Table 8. Contents[a] of anthocyanidin glycosides in whole berries and juices of blackcurrant

Treatment	Delphinidin glycosides		Cyanidin glycosides		Total anthocyanins	
	Glc	Rut	Glc	Rut	Glc	Rut
Blackcurrant (whole berry)	347	810	241	949	589	1759
Reference (10 mL H_2O)	198	587	133	635	331	1222
Pectinex Ultra SP-L	392	651	350	606	743	1258
Pectinex Smash	337	650	285	604	622	1254
Pectinex BE-3L	321	650	229	634	550	1284
Biopectinase CCM	304	707	204	756	508	1463
Reference (39 mL H_2O)[b]	143	421	95	439	238	860
Econase CE	115	520	66	541	181	1061

Glc = glucoside; Rut = rutinoside.
[a] Expressed as means of duplicate samples in $mg\,kg^{-1}$ for the weight of the aglycon.
[b] Reference to Econase CE treatment.

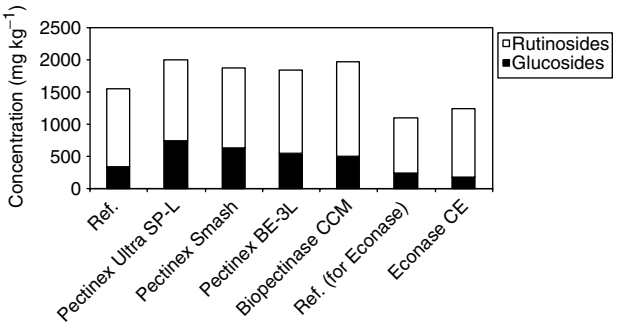


Figure 5. Distribution of anthocyanidin glycosides in blackcurrant juices.

into the juice (Table 8). The anthocyanin yields were increased from 1550 $mg\,kg^{-1}$ to 2000 $mg\,kg^{-1}$ and 1970 $mg\,kg^{-1}$, ie by 27–29%, by Pectinex Ultra SP-L and Biopectinase CCM, respectively (Fig 5). The relative ratio of delphinidin glycosides *versus* cyanidin glycosides remained about the same in the juices as compared with the whole berry. Of the glycosides, the rutinosides of delphinidin and cyanidin were more abundant than the corresponding glucosides in all cases (Table 8, Fig 5). With all other enzymes with the exception of Econase CE, the anthocyanidin glucoside

extraction was increased more pronouncedly than the rutinoside extraction. Landbo and Meyer[40] found that the relative quantitative distribution of the four major blackcurrant anthocyanins did not change with enzymatic maceration of the berries. In the present study, however, the relative contents of delphinidin and cyanidin glucosides were increased (total glucosides 21% in reference juice *versus* 26–37% in pectinase-treated juices) and those of rutinosides decreased (total rutinosides 79% in reference juice *versus* 63–74% in pectinase-treated juices). No hydrolysis of extracted rutinosides was observed by either of the five enzyme mixtures used, indicating that rutinosidase activity or rhamnosidase activities were not present in the preparations used. In the Econase CE treatment a partial hydrolysis of the extracted delphinidin and cyanidin glucosides was observed, whereas the other enzymes did not have any activity against glucosides. Similarly as in the case of bilberries, the glucosidase present in Pectinex BE-3L was unable to hydrolyse the glucosides present in blackcurrant (Table 8). The enzyme treatments clearly had less significant effects on the anthocyanin profiles in blackcurrant juices than in bilberry juices (Figs 2–5).

CONCLUSION

The results indicate that the enzyme product used in pressing strongly affects both juice and anthocyanin yields. Careful understanding of enzyme activities is required as maximisation of anthocyanin extraction and juice yield may not be obtained with the same enzyme mixture. The anthocyanin yield is affected by potential improvement in the extractability, but exoglycosidases may also cause hydrolysis of the liberated anthocyanins.

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