Food Chemistry 111 (2008) 1043–1049

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Berry seed press residues and their valuable ingredients with special regard to black currant seed press residues

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ARTICLE INFO

Article history:
Received 23 November 2007
Received in revised form 8 April 2008
Accepted 5 May 2008

Keywords:
Berry seed
Press residue
Black currant
Tocopherols
Total phenols
TEAC
Fatty acids

ABSTRACT

Berry seeds are distinguished by longevity though clear scientific appraisals cannot be made. Besides a hard seed coat other protecting substances are presumed in the seeds. Commonly the seeds are utilized as a source of oils. After pressing, there is a residue left that is still rich in bioactive ingredients. This paper gives an overview of the health-beneficial ingredients remaining in the residue of various berry seeds (bilberry, cranberry, rose hip, strawberry, elder, and black currant) with special focus on black currant. The fatty acid distribution and the content of fat, tocopherols and tocotrienols, phytosterols, carotenoids, vitamin C, fibre, protein, amino acids, dry matter, ashes, minerals, total phenols (gallic acid equivalent) and antioxidant capacity (TEAC) were determined. The investigation of berry seed press residues revealed that the total phenols and tocopherols were quantitatively the most important features of this material but there were significant differences between batches and cultures.

1. Introduction

In the production of berry-based juices, jellies and jams, most processors consider the seeds of the berries to be removed from the berry pulp during processing to improve the end product. The berry seed materials removed as waste by-products may contain health-beneficial compounds. As a consequence, commercial uses should be found. Since little, hard seeds are usually excreted undigested, assumed health-promoting substances inside the seeds cannot develop their potential. Therefore, the processed seeds might be beneficial. Investigations were undertaken to determine the chemical composition of berry seeds and to characterize some of the potentially health-beneficial substances. Previous research focused mainly on the seed oil fatty acid (FA) composition (Johansson, Laine, Linna, & Kallio, 2000; Ruiz del Castillo, Dobson, Brennan, & Gordon, 2002), triacylglycerols, and glycerophospholipids (Kallio, Yang, Peippo, Tahvonen, & Pan, 2002b) or tocopherols and tocotrienols (Kallio et al., 2002b; Bushman et al., 2004). Oil seeds have been examined for FAs to promote health and to prevent disease, e.g., atopic dermatitis (Noli et al., 2007), gastric ulcer (Xing et al., 2002), immune response (Wu et al., 1999) or effects on plasma lipids (Tahvonen, Schwab, Linderborg, Mykkänen, & Kallio, 2005). Furthermore, research was conducted on the fibre (Nawirska & Kwaśniewska, 2005), phytosterols (Yang, Koponen, Tahvonen, & Kallio, 2003), polyphenols (Lu & Foo, 2003) and the antioxidant potential of the seeds or the seed oils (Bushman et al., 2004; Parry et al., 2005). The protective effect of black currant seeds towards helicobacter pylori adhesion to the gastric mucosa of human was investigated by Lengsfeld, Deters, Faller, and Hensel (2004). Black currant seeds are recommended as edible inclusion in breakfast cereals (Tahvonen et al., 1998). High nut and seed intakes (>6.2 g/d, e.g., sunflower, pumpkin) were associated with a decreased risk of colon cancer in women, but this effect could not be demonstrated in men (Jenab et al., 2004). The above mentioned literature does not specifically indicate whether health-promoting constituents are also present in the berry seeds. The purpose of the present investigation was to quantify and to characterize the berry press residue overall attributes.

2. Material and methods

2.1. Treatment of the berry seeds

Seeds of bilberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium oxycoccus* L.), rose hip (*Rosa canina* L.), strawberry (*Fragaria x ananassa* L.), elder (*Sambucus nigra* L.), and black currant (batch I and II) (*Ribes nigrum* L.) were obtained from a wholesale trader who

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doi:10.1016/j.foodchem.2008.05.017

provided seeds in large amounts. This trader buys seeds throughout countries of the European Community. As a consequence, seeds of different growing areas can be pooled and heritage may vary from year to year. Detailed growing conditions of berry plants cannot be verified. Berry seed samples were pressed at approximate 60 °C with varying temperatures in different segments of the press procedure. Frictional heating was an appreciable contributor to the heat development. Oil was removed for purposes not mentioned in this paper. The seed press residue (PR) was ground and sieved to a particle size <500 μm (IGV Potsdam). Thus, hard coat fractions were separated and PRs could be utilized as a fortification in edible products. PRs were stored at −20 °C until analyses.

2.2. Fat content and FA distribution

An aliquot of the sample was boiled with 4 N HCl for 1 h, filtrated, washed with HPLC-grade water and extracted twice with petroleum ether using a Soxtherm®. The total fat content was gravimetrically determined. For determination of total lipids, the extracted fat was methylated using sodium methoxide in the presence of hexane, then mixed with sodium hydrosulphate, and centrifuged. The supernatant fraction was analyzed by GC/FID using H_2 as carrier gas (Kraft, Collomb, Möckel, Sieber, & Jahreis, 2003). The used column is qualified to separate fatty acid methyl esters (FAME) between C_4 and C_{22}. Results are given as percent of total FAME. Calibration standard for FAME (no. 18919-1AMP) was purchased at Supelco, Taufkirchen, Germany.

2.3. Tocopherols and tocotrienols

Vitamin C was added to an aliquot of the sample followed by a hydrolysis with 2 M ethanolic KOH (80%) at 80 °C for 30 min. The tocopherols/tocotrienols were extracted with *n*-hexane/2,6-di-*tert*-butyl-*p*-kresol and determined using HPLC/fluorescence (Kuhnt, Wagner, Kraft, Basu, & Jahreis, 2006). A standardized milk powder (BCR-421, Promochem, Wesel, Germany) with a known content of tocopherol was analyzed in parallel with each sample batch. Standards for tocopherol calibration (Isomer kit no. 613424) were purchased at Calbiochem, Darmstadt, Germany and for tocotrienol (Isomer kit no. 8524) at Merck, Darmstadt, Germany.

2.4. Phytosterols

An aliquot of the sample with an internal standard (5α-Cholestane, no. C8003, Sigma–Aldrich, Taufkirchen, Germany) was hydrolysed at 70 °C for 1 h in 1 M ethanolic NaOH (90%) followed by three extractions with cyclohexane. Combined extracts were concentrated under a stream of nitrogen and redissolved in *n*-decane for measurement of phytosterols using GC/FID under isocratic conditions with H_2 as carrier gas (Keller, Helbig, Härtl, & Jahreis, 2007). Sitosterol (no. C1270) and campesterol (no. C5157) for calibration were purchased at Sigma–Aldrich, Taufkirchen, Germany.

2.5. Carotenoids

An aliquot of the sample and internal standard (echinenone, CaroteNature, Lupsingen, Switzerland) was extracted after addition of MgO with tetrahydrofuran/methanol with 0.1% butylated hydroxytoluene (1 + 1 v/v) according to Seybold, Fröhlich, Bitsch, Otto, and Böhm (2004). The extract was vacuum-dried at 30 °C and redissolved in tetrahydrofuran/methanol with 0.1% butylated hydroxytoluene (1 + 1, v/v). The content of carotenoids was measured using gradient procedure at HPLC/DAD. Standards for all carotenoids investigated were purchased at CaroteNature, Lupsingen, Switzerland.

2.6. Antioxidant capacity (TEAC), total phenols (gallic acid equivalent)

Antioxidant capacity was assessed by the TEAC-assay (Trolox equivalent antioxidant capacity) according to Schlesier, Harwat, Böhm, and Bitsch (2002). After an aliquot of the sample was extracted with *n*-hexane (TEAC II) or distilled water (TEAC III), ABTS (2,2′-azinobis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt) was added which was prior to the test oxidised to a radical with MnO_2 (TEAC II) or $K_2O_8S_8$ (TEAC III). The sample was photometrically measured at $\lambda = 734$ nm using Trolox® (Sigma–Aldrich, Taufkirchen, Germany) standard solutions for quantification.

Total phenols were determined by the Folin-Ciocalteu method (Schlesier et al., 2002). An aliquot of the sample was dissolved in HPLC-grade water. To the supernatant fraction Folin-Ciocalteu reagent (Fluka, Buchs, Switzerland, 1:10 diluted) and $Na_2CO_3 \cdot 10H_2O$ was added and mixed. After 2 h reaction time, absorbance at $\lambda = 750$ nm was determined. Results are presented as gallic acid equivalents (GAE) with gallic acid monohydrate as standard (Riedel-de Haën, Seelze, Germany).

2.7. Vitamin C

Dehydroascorbic acid, the oxidised form of vitamin C, was determined by photometric analysis according to Schlesier et al. (2002). In brief, a sample aliquot diluted in phosphoric acid was mixed with trichloracetic acid and centrifuged. Supernatant fraction was heated with dinitrophenylhydrazine reagent at 60 °C for 1 h. Samples were cooled in an ice bath and mixed with sulfuric acid before measurement. Results were calculated using a standard curve with different concentrations of ascorbic acid (Fluka, Buchs, Switzerland).

2.8. Fibre

Total fibre was determined enzymatically with BIOQUANT® Total Dietary Fibre (Merck, Darmstadt, Germany) employing α-amylase, protease, and amyloglucosidase (AOAC International, 1995). Detergent analysis was carried out according to the Van Soest method estimating NDF (neutral detergent fibre), ADF (acid detergent fibre) and ADL (lignin) (Van Soest, Robinson, & Lewis 1991). In brief, aliquots of the sample were boiled with the NDF and ADF solutions, respectively, and subsequently washed with distilled water. The respective fibre fractions were determined gravimetrically after drying and incinerating. For ADL, another ADF-fraction was hydrolyzed with 72% H_2SO_4, then washed with distilled water and acetone and dried. Ash content was determined and mass loss was recorded. Hemicelluloses were calculated as NDF–ADF, cellulose as ADF–ADL.

2.9. Protein and amino acids

For crude protein analysis the Kjeldahl method was applied ($N \times 6.25$).

For the determination of acid (Asp, Glu), neutral (Thr, Ser, Pro, Hyp, Ala, Gly, Val, Ile, Leu, Tyr, Phe) and alkaline (Lys, His, Arg) amino acids (AAs), a prior acid hydrolysis was used with 6 N HCl. Due to their instability, sulphur-containing AAs (Met, Cys) needed to be oxidised to methionine sulfon and cysteic acid using performic acid, before acid hydrolysis followed. Since the neutral AA tryptophan would be destroyed at those conditions, the sample was hydrolysed with 4 N LiOH at 110 °C for 20 h under anaerobic conditions (Landry, Delhaye, & Jones 1992). AAs were verified by ion exchange chromatography with ninhydrin post column derivatization (Amino Acid Analyzer LC 3000; Eppendorf/Biotronik). Calibration standards were purchased at Onken GmbH, Gründau, Germany.

To evaluate the protein quality, the amino acid ratio (AAR), the essential amino acid-index (EAAI) and the biological quality (BQ) were calculated as written below

- AAR = g of AA per 16 g N of test protein ÷ requirement [g] of AA per 16 g N.

  (AAR is based on the AA requirements from the FAO/WHO/UNU, 1985).
- EAAI = geometric mean of the AAR of all essential AAs.
- BQ = −15.71 + 1.0975 × EAAI (approximated BQ).

EAAI and BQ are based on the pattern of reference protein from whole egg.

2.10. Dry matter, ash content, and elements

Dry matter was determined gravimetrically after drying at 105 °C for 24 h. Ash content was determined after sample conditioning at 525 °C for 4 h. For mineral determination 2.5 mL 25% HCl were added to the ash sample, incubated for 5 min and the flask was filled up with H_2O to a final volume of 25 mL. The sample was heated, filtrated and acidified with ultrapure HNO_3 to a final concentration of 2% of the sample. For measuring with inductively coupled plasma optical emission spectrometry (ICP-OES; Spectroflame, Spectro, Kleve, Germany), samples were 1:2 diluted with ultrapure water before injection. Analyses were conducted according to DIN 38406 (E22) except for Al, which was determined at λ = 167.08 nm. Dissenting from the DIN, calibration ranges for the elements were adjusted according to the expected concentrations. Calibration standards were purchased at Merck, Darmstadt, Germany (multielement standard Merck IV; As-, Mo-, Se-, and Ti-ICP standard) and Alfa Aesar, Karlsruhe, Germany (P-, S-, V-plasma standard).

3. Results and discussion

3.1. FAs

Seeds were pressed in order to be removed from as much oil as possible with concomitantly high saving of the arising PR. This means, higher temperatures might have separated more oil but would have damaged sensitive ingredients of the PR like antioxidants. This feature originated different extraction efficiencies of approximately 50%. For all seeds the same pressing process was used. The ground and sieved black currant PRs contained 16.5% or 25.7% oil depending on the batch of black currant seeds (Table 1). Unprocessed black currant seeds contained 29.3% oil. The high variation in the oil contents of the PRs was possibly due to variations in the oil content from different seed batches (Ruiz del Castillo et al., 2002). The FA distribution in black currant PRs showed a high level of linoleic acid (C18:2, $n-6$, 48%), α-linolenic (C18:3, $n-3$, 15%) and γ-linolenic acid (C18:3, $n-6$, 13%) (Table 2) comparable with the results reported by Stránský, Zarevúcka, and Wimmer (2005). The predominating FA in the PRs investigated was linoleic acid with a mean of 41 ± 7%, followed by α-linolenic acid with 29 ± 9% and oleic acid (C18:1, $n-9$) with 17 ± 5%. Only black currant contained appreciable amounts of γ-linolenic acid, with approximately 13 ± 0%. All other FAs consisted of less than 10% in all PRs. According to Ruiz del Castillo et al. (2002), higher contents of γ-linolenic acid were negatively correlated with α-linolenic acid indicating a competitive inhibition of the Δ6 and Δ15 desaturases. Both of these enzymes are responsible for the conversion of the precursor linoleic acid into α-linolenic acid or γ-linolenic acid, respectively. The sum of C18:3 ($n-3 + n-6$) of all PRs ranged between 23% (rose hip) and 43% (cranberry) with an overall mean of 26 ± 10%. There was a large variation in the ratio of α-linolenic acid to γ-linolenic acid. The PR of each of the berry seeds contained α-linolenic acid, while γ-linolenic acid was only detected in black currant. Among the species, the physiologically important stearidonic acid (C18:4, $n-3$) was only found in black currant (2.9%). Stearidonic acid mainly exists in the seed oils of black currant, evening primrose and borage (Ursin, 2003) and is a precursor of EPA in humans (Miles, Banerjee, & Calder, 2004). No differences were observed in the FA distribution of whole black currant seeds and black currant PRs, processing does not change the FA distribution.

In the PRs, the content of saturated fatty acids amounted for less than 10% of the total FAME detected. The content of monounsaturated fatty acids (MUFA) varied between 13% (black currant I) and 24% of total FAME (bilberry and cranberry), while the polyunsaturated fatty acids (PUFA) content varied between 64.2% (bilberry) and 79.2% of total FAME (black currant II). More than 94% of the detected FAs were long-chain fatty acids (C15–C24) while medium-chain fatty acids (C11–C14) represented less than 0.1% of total FAME. Nutrition societies recommend an $n-6/n-3$ FA ratio of 5:1 for a healthy diet (DACH, 2002). All tested berry PRs demonstrated a lower ratio (<3.6:1) with cranberry having the lowest ratio of 0.7:1. Therefore PRs can improve the average human diet in western countries, since the $n-6/n-3$ ratio in most industrialized countries is generally as high as 15:1 (Simopoulos, 2002).

3.2. Tocopherols and tocotrienols

Differences between batches within one species may result from environmental influences, e.g., geographical location or harvesting time (Kallio, Yang, & Peippo, 2002a). Black currant PR I and II contained not only different tocopherol concentrations, but also different tocopherol profiles (Table 1). The largest amounts of tocopherols were found in elder and black currant PR. The main tocopherol isomers in the species tested were α- and γ-tocopherol, whereas α-tocopherol was the principle isomer in black currant I

Table 1
Tocopherol and tocotrienol concentrations in various berry seed press residues given as means of duplicate or triplicate measurements [mg/100 g residue]

PR sample	Tocopherol [mg/100 g PR]				Tocotrienol [mg/100 g PR]				Oil content [%]	Tocopherol	Tocotrienol
	α	β	γ	δ	α	β	γ	δ		[mg/100 g oil of PR]	
Bilberry	0.89	0.04	0.33	–	0.24	–	1.57	–	5.2	24.6	33.0
Cranberry	0.87	0.16	0.15	–	1.01	–	3.92	–	5.6	21.0	93.0
Rose hip	0.78	–	0.87	–	–	–	–	–	4.0	40.7	–
Strawberry	0.49	–	0.91	–	–	–	–	–	6.2	22.7	–
Elder	0.96	0.31	12.44	0.16	0.89	–	–	–	12.0	115.3	7.1
Black currant I	8.94	0.34	7.99	0.26	–	–	–	–	16.5	106.6	–
Black currant II	2.82	0.14	5.97	0.18	–	–	0.12	–	25.7	35.5	0.5

PR: Berry seed press residue.

Table 2
Fatty acid distribution of various seed press residues given as means of duplicate or triplicate measurements [mol% of FAME]

Fatty acid	Bilberry	Cranberry	Rose hip	Strawberry	Black currant I	Black currant II	Black currant (whole seeds)
C12:0	0.01	n.d.	0.04	0.03	0.01	n.d.	0.01
C14:0	0.06	0.04	0.06	0.06	0.04	0.04	0.03
C16:0	5.18	1.10	3.28	5.34	6.26	6.11	6.16
C18:0	1.30	0.23	1.98	1.68	1.26	1.31	1.17
C20:0	0.19	0.04	0.91	0.59	0.08	0.09	0.11
C21:0	0.15	n.d.	0.23	0.19	0.03	n.d.	n.d.
C22:0	0.16	n.d.	0.29	0.27	0.04	n.d.	0.06
C24:0	0.14	n.d.	0.24	0.22	n.d.	n.d.	0.06
$C18:1_{n-9}$	22.12	22.52	14.84	19.91	10.79	11.00	11.09
$C18:1_{n-7}$	0.67	0.64	0.49	0.63	0.77	0.75	0.76
$C20:1_{n-9}$	0.22	0.25	0.51	0.29	0.76	0.89	0.90
$C18:2_{n-6}$	33.59	31.02	47.68	36.19	47.91	47.86	47.78
$C20:2_{n-6}$	0.04	0.23	0.15	0.06	0.23	0.26	0.27
$C18:3_{n-6}$	0.07	n.d.	n.d.	0.02	13.03	12.94	12.55
$C18:3_{n-3}$	29.84	43.54	23.09	28.18	14.58	15.13	14.75
$C20:3_{n-3}$	0.19	0.06	0.99	0.16	n.d.	0.03	n.d.
$C18:4_{n-3}$	0.04	n.d.	0.03	0.04	2.85	2.94	2.71
Minor FA	6.03	0.33	5.19	6.14	1.36	0.65	1.59
SFA	7.25	1.78	7.57	8.48	7.81	7.63	7.60
MUFA	23.55	23.41	16.42	21.53	12.46	12.64	13.01
PUFA	64.16	74.85	72.21	64.96	78.62	79.18	77.79
LCFA	94.85	99.79	96.07	94.87	98.85	99.59	98.36
$n-3$	30.01	43.60	23.20	28.31	17.43	18.09	17.46
$n-6$	34.75	31.25	49.51	37.29	61.96	61.81	60.59
$n-9$	22.47	22.77	15.47	20.38	11.55	11.89	11.99
$n-6/n-3$	1.16	0.72	2.13	1.33	3.56	3.42	3.47

n.d.: Below detection limit (values <0.001% of FAME).
FAME: Fatty acid methyl esters; FA: Fatty acids; SFA: Saturated fatty acids, includes not separately listed SFA; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; LCFA: Long-chain fatty acids (C15–C24), the value includes minor fatty acids determined but not separately listed.

PR, and γ-tocopherol primarily in elder and black currant II PR. Tocotrienols were detected in high concentrations in cranberry and bilberry PR where they dominated over tocopherols. Nevertheless, compared to the total tocopherol concentrations of the other species they exhibited lower total tocotrienol concentrations. Neither rose hip nor strawberry PRs contained measurable amounts of tocotrienols. The tocopherol and tocotrienol related to the remaining oil concentrations within the PR demonstrated high tocopherol concentrations, especially in elder and black currant I (115 and 107 mg/100 g oil, respectively). The tocopherol concentration of the PR of elder and black currant was comparable to other tocopherol-rich commercial seed oils namely maize and soybean oil (162 and 180 mg/100 g oil, respectively) (Tuberoso, Kowalczyk, Sarritzu, & Cabras, 2007).

Statistical analysis basing on the tocopherols and FAs in PR revealed a positive correlation between γ-tocopherol and the sum of $n-6$ FAs (Table 3). For α-tocopherol this relation was not significant. The $n-3$ FAs demonstrated a negative but insignificant association to the PR α- and γ-tocopherol concentrations. In contrast, $n-3$ FAs and α- and γ-tocotrienols correlated positively. These results support a positive correlation of the $n-6/n-3$ ratio and the α- and γ-tocopherol concentrations. Comparing the coefficients of correlation of tocotrienol and tocopherol concentrations towards the $n-3$ FAs, $n-6$ FAs, $n-9$ FAs, or $n-6/n-3$ ratio, the coefficients exhibited contrary behaviour, i.e., for example, tocopherols correlating negatively to $n-3$ FAs are associated with tocotrienols correlating positively to $n-3$ FAs and *vice versa*. Positive associations of PUFAs could be verified to β-tocopherol with $R = 0.734$, $p = 0.097$, and γ-tocopherol with $R = 0.740$, $p = 0.092$ (α-tocopherol: $R = 0.622$, $p = 0.187$). In contrast, MUFAS were basically negatively associated to α- and γ-tocopherols.

3.3. Phytosterols and carotenoids

Only moderate amounts of phytosterols (sitosterol: 76.6 mg/100 g, campesterol: 6.24 mg/100 g) and carotenoids (lutein: 0.778 mg/100 g, α-carotene: 0.537 mg/100 g, (*all-E*)-ß-carotene: 0.223 mg/100 g, (*9Z*)-ß-carotene: 0.042 mg/100 g) were detected in black currant I PR. In comparison, carotenoid-rich plants such as spinach or carrots contain approximately 17 and 16 mg carotenoids/100 g, respectively (Müller, 1997). Saito et al. (2006) reported a positive health effect in mild hypercholesterolemic subjects when a mayonnaise enriched with 0.3 g/d phytosterol esters was consumed for 4 weeks. The phytosterol concentration in the black currant PR was generally too low to reduce serum low-density lipoprotein cholesterol. Phytosterol concentrations of sea

Table 3
Correlation coefficients of tocopherol or tocotrienol concentrations and fatty acid parameters of seed press residues[a]

	Tocopherol				Tocotrienol			
	α		γ		α		γ	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*
MUFA	−0.701	0.121	−0.881	0.020	0.611	0.198	0.667	0.148
PUFA	0.622	0.187	0.740	0.092	0.043	0.935	−0.041	0.938
$n-3$	−0.581	0.227	−0.758	0.081	0.906	0.013	0.908	0.012
$n-6$	0.719	0.107	0.904	0.013	−0.641	0.170	−0.682	0.135
$n-9$	−0.701	0.121	−0.880	0.021	0.638	0.173	0.693	0.127
$n-6/n-3$	0.764	0.077	0.928	0.006	−0.642	0.169	−0.677	0.140

[a] Press residues of bilberry, cranberry, rose hip, strawberry, and black currant I and II were used for correlation analyses.

buckthorn (*Hippophae rhamnoides* L.) were reported to be in the same concentration range (1.2–1.8 mg/g whole seeds) as of the black currant PR (Yang, Karlsson, Oksman, & Kallio, 2001). The black currant PR phytosterol profile was dominated by the sitosterol fraction with 92.5% of total phytosterols. The sitosterol fraction was lower in blueberry and lingonberry (85 and 80% in free sterols, 57 and 42% in esterified sterols, respectively), and also the campesterol fraction (7 and 6% in free sterols, 5 and 3% in esterified sterols, respectively) was lower in these two berries (Yang et al., 2003). Our analysis quantified the most dominating phytosterol peaks, leading to different relative portions compared to the literature. In general, phytosterols in seeds range between 22 and 714 mg/100 g seeds (Pegel, 1997).

3.4. Antioxidant parameters (TEACs, total phenols as GAE, vitamin C)

The TEAC II values varied between 5.8 (bilberry) and 84.4 (elder) μmol/100 g (Fig. 1). Black currant showed higher TEAC II values (74.7 and 67.2 μmol/100 g) compared to the other species PRs tested. TEAC III values ranged between 19.5 and 83.9 μmol/g and thus were approximately 150 times higher than the TEAC II values. Both TEAC assays measured the ability of berry seed PR extracts to reduce *in vitro* formed radicals (Böhm & Schlesier, 2004). The TEAC value of an extract is the product of a sum of antioxidant compounds that will depend on the solvent used to extract the test matrix (Schlesier et al., 2002). TEAC II measurements performed using hexane comprised tocopherols and lipophilic compounds that are not included when extracting with water (TEAC III) that includes only hydrophilic compounds (Schlesier et al., 2002). The latter are obviously more abundant in the PRs than lipophilic compounds. These characteristic features of the TEAC tests were reflected in the correlation coefficients of the experimental results: the TEAC II values of the PRs correlated significantly with the PR tocopherols ($R = 0.893$, $p = 0.017$). TEAC III demonstrated no significant correlation with the total tocopherol content ($R = 0.033$, $p = 0.945$), TEAC II values ($R = -0.371$, $p = 0.469$), vitamin C ($R = 0.533$, $p = 0.173$) or GAE ($R = 0.231$, $p = 0.582$). One group of hydrophilic compounds responsible for high TEAC III values can be flavonoids that exist in the PRs. Anthocyanins as delphinidin and cyanidin bound as rutinosides and some glucosides are the major forms found within the black currant PR, where the whole berry showed a similar composition like the seed (Kapasakalidis, Rastall, & Gordon, 2006; Lu & Foo, 2003). In addition black currant berries contained high concentrations of proanthocyanidines, mostly found as procyanidin and prodelphinidin polymers (Wu, Gu, Prior, & McKay, 2004). Commonly utilized methods are incapable of extracting these compounds from the sample and proanthocyanidines were not determined in this work. Most of the antioxidant capacity determinations revealed higher antioxidant capacity for elder berries than for black currant berries (Wu et al., 2004). Our results of TEAC III and GAE for the PRs in part verified these differences (Figs. 1 and 2). The comparison of TEAC III values of the PRs (19.5–83.9 μmol/g) to other fruits like prunes (14.8 μmol/g) and figs (5.02 μmol/g) revealed that PRs contained significant levels of antioxidants similar to strong antioxidant herbs (rosemary, 43.95 μmol/g) (Pellegrini et al., 2006).

As demonstrated in black currant, there is an enormous variation in the GAE within one species (Fig. 2). The analysis of four different *Ribes nigrum* cultivars revealed GAE-concentrations between 0.72 and 1.16 g/100 g of berries (Costantino, Albasini, Rastelli, & Benvenuti, 1992). Vinson, Su, Zubik, and Bose (2001) quantified 0.256 g/100 g in fresh bilberry, and 0.221 g/100 g in bilberry PR. Grapes known as a rich source of polyphenols contained a GAE of 0.294 g/100 g (Vinson et al., 2001). Generally, GAE represents the total phenolic content of a sample and utilizes gallic acid as a standard phenol which exhibits the strongest antioxidant capacity in several test systems when compared with other antioxidants like uric acid, Trolox®, and ascorbic acid (Schlesier et al., 2002; Stratil, Klejdus, & Kubáň, 2006). GAE was associated with the vitamin C ($R = 0.661$, $p = 0.075$), the TEAC II values ($R = -0.755$, $p = 0.140$), and the tocopherol concentrations ($R = -0.783$, $p = 0.066$) in the PRs. Thus, PRs with a high tocopherol content and TEAC II values possessed low levels of vitamin C and GAE, and *vice versa*. Vitamin C concentrations varied between 15.0 mg/100 g (strawberry) and 68.6 mg/100 g (bilberry), and were observed in a similar range as fresh black currant juice (39.2 mg/100 mL, Young et al., 1999). The Folin-Ciocalteu reagent reacts also with ascorbic acid, leading to the correlation between vitamin C and GAE (Stratil et al., 2006).

3.5. Fibre

The mean total fibre content in the tested PRs was 60 mg/100 g, being highest in rose hip (71.3 mg/100 g) and lowest in black currant II (48.2 mg/100 g) and cranberry (48.5 mg/100 g). Black currant I, bilberry, strawberry and elder contained 58.6–59.4 mg/100 g. For a better characterization of the fibre, a detergent fibre analysis was performed with black currant PR. The NDF (insoluble fibres) was 42.5 g/100 g, the soluble fibres were 57.5 g/100 g and the ADF was 18.5 g/100 g. The insoluble fibre consisted of 8.2 g/100 g lignin (cutin was not determined and removed from the

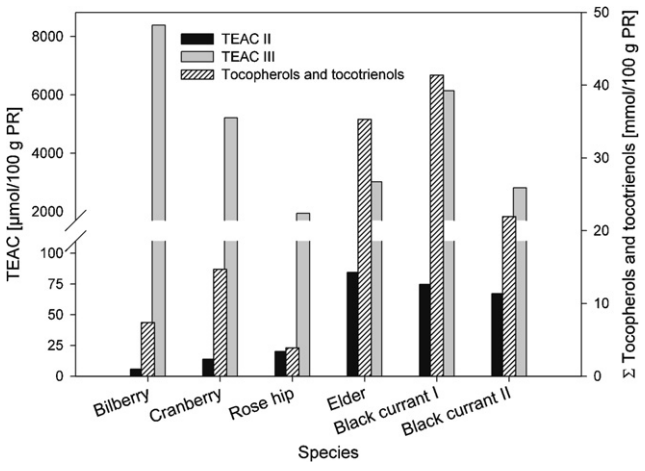

Fig. 1. Trolox equivalent antioxidant capacities (TEAC II and III) and total tocopherol concentrations of various species of berry seed press residues (PR) given as means of duplicate or triplicate measurements.

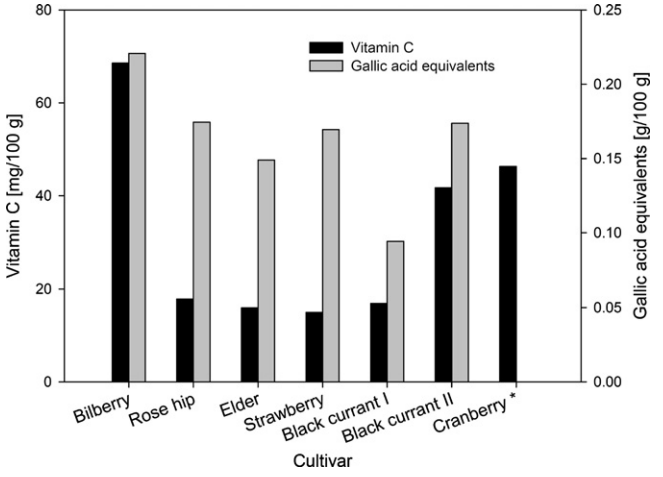

Fig. 2. Gallic acid equivalent (GAE) and vitamin C concentrations in various berry seed press residues (PR) given as means of duplicate or triplicate measurements
* GAE not determined.

ADL fraction), 10.3 g/100 g cellulose, and 24.0 g/100 g hemicellulose. Nawirska and Kwaśniewska (2005) determined 91% total fibre in the dry matter of black currant pomace after fruit processing. The fresh fruit pomace and the seed PR had same levels of hemicellulose (25% and 24%, respectively) and cellulose (12% and 10%, respectively) suggesting that fruit pomace and PRs had similar fibre content. Both matrices had a dry matter content of about 93%. The lignin content in fruit pomace was 73.8 g/100 g of dry matter which was much greater compared to the seed residue with 8.2 g/100 g (Nawirska & Kwaśniewska 2005).

3.6. Protein and amino acids

The protein is a quantitatively important fraction in the PRs (Table 4). The most frequent AA was glutamine (17%), followed by aspartic acid (9%) and arginine (8%).

Based on the WHO AA requirements, in the tested matrices (black currant PR I, II, and whole seeds) tryptophan was the limiting AA for adults (AAR 2.0/2.2/2.2). For the 10–12-year-old adolescents lysine was the limiting AA in each matrix (AAR 1.1), and additionally tryptophan in black currant PR I (AAR 1.1). The ratio of essential AAs to whole egg AAs estimates the BQ. Compared to whole egg, the lower BQ values were due to the imbalance between the concentrations of the essential AAs in the PR and seeds. The EAAI is another classification parameter for protein which is closely associated to the BQ. The EAAI reflected the same quality as the BQ values (Table 4). The protein of the black currant seeds is of good quality, evident by the AAR of >1 for all the essential AAs. These AAs features evaluated do not take protein digestibility into consideration which is thought to be as important as the protein quality, but which was not investigated in this study.

3.7. Dry matter, ash content, and elements

PR of black currant was rich in dry matter (93.5%) of which 3.96% was ash containing the elements K (6.99 mg/g) > P (6.12 mg/g) > Ca (4.83 mg/g) > S (2.51 mg/g) > Mg (1.89 mg/g). Moderate concentrations of the following elements were detected: Fe at 0.19 mg/g, and Na, Al, Zn, Mn, and Cu at <0.1 mg/g. The concentrations of As, Ba, Cd, Co, Cr, Ni, Mo, Pb, Se, Sr, Ti, and V were <0.01 mg/g.

The PRs had a high concentration of iron relative to other plants. In comparison, oat and wheat flour contained 0.023 and 0.011 mg/g, respectively (Cook, Reddy, Burri, Juillerat, & Hurrell, 1997). The concentration of iron in the PR may be elevated due to abrasions of the mill during processing.

Table 4
Content of amino acids, crude protein and parameters of protein quality* in black currant seed press residues and whole seeds given as means of duplicate measurements [g/16 g N]

		Black currant I	Black currant II	Black currant (whole seeds)
Amino acid [g/16 g N]	Cys	2.3	2.7	2.8
	Asp	8.6	9.1	9.4
	Met	1.9	2.1	1.7
	Thr	3.6	4.0	3.3
	Ser	3.9	3.8	3.8
	Glu	17.4	17.8	17.8
	Gly	4.8	5.0	5.2
	Ala	4.0	3.9	4.2
	Val	2.9	2.9	3.1
	Ile	4.5	4.6	4.9
	Leu	5.6	5.8	6.1
	Tyr	2.2	2.3	2.5
	Phe	3.8	3.9	4.1
	His	3.5	3.5	3.8
	Lys	4.4	4.7	5.0
	Arg	7.5	8.0	8.3
	Pro	3.3	3.7	3.8
	Hypro	0.0	0.0	0.0
	Trp	1.1	1.1	1.1
	Sum	85.3	88.9	90.9
Crude protein [mg/100 g residue]		21.5	22.5	19.4
EAAI		0.72	0.75	0.76
BQ*		63	67	68

EAAI: Essential amino acid-index.
BQ: Biological quality (approximated).
* Ratio of essential amino acids to whole egg amino acids

4. Conclusions

The PRs tested contained moderate to high concentrations of tocopherols and some tocotrienols. In addition, there was a favourable $n-6/n-3$ ratio and a high PUFA content of >65% with linoleic acid predominating (>34%). The content of stearidonic acid in black currant was conspicuously high.

Some of the PRs were distinguished by moderate to high concentrations of GAE, comparable with fresh grapes for example. The water soluble fraction of the PRs showed high TEAC values that were at least as high as that of herbs. These results need to be put into perspective, because PRs cannot be consumed in the same amount or manner as grapes or apples. The protein quality of black currant seeds was of good grade, and contained all essential AAs in fairly balanced proportions. Phytosterols and carotenoids were present only in trace amounts.

In summary, berry PRs contained several components with health-promoting attributes. Future processing may enable us to convert these residues into edible products so their potential can be utilized.

Acknowledgements

We would like to thank the BMBF (Federal Ministry of Education and Research) which provided financial support for this project in the Rephyna® group. We would also like to thank the IGV GmbH Bergholz-Rehbrücke who provided the test materials.

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