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Research Article

Supercritical Fluid Extraction of Berry Seeds: Chemical Composition and Antioxidant Activity

Graziele Gustinelli ^{1,2}, Lovisa Eliasson,¹ Cecilia Svelander,² Thomas Andlid,² Leif Lundin,¹ Lilia Ahrné,^{1,2,3} and Marie Alminger²

¹Department of Bioscience and Materials, Research Institutes of Sweden, Gothenburg 402 29, Sweden

²Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg 412 96, Sweden

³Department of Food Science, Ingredient and Dairy Technology, University of Copenhagen, Copenhagen 1958, Denmark

Correspondence should be addressed to Graziele Gustinelli; graziele.gustinelli@ri.se

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The influence of supercritical fluid extraction (SFE) and solvent extraction of oils from cloudberry, bilberry, and black currant seeds on the yield, chemical properties, and recovery of antioxidant compounds was investigated. SFE was performed for 1 h at 350 bar and at 50°C and 80°C. Fatty acids, vitamin E, carotenoids, and free radical-scavenging activity (DPPH) were assayed. SFE at 80°C resulted in higher oil yields for cloudberry and black currant seeds. The oils were rich in polyunsaturated fatty acids (PUFAs) (66.8%–75.9% w/w), with high percentages of linoleic and α -linolenic acids. The black currant seed extracts had the highest concentrations of vitamin E (range, 113.0–241.8 mg/100 g oil) and carotenoids (range, 11.5–32.3 mg/100 g oil) and the highest antioxidant activity. The cloudberry seed oils also had high antioxidant content and activity. These findings indicate the potential of SFE for the recovery of PUFA and antioxidant compounds in berry by-products.

1. Introduction

Berries are a common raw material that is processed in the food and juice industry. However, the processing of berries generates large amounts of solid by-products in the forms of peel, pulp, and seeds, which are mostly under utilised [1]. The wastes are usually burnt or deposited in landfills and hence, cause significant impact on the environment [2]. An alternative approach that reduces the amount of waste produced and promotes socioeconomic and environmental benefits is the valorisation of by-products through the extraction of valuable compounds [3].

Berry seeds are rich in oil, and their utilisation offers several advantages as their lipids generally have a high content of polyunsaturated fatty acids (PUFAs) and a favourable $n-6/n-3$ ratio compared to other vegetable oils [4, 5]. In addition, these oils are rich in vitamin E, carotenoids, and bioactive compounds with antioxidant activities [5].

Supercritical fluid extraction (SFE) is a green method for the extraction of valuable compounds from berry seeds, in contrast to conventional extraction methods that use hazardous organic solvents. The advantages of working with SFE are reduced solvent use, shorter extraction time, and lower energy consumption, as compared with the conventional extraction methods, such as solvent extraction and cold-pressing. SFE also allows the fractionation of compounds by adjusting the pressure and temperature based on the solubilities of the targeted compounds [6]. Moreover, the solvent is easily separated from the extract by depressurisation of the supercritical fluid, thereby saving time, and the fact that organic solvents are not used makes the extract suitable for use in the food industry [7]. The selectivity of SFE for the extraction of target compounds has previously been reported for the extraction of antioxidants in oil from grape seeds [3, 8] and vitamin E and carotenoids from other berries [4, 9]. However, there is little information available

regarding the recovery using SFE of antioxidants from berry seeds.

The plant matrix is an important parameter for extraction [7]. Matrix pretreatments, such as milling, can increase the extraction efficiency by breaking the cells, thereby increasing the surface area for mass transfer [10]. The oil at the surface and in the shallow subsurface is quickly extracted, whereas the oil inside the intact cells, in the particle core, has stronger mass transfer resistance [11]. A better understanding of how the different kinds of berry matrices and cell structure morphologies are affected by milling is essential to improve the extraction efficiency.

The objective of this study was to investigate the yields, chemical compositions, and recovery of antioxidant compounds of SFE-extracted oils from the seeds of cloudbberries, black currants, and bilberries. The oils were extracted by SFE at different temperatures (50°C and 80°C) and compared with oils that were obtained from the same seeds using conventional solvent extraction with hexane. In addition, the microstructures of seed particles were visualised using light microscopy to elucidate the influence of cell morphology on the extraction efficacy.

2. Materials and Methods

2.1. Chemicals. Carbon dioxide (>99.99%) was purchased from AGA Gas AB (Växjö, Sweden). The internal standard heptanoic (margaric) acid 17:0 (≥99%) and the external standards of FAME mixture GLC 463 and stearidonic acid 18:4 n-3 were purchased from Nu-Chek Prep Inc. (Elysian, USA). DL α -tocopherol (≥97%) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Alfa Aesar GmbH (Karlsruhe, Germany). The standards δ -tocopherol (95.5%) and γ -tocopherol (97.3%) were from Supelco (Bellefonte, PA, USA), and α -tocotrienol (97%), (*R*)- γ -tocotrienol (≥97.0%), and acetyl chloride (≥99.0%) were obtained from Fluka, Sigma-Aldrich (Stockholm, Sweden). Methanol (LC-MS grade ≥99.9%), toluene (HPLC grade 99.9%), cyclohexane (HPLC grade ≥99.7%), petroleum ether (ACS reagent, ≥95.0%), *tert*-butyl methyl ether (MTBE) (HPLC grade ≥99.8%), and Tween 80 were all from Sigma-Aldrich (Stockholm, Sweden). 2,2,4-Trimethylpentane (HPLC grade 99.5%) was purchased from Labscan (Dublin, Ireland). Ethanol absolute (AnalaR NORMAPUR) was from VWR International (Spånga, Sweden).

2.2. Berry Seeds. The berries were picked in Norrbotten, northern Sweden. Cloudbberries (*Rubus chamaemorus*) and bilberries (*Vaccinium myrtillus* L.) were grown wild, and the black currants (*Ribes nigrum* L.) were cultivated. The seeds were separated from the press cake (obtained after juice removal) in a pureeing machine (Robot Coupe C200) and stored at -40°C. The seeds were thawed at 4°C and dried in a hot-air oven at 40°C (Garomat 142; Electrolux AB, Stockholm, Sweden) until a moisture content of approximately 6.5% was obtained. After drying, the seeds were kept in the freezer at -40°C until use in the extraction experiments. Immediately before extraction, the seeds were

ground for 30 s in a coffee mill (model 2393; OBH Nordica, Stockholm, Sweden).

2.3. Solvent Extraction. The extractions were performed with a mixture of 1 g of milled seeds and 10 mL of hexane. The mixtures were mixed for 2 h in an orbital rotary plate (Reax-2; Heidolph GmbH, Schwabach, Germany) at 150 rpm. After shaking, the mixtures were centrifuged in a Heraeus-Kendro Multifuge 1S (Heraeus GmbH, Hanau, Germany) for 10 min (3000 × *g*). The supernatants were collected, and the procedure was repeated. The supernatants were pooled, and the solvent was evaporated by flushing the samples with N₂ at 40°C. The evaporated extracts were stored at -18°C until the analysis. The extractions were performed in 18 replicates for bilberry seeds and 30 replicates for cloudberry and black currant seeds. A high amount of extractions were carried out to ensure that there was sufficient oil for the analysis.

2.4. Supercritical Fluid Extraction (SFE) of Berry Seeds. The supercritical fluid extractions were carried out in triplicate, using a laboratory-scale supercritical fluid system (SFE-500M1-2-C50; Waters Corp., Milford, MA, USA). The unit consisted of a CO₂ pump connected to a cooling bath (F32-HD; Julabo GmbH, Seelbach, Germany), an automated back-pressure regulator, a cylindrical extractor vessel of volume 500 mL equipped with a heating jacket, and one separation vessel (500 mL) kept at 10 bar and 25°C. Ground seeds (50 ± 0.5 g) were loaded into the extraction vessel. For each extraction, 2 g of glass wool was used to protect the vessel filters and to fill up the empty space of the vessel. The extractions were carried out for 60 min at a flow rate of 30 g CO₂/min. The extraction pressure was 350 bar, and the extraction temperature was 50°C or 80°C. The extracts obtained after SFE were used for estimation of fatty acids, tocopherols, tocotrienols, carotenoids, and DPPH. Due to the small volumes obtained from each extraction, the triplicates were pooled; hence, this limited the interpretation of the results. The extracts were stored at -18°C until the analysis.

2.5. Light Microscopy (LM) Analysis. The milled seeds were smeared on the surface of a microscope slide, and a droplet of water was added to disperse the fragments of seeds. The microstructures of the berry seeds were examined under a Microphot FXA microscope (Nikon, Tokyo, Japan). Images were acquired with an Altra 20 camera (Olympus, Tokyo, Japan), and the objective lenses ×10 and ×20 were used.

2.6. Fatty Acid Analysis by Gas Chromatography (GC). The methylation of fatty acids from bilberry, black currant, and cloudberry seed oils was based on the method of Cavonius et al. [12]. Toluene (1 mL) was added to the extracts (0.025 ± 0.005 g) and then mixed with 1 mL of freshly prepared 10% (v/v) acetyl chloride in methanol. The solutions were incubated for 2 h at 70°C. After cooling to room temperature, 1 mL of Milli-Q water was added, followed by

2 mL of petroleum ether. The solutions were vortexed and centrifuged for 5 min at 2500 $\times g$. The supernatant that contained the methyl esters of fatty acids (FAMES) was collected in a new tube, evaporated under N_2 at 40°C, and redissolved in 1 mL of 2,2,4-trimethylpentane. The samples were analysed by using a GC (7890 A; Agilent Technologies, Santa Clara, CA, USA) with a triple-axis mass spectrometric (MS) detector in the electron impact mode (5975 C; Agilent Technologies) and equipped with a J&W DB-wax column (30 m \times 0.250 mm \times 0.25 μ m). Helium was used as the carrier gas. External standards were used for the identification of the peaks, and an internal standard (17:0) was used for the quantification of fatty acids. The limit of detection was 50 μ g/mL for 17:0. The results were calculated in grams of FA per 100 g of oil.

2.7. Determination of Tocopherols and Tocotrienols by High-Performance Liquid Chromatography (HPLC). Methanol (2 mL) was added to 0.04 \pm 0.005 g of extracts and vortexed. The samples were sonicated for 15 min. A 1 mL aliquot of the upper phase was collected and transferred to a vial. The samples were analysed by HPLC coupled with an on-line fluorescence detector (RF-551; Shimadzu, Kyoto, Japan). A reverse phase C18 Kromasil column (pore size, 100 Å; L \times ID, 250 mm \times 2.1 mm; and particle size, 5 μ m) was used to separate the tocopherols and tocotrienols. The mobile phase was composed of a methanol:water mix (95:5 v/v). Tocopherols and tocotrienols were detected at 295 nm excitation and 330 nm emission wavelengths. The limits of detection were 2.5 μ g/mL for α -tocopherol, 1.25 μ g/mL for γ -tocopherol, 0.3135 μ g/mL for δ -tocopherol, 0.1 μ g/mL for α -tocotrienol, and 0.3125 μ g/mL for γ -tocotrienol. The peaks were identified using standards and quantified by calibration curves using their linear regression equations. The correlation coefficients (R^2) of the standard curves were 0.9989, 0.9996, 0.9991, 0.9972, and 0.9992 for α -tocopherol, γ -tocopherol, δ -tocopherol, α -tocotrienol, and γ -tocotrienol, respectively. The concentrations were confirmed spectrophotometrically based on the UV absorption profiles of pure tocopherols at their maximum wavelengths [13]. The results are expressed in mg of vitamin E/100 g berry seed oil.

2.8. Determination of Carotenoids by HPLC. *Tert*-butyl methyl ether (MTBE; 1 mL) was added to 0.04 \pm 0.005 g of extracts and vortexed. The samples were transferred to amber vials and analysed by using HPLC equipped with a UV-visible photodiode array detector (996, Waters; Millipore, MA, USA). The carotenoids were separated using reverse-phase elution on a C30 column (5 μ m, 250 \times 4.6 mm ID; YMC Europe GmbH, Schermbach, Germany). The absorption spectrum from 250 nm to 550 nm was measured. The limits of detection were 62.2 μ g/mL for β -carotene and 312.5 μ g/mL for lutein. The mobile phase consisted of methanol and MTBE. The following gradient mixture was used for black currant and bilberry seed oils: isocratic elution with 85% (v/v) MeOH and 15% (v/v) MTBE for 2 min; the gradient was then built up over 9 min with 75% MeOH and 25% MTBE; for the next 12 min, the gradient attained 10%

MeOH and 90% MTBE; in the following 3 min, the gradient reached the initial composition of 85% MeOH and 15% MTBE, followed by isocratic elution for the final 4 min. For cloudberry seed oil, the gradient mixture was obtained as follows: the initial conditions of 85% MeOH and 15% MTBE were established over 6 min as 75% MeOH and 25% MTBE, progressing to 60% MeOH and 40% MTBE over 1 min and 10% MeOH and 90% MTBE for 16 min, and the gradient was reset to the initial conditions over 3 min and equilibrated for an additional 6 min. Lutein and β -carotene were identified on the basis of their retention times and the spectral characteristics of pure standards, as described by Svelander et al. [14]. Quantifications were made using the linear regression equations from standard curves. The R^2 was 0.9991 for β -carotene and 0.9999 for lutein, for the analysis of black currant and bilberry seed oils, and 0.9978 for β -carotene and 1 for lutein, for cloudberry seed oils. The concentration of the standard solutions was previously determined spectrophotometrically based on the UV absorption profiles of pure β -carotene and lutein at their maximum wavelengths [15]. The results are expressed in mg of carotenoids/100 g of berry seed oil.

2.9. Free Radical-Scavenging Activity (DPPH Method). The antioxidant activities of the extracts were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, according to the procedure of Brand-Williams et al. [16]. All the solutions were prepared in ethanol. The samples were diluted to obtain five different concentrations, so as to determine the effective concentration (EC_{50}). An aliquot of 1 mL of each dilution was mixed with 1 mL of freshly prepared DPPH solution (100 μ M). Solutions of α -tocopherol were used as the positive control [17]. The mixtures were incubated in the dark at room temperature. After 1 h, the change in the absorbance after the reaction between radical scavengers and DPPH was measured at 517 nm in a spectrophotometer (Ultrospec 1000; Pharmacia Biotech (Biochrom) Ltd., Cambridge, England). The results were expressed in mg oil/mL of ethanol.

2.10. Statistical Analysis. The analyses were carried out in triplicate. Statistical significance was tested by performing analysis of variance (ANOVA), followed by a Tukey post hoc test (level of significance of $p = 0.05$). Correlation among EC_{50} and antioxidants (vitamin E and carotenoids) was calculated using Pearson's correlation coefficient (r) (level of significance of $p = 0.01$). The correlations were analysed for each antioxidant separately and for the sum of the EC_{50} (mg antioxidant/mL ethanol) and concentrations (mg antioxidant/100 g oil) of vitamin E and carotenoids.

3. Results and Discussion

3.1. Light Microscopy Analysis of Berry Seed Particles. The extraction process involves two different extraction periods; the first period is characterised by the rapid extraction of surface and shallow subsurface oil, while the second period proceeds more slowly and is controlled by diffusion of the

fluid into the matrix [11]. Figures 1(a)–1(c) show the LM images of the dry and ground seeds of cloudberry (a), black currant (b), and bilberry (c) before extraction. The pretreatments disrupted the seeds, revealing the interior part divided by the lamellar structure. The milling step broke several of the cells, releasing immediately some of the oil from the cells, which could be easily extracted. The oil droplets spread around the seed particles were dispersed from the particle surface by the addition of a water droplet to the objective glass. In Figure 1(a), the presence of a big oil droplet is shown (indicated by a white arrow). The milling step disrupted each berry seed in a different way, depending on the size of the seed and the cell shape. The seeds of cloudberry are larger and have higher density than the bilberry and black currant seeds [18]. Differences in the weights and sizes of seeds can produce heterogeneous particles after milling, with different shapes and sizes. The disrupted cells are indicated by arrows (black) in Figures 1(a)–1(c). Cloudberry seeds have the largest cells of the three berry types, while bilberry seeds have the longest and narrowest cells (Figures 1(a) and 1(c)), which may have facilitated the extraction from the inner part of the broken bilberry seed cells. Black currant seeds (Figure 1(b)) have the smallest cells and particles. Longer milling times might break the cloudberry and bilberry seeds into smaller particles with larger surface areas, resulting in more efficient extractions. Further investigations into the effects of pretreatments of the investigated berries are necessary to optimise the extraction.

The small cells and seed particles present a larger membrane surface area, facilitating the extraction of non-polar compounds from the membranes, for example, of the vitamin E located in the cell membranes [19]. Milling of the seeds broke the cell membranes and released to the surface the oil than contains vitamin E. This may explain why a major fraction of the vitamin E was extracted during the first part of the extraction, which was followed by a decrease in the extraction rate due to mass transfer resistance from the cell membrane. As shown in Figures 1(a)–1(c), the particle cores have many intact cells, which may have decreased the extraction rates of other compounds present in the oil.

3.2. Extraction Yields. The yields and chemical compositions of the cloudberry, black currant, and bilberry seed oils were assessed after SFE at 50°C and 80°C or after hexane extraction (which was performed at room temperature and evaporated at 40°C). Figure 2 presents the yield% (w/w) values of the cloudberry, black currant, and bilberry seed oils. Significantly higher yields were obtained with hexane extraction compared to SFE. Studies comparing SFE and conventional extraction using hexane as solvent often report higher extraction yields with the conventional extraction method [20, 21]. However, since hexane is hazardous to workers and the environment, more gentle extraction procedures are needed [22]. In addition, a higher yield may not reflect the quality of the extracts. Johansson et al. [18] assessed the oil contents of berry seeds with moisture contents of 6%, similar to the current work, using the Folch

procedure, which is another conventional extraction method. They reported for cloudberry, black currant, and bilberry seeds oil content of 11.9%, 15.9%, and 30.5%, respectively. In the current work, lower oil levels were obtained with hexane extraction, which might be due to the differences in the type of cultivar, geographical origin, and the genotype [23]. The highest yields were obtained for bilberry seeds. Gustinelli et al. [24] have reported a higher extraction yield (21.78%) for bilberry seed oil extracted at 350 bar and 50°C. Since extraction time and flow rate are important parameters for SFE, the higher value seen in the present study might be explained by the longer extraction time (80 min) and higher flow rate (40 g CO₂/min). The measured yields were 2.0% and 18.8% for black currant seeds extracted by SFE at 50°C and for bilberry seeds extracted by hexane, respectively. For SFE, the yields of cloudberry and black currant seed oils were higher at 80°C than at 50°C. The temperature is considered to influence the yield of oil based on two counteracting forces [25]. At constant pressure, an increase in temperature decreases the solvent density, leading to a decrease in solubility. However, an increase in temperature increases the vapour pressure of the solutes, thereby improving the solubility of the oil during exposure to SC-CO₂. The effect of the increase in temperature depends on which one of the two forces is predominant. In the present study, the vapour pressure predominated during SFE of the black currant and cloudberry seeds at 350 bar. In mixtures of similar compounds, the intermolecular interactions are similar, and the vapour pressure depends on the molecular weights of the compounds [26]. However, in complex mixtures such as berry seed oils, complex intermolecular interactions result in significant differences compared with solubilities of the pure lipids (fatty acids and mono-, di-, and triacylglycerols) [27]. Therefore, the vapour pressure and its effects on the extraction yields of different berry seeds extracted under the same conditions are more difficult to predict.

3.3. Fatty Acid Composition. Table 1 shows the fatty acid compositions of cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction. The major fatty acids were linoleic (18:2 $n-6$) and α -linolenic acid (18:3 $n-3$). Only the bilberry seed oil had $n-6/n-3$ ratios ≤ 1 . The sum of the PUFAs ranged from 66.8% to 75.9% of the total fatty acids for bilberry seeds extracted by hexane and for black currant seeds extracted by SFE at 50°C, respectively. There were small but significant differences in the fatty acid compositions of the oils obtained by either SFE or hexane extraction. The largest relative compositional difference measured was the concentration α -linolenic acid (18:3 $n-3$), which was significantly lower in the black currant seed oils extracted by hexane. The α -linolenic acid (18:3 $n-3$) is mostly in the triacylglycerol fraction and therefore, its concentration relies on the solubility of the triacylglycerols, which depends on their molecular weights [28]. Johansson et al. [28] have reported 26 different triacylglycerol species for black currant seed oil.

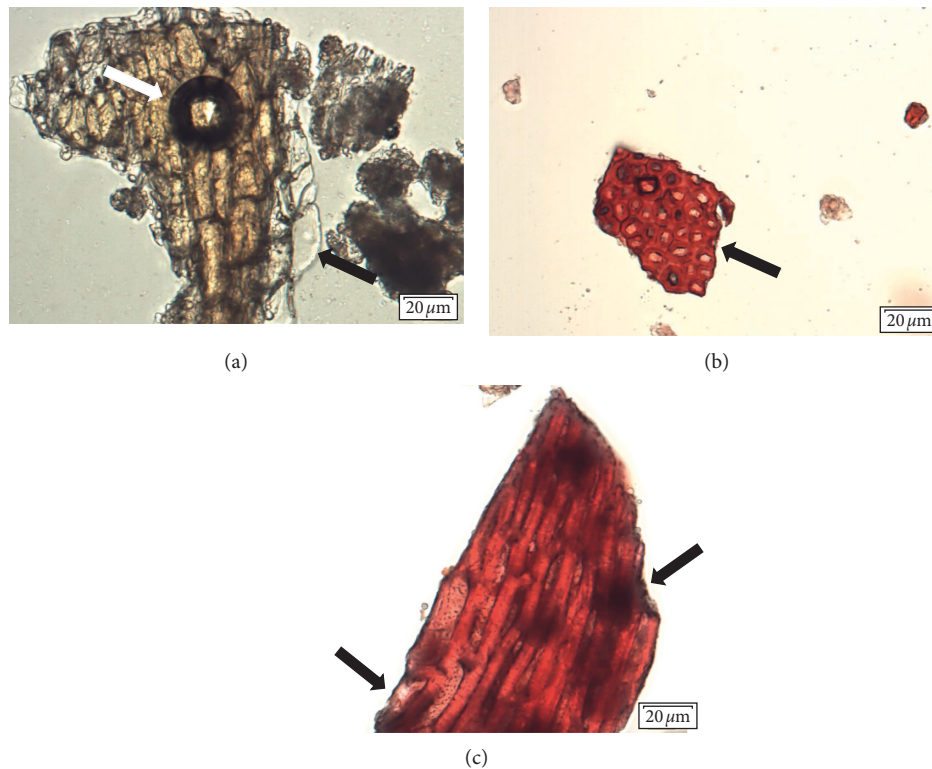


FIGURE 1: Seeds of cloudberry (a), black currant (b), and bilberry (c) dried and milled for 30 s. White arrow indicates oil droplet released after milling. Black arrows indicate disrupted cells.

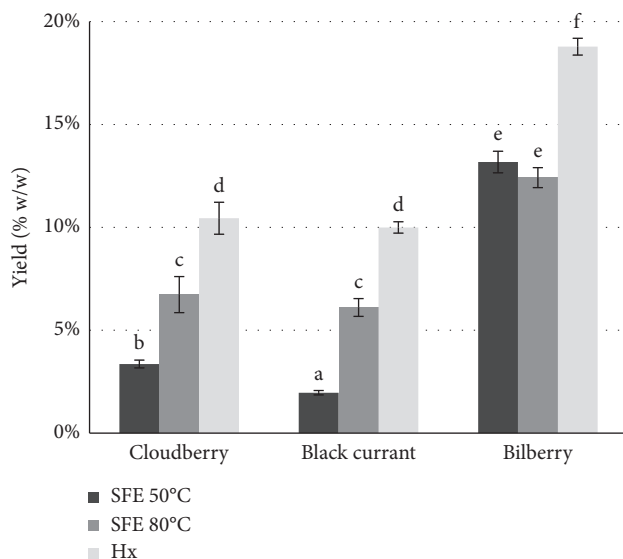


FIGURE 2: Extraction yield (% w/w) of cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction. Results are mean values \pm standard deviation of triplicate extractions. Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test.

All the oils analysed had high percentages of PUFA and the major fatty acids were linoleic and α -linolenic acid. Furthermore, the black currant seed oil had a high level of

γ -linolenic acid (18:3 $n-6$) and was the only berry oil in which stearidonic acid (18:4 $n-3$) was detected. Both γ -linolenic and stearidonic acids are associated with reduced inflammation [29, 30]. Similar results for the fatty acid profile have been reported in previous studies of SFE of cloudberry, black currant, and bilberry seed oils [4, 24, 31, 32].

3.4. Recovery of Antioxidants and Antioxidant Activity. Table 2 lists the contents of tocopherols and tocotrienols in the berry seed oils. The major vitamin E isomers detected in the seed oils from cloudberry, black currant, and bilberry were γ -tocopherol, α -tocopherol, and γ -tocotrienol, respectively. The highest concentration of vitamin E was obtained in black currant seed oil extracted by SFE at 50°C, while bilberry seed oil extracted by hexane extraction had the lowest vitamin E content. For cloudberry seeds, hexane extraction resulted in the highest recovery of vitamin E. Increasing the temperature of SFE enhanced the extraction of vitamin E for cloudberry and bilberry seeds; although for black currant seeds, increasing the temperature was found to decrease the extraction yield. In general, the oils with lower yields contained higher concentrations of vitamin E. A strong negative correlation between the oil yield and the concentration of vitamin E was found ($r = -0.846$, $p = 0.01$), which suggests that most of the vitamin E could be extracted. Vitamin E is soluble in triacylglycerols and can be easily extracted during SFE of seeds [25]. In plant seeds, the vitamin E is mostly located in the membranes [33]. The

TABLE 1: Fatty acid composition of cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction.

Oils	Fatty acids% (w/w)											$n-6/n-3$ ratio
	16:0	18:0	18:1 $n-9$	18:1 $n-7$	18:2 $n-6$	18:3 $n-3$	18:3 $n-6$	18:4 $n-3$	20:0	20:1 $n-9$	$n-6/n-3$ ratio	
Cloudberry 50°C	3.7 ± 0.06 ^a	1.8 ± 0.04 ^{ab}	16.0 ± 0.15 ^b	1.0 ± 0.05 ^a	41.6 ± 0.21 ^{bc}	32.4 ± 0.08 ^d	1.5 ± 0.02 ^a	n.d.	1.1 ± 0.04 ^a	0.9 ± 0.03 ^c	1.3 ± 0.00 ^b	
Cloudberry 80°C	3.4 ± 0.04 ^a	1.8 ± 0.03 ^{ab}	16.6 ± 0.04 ^b	1.0 ± 0.01 ^a	41.3 ± 0.12 ^{bc}	33.8 ± 0.04 ^{ef}	n.d.	n.d.	1.2 ± 0.03 ^b	0.9 ± 0.03 ^c	1.2 ± 0.00 ^b	
Cloudberry Hx	3.4 ± 0.01 ^a	1.9 ± 0.01 ^{abc}	16.6 ± 0.04 ^b	1.0 ± 0.01 ^a	40.8 ± 0.04 ^b	34.1 ± 0.12 ^{ef}	n.d.	n.d.	1.2 ± 0.01 ^b	1.0 ± 0.01 ^d	1.2 ± 0.01 ^b	
Black currant 50°C	7.9 ± 0.04 ^c	2.1 ± 0.02 ^{bc}	12.3 ± 0.04 ^a	1.0 ± 0.03 ^a	42.5 ± 0.04 ^{cd}	16.4 ± 0.01 ^b	13.4 ± 0.02 ^c	3.5 ± 0.07 ^a	n.d.	0.8 ± 0.01 ^b	2.8 ± 0.01 ^c	
Black currant 80°C	7.7 ± 0.08 ^c	2.1 ± 0.06 ^{bc}	13.0 ± 0.08 ^a	1.1 ± 0.02 ^a	42.1 ± 0.42 ^{bc}	17.5 ± 0.11 ^c	12.7 ± 0.04 ^b	3.2 ± 0.22 ^a	n.d.	0.7 ± 0.03 ^a	2.7 ± 0.06 ^c	
Black currant Hx	7.7 ± 0.64 ^c	2.4 ± 0.05 ^c	12.9 ± 0.12 ^a	1.1 ± 0.05 ^a	44.1 ± 0.09 ^d	14.5 ± 0.60 ^a	12.8 ± 0.26 ^b	3.6 ± 0.39 ^a	n.d.	0.8 ± 0.02 ^b	3.2 ± 0.18 ^d	
Bilberry 50°C	5.9 ± 0.14 ^b	1.6 ± 0.03 ^a	24.2 ± 0.85 ^c	1.0 ± 0.12 ^a	32.6 ± 15.6 ^a	34.7 ± 0.59 ^f	n.d.	n.d.	n.d.	n.d.	0.9 ± 0.06 ^a	
Bilberry 80°C	6.1 ± 0.50 ^b	1.8 ± 0.49 ^{ab}	23.9 ± 0.05 ^c	1.1 ± 0.06 ^a	33.4 ± 0.46 ^a	33.7 ± 0.43 ^e	n.d.	n.d.	n.d.	n.d.	1.0 ± 0.00 ^a	
Bilberry Hx	6.1 ± 0.16 ^b	1.7 ± 0.10 ^{ab}	24.3 ± 0.13 ^c	1.1 ± 0.04 ^a	32.9 ± 0.63 ^a	34.0 ± 0.22 ^{ef}	n.d.	n.d.	n.d.	n.d.	1.0 ± 0.02 ^a	

Results are mean values ± standard deviation of triplicates, and different letters within the same column indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test. n.d. = not detected.

TABLE 2: Content of tocopherols (T) and tocotrienols (Tr) in cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction.

Oils	mg/100 g oil						mg/100 g dry seeds
	α -T	γ -T	δ -T	α -Tr	γ -Tr	Total	Total
Cloudberry 50°C	38.2 ± 1.44 ^d	67.1 ± 2.95 ^b	4.9 ± 0.01 ^b	n.d.	0.8 ± 0.03 ^a	111.0 ± 5.38 ^c	3.7 ± 0.18 ^a
Cloudberry 80°C	40.2 ± 1.34 ^d	74.9 ± 1.91 ^{bc}	4.9 ± 0.03 ^b	0.2 ± 0.00 ^a	1.1 ± 0.01 ^a	121.3 ± 4.67 ^{cd}	8.2 ± 0.31 ^c
Cloudberry Hx	40.3 ± 0.13 ^d	84.6 ± 1.53 ^c	5.5 ± 0.05 ^b	n.d.	1.0 ± 0.06 ^a	131.5 ± 2.37 ^d	13.7 ± 0.25 ^e
Black currant 50°C	122.1 ± 1.24 ^f	102.7 ± 6.10 ^d	16.0 ± 0.67 ^d	0.3 ± 0.02 ^b	0.7 ± 0.02 ^a	241.8 ± 9.65 ^f	4.7 ± 0.19 ^b
Black currant 80°C	93.2 ± 1.21 ^e	87.4 ± 0.02 ^c	13.7 ± 0.06 ^c	0.3 ± 0.01 ^b	2.4 ± 0.01 ^a	197.0 ± 1.58 ^e	12.0 ± 0.09 ^d
Black currant Hx	30.7 ± 1.11 ^c	69.7 ± 1.78 ^b	12.5 ± 0.17 ^c	n.d.	0.1 ± 0.02 ^a	113.0 ± 4.34 ^{cd}	11.3 ± 0.43 ^d
Bilberry 50°C	18.1 ± 0.12 ^b	3.6 ± 0.17 ^a	0.3 ± 0.01 ^a	0.6 ± 0.00 ^c	36.4 ± 2.15 ^c	59.1 ± 3.14 ^b	7.8 ± 0.41 ^c
Bilberry 80°C	25.8 ± 0.32 ^c	4.8 ± 0.00 ^a	0.5 ± 0.01 ^a	0.7 ± 0.01 ^d	37.4 ± 1.84 ^c	69.3 ± 3.06 ^b	8.6 ± 0.38 ^c
Bilberry Hx	4.7 ± 0.19 ^a	1.6 ± 0.03 ^a	0.2 ± 0.01 ^a	n.d.	10.7 ± 0.39 ^b	17.2 ± 0.85 ^a	3.2 ± 0.16 ^a

Results are mean values ± standard deviation of triplicates, and different letters within the same column indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test; n.d. = not detected.

milling damages the cells in the membranes (Figure 3), which could facilitate the extraction of the vitamin E in the initial phase of extraction. Shen et al. have reported a higher extraction rate at the beginning of extraction of α -tocopherol at similar pressure (310 bar) and low temperature (40°C) [34]. In the present work, oil samples with high yields had lower vitamin E concentrations because of a dilution effect from the other nonpolar compounds, such as mono-, di-, and tri-acylglycerols, which continued being recovered during the final part of the extraction.

Previous studies have reported the vitamin E concentrations for cloudberry, black currant, and bilberry seed oils extracted at 350 bar and 50°C [4, 24]. Yang et al. [4] reported a higher concentration of vitamin E for cloudberry seed oil (260 mg/100 g oil) and lower concentrations for black currant and bilberry seed oils (110 and 40 mg/100 g oil, resp.). Gustinelli et al. [24] have reported a higher concentration of vitamin E for bilberry seed oil (62.5 mg/100 g oil). The longer extraction time used in both studies (120 min and 80 min) may have influenced the recovery rates of antioxidants. Moreover, variations in the chemical composition of the initial material could also have resulted in differences in the eventual extracts.

The use of SFE at 350 bar and 50°C for the extraction of black currant seed oil was advantageous for the recovery of α -tocopherol (122 mg/100 g oil), whereas for bilberry seeds, extraction at 80°C was more efficient. Temperature is an important parameter for extraction, as it influences the solubility as well as the cellular matrix that contains the targeted compound. In plant matrices, the vitamin E is located in the cell membranes and forms complexes with lysophospholipids and free fatty acids [19]. As vitamin E is fat-soluble, it is concentrated in domains that are rich in lipids, thereby protecting the lipids from oxidation [35]. In plant seeds, the lipids (predominantly triacylglycerols) are also stored in organelles, called "oil bodies" [36]. When the seeds are dried and ground, the cell membranes and walls are broken and the oil bodies are easily accessed by the SC-CO₂ or the hexane, increasing their extractability.

Analyses of the levels of vitamin E in bilberry seed oil extracted by SFE (59 to 69 mg/100 g oil) and black currant

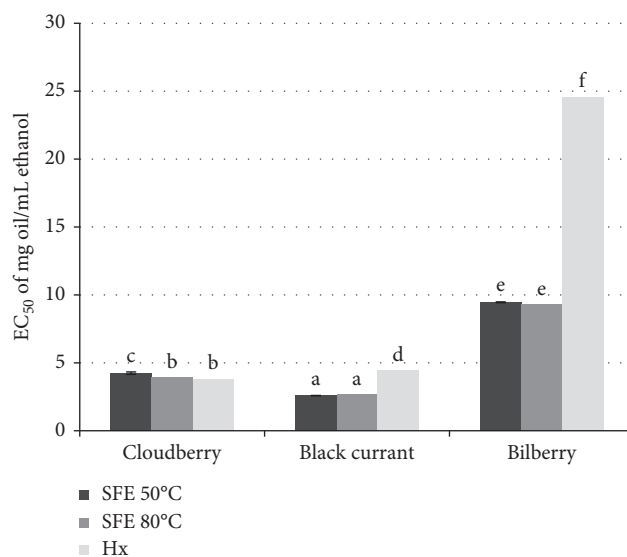


FIGURE 3: Values of EC₅₀ of cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction. Results are mean values ± standard deviation of triplicates. Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test.

seed oil extracted by SFE (242 to 113 mg/100 g oil) showed that the concentrations were higher than those reported in the literature for oils extracted with SFE (40 and 110 mg/100 g oil, resp.) [4]. In contrast, the concentration of vitamin E in cloudberry seed oil extracted by SFE (111 to 132 mg/100 g oil) was considerably lower than that described in the literature for cloudberry seed oils extracted by SFE (260 to 270 mg/100 g oil) [4, 9]. The differences in the concentrations of vitamin E compared to other studies may reflect differences in extraction efficiency between the different extraction methods, differences in drying and milling of the seeds before extraction and environmental and physical factors, such as weather conditions, geographical region, ripeness, and berry variety, all of which are known to affect the chemical composition of the raw material [37, 38].

TABLE 3: Content of carotenoids in cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction.

Oils	mg/100 g oil			mg/100 g seeds	
	Lutein	β -carotene	Carotene equivalents	Total	Total
Cloudberry 50°C	0.9 ± 0.00 ^a	4.3 ± 0.10 ^g	31.3 ± 0.09 ^f	36.5 ± 0.18 ^f	1.2 ± 0.01 ^d
Cloudberry 80°C	0.2 ± 0.00 ^a	2.6 ± 0.03 ^e	34.9 ± 0.14 ^g	37.7 ± 0.16 ^g	2.5 ± 0.01 ^e
Cloudberry Hx	n.d.	3.4 ± 0.04 ^f	53.5 ± 0.09 ^h	57.0 ± 0.06 ^h	5.9 ± 0.01 ^h
Black currant 50°C	12.1 ± 0.02 ^e	n.d.	4.8 ± 0.02 ^b	16.9 ± 0.02 ^e	0.3 ± 0.00 ^a
Black currant 80°C	10.1 ± 0.07 ^d	1.4 ± 0.0 ^d	1.7 ± 0.01 ^d	13.2 ± 0.09 ^c	0.8 ± 0.01 ^c
Black currant Hx	30.8 ± 0.12 ^f	1.5 ± 0.02 ^d	5.6 ± 0.13 ^e	38.0 ± 0.23 ^g	3.8 ± 0.03 ^g
Bilberry 50°C	2.4 ± 0.01 ^b	0.1 ± 0.00 ^a	0.4 ± 0.00 ^a	2.8 ± 0.02 ^a	0.4 ± 0.00 ^{ab}
Bilberry 80°C	3.2 ± 0.03 ^c	0.3 ± 0.01 ^b	0.6 ± 0.01 ^a	4.1 ± 0.05 ^b	0.5 ± 0.01 ^b
Bilberry Hx	12.1 ± 0.56 ^e	0.9 ± 0.03 ^c	2.6 ± 0.02 ^c	15.6 ± 0.57 ^d	2.9 ± 0.13 ^f

Results are mean values ± standard deviation of triplicates, and different letters within the same column indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test; n.d. = not detected.

Cloudberry seed oil extracted by hexane gave the highest recovery of vitamin E, when analysing the normalised data, expressed as mg of vitamin E/100 g of dry seeds (Table 2). The normalised data were derived by the vitamin E concentration (mg vitamin E/100 g oil) in relation to the extraction yield and represent the total amount of vitamin E extracted, independently of its final oil concentration. Black currant seed oil extracted by SFE at 50°C, which had the highest recovery of vitamin E per oil, had a comparatively low normalised concentration. This indicates that at 80°C a higher level of vitamin E can be extracted from black currant seeds, although the high extraction yield under this condition exerts a dilution effect. The pure lipids belonging to the major lipid classes act as cosolvents during SFE and might influence the extraction of minor compounds, such as vitamin E and carotenoids [26].

Table 3 gives the concentrations of carotenoids in the berry seed oils. Lutein and β -carotene were identified in all the berry seed oils. Significantly higher recoveries (16 to 57 mg/100 g oil) of carotenoids were obtained by hexane extraction than by SFE for all three berry seeds. This was also the case when analysing the normalised data, expressed as mg of carotenoids/100 g of dry seeds. Lutein was the major carotenoid in the black currant and bilberry seed oils. The cloudberry seed oils had the highest concentrations of β -carotene and carotene equivalents, which contributed to the intense orange colour of the extracted oils. The concentrations of carotenoids were slightly lower than the value reported by Manninen et al. for the sum of α -carotene and β -carotene in cloudberry seed oil extracted at 300 bar and 40°C [9]. Black currant seed oil extracted by hexane showed a high recovery of carotenoids, similar to that of the cloudberry seed oils extracted by SFE.

The EC₅₀ value is the concentration at which an extract reduces the DPPH absorbance by 50%. The lower the value is for an extract, the stronger is its antioxidant activity. As is evident from Figure 3, bilberry seed oils have the highest EC₅₀ values, and thus, the lowest antioxidant activity compared with the oils extracted from cloudberry and black currant seeds. This is probably because of its low vitamin E and carotenoid contents. A significant negative correlation was found for the EC₅₀ values and vitamin E content ($r = -0.763$, $p = 0.01$). The lowest EC₅₀ values were obtained

for black currant seed oils extracted by SFE (at 50°C and 80°C), which also had the highest content of vitamin E. The correlation of the EC₅₀ values and carotenoid contents was also negative, albeit not statistically significant ($r = -0.377$), indicating only a trend. This is probably explained by the fact that the antioxidant activities of carotenoids are not only restricted to scavenging activities, but these compounds also quench singlet oxygen [39]. However, when the values for vitamin E and carotenoids were combined, a higher negative correlation was found ($r = -0.802$), indicating a synergistic effect between vitamin E and carotenoids. Carotenoids can transfer electrons to the α -tocopheroxyl radical to regenerate tocopherol, which can then continue acting as an antioxidant [40]. A previous study has also reported a synergistic contribution of vitamin E and carotenoids to the antioxidant activities of lentils [23].

The reason for the variability of the extraction yields and recovery rates of antioxidants obtained for hexane extraction and for SFE at the two different temperatures may be related to differences in the structures and compositions of the three berry seeds. Therefore, the SFE conditions need to be optimised for each individual raw material. In addition, pretreatments (e.g., drying and milling) of the seeds should be taken into account. Milling seeds of different sizes and with different structural features lead to different particle sizes and surface areas for mass transfer, both of which influence the yield and composition of the oil.

4. Conclusions

This study investigated the yields, chemical compositions, and recovery rates of antioxidant compounds of seed oils extracted from cloudberry, bilberry, and black currants by solvent extraction and by SFE at 350 bar and at 50°C and 80°C. Higher extraction yields were obtained with solvent extraction than SFE. The oils extracted from cloudberry, bilberry, and black currant seeds are excellent sources of PUFAs. Oils extracted by solvent extraction had better recovery of carotenoids than SFE while SFE showed higher vitamin E content and better antioxidant activity for black currant and bilberry seed oils. The lower EC₅₀ of black currant seed oils is likely correlated to its high content of antioxidants. Black currant seed oils had the highest

antioxidant activities, as seen by the low EC₅₀ values and also their high concentrations of vitamin E and carotenoids. The results suggest that SFE extraction can be used as an efficient alternative to conventional extraction for concentration of tocopherols and carotenoids but due to the limited sample size, further studies are needed to support the findings. The seed cells differed in size and shape, and the milling step led to differences in the numbers of broken cells and particle sizes. These aspects warrant further investigation for optimisation of the pretreatments, so as to increase the total surface area of the cell wall particles and the number of broken cells.

Data Availability

The data (carotenoids, vitamin E, DPPH, and fatty acids) used to support the findings of this study are currently under embargo, while the research findings are commercialized. Requests for data (6 months), after publication of this article, will be considered by the corresponding author.

Disclosure

The results of this manuscript were partially presented at the 15th Euro Fed Lipid Congress at the European Federation of Food Science and Technology (EFFoST).

Conflicts of Interest

The authors declare no conflicts of interest.

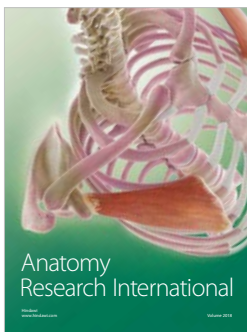
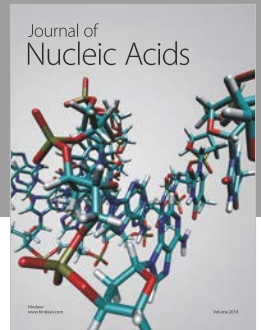
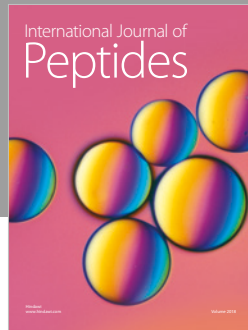
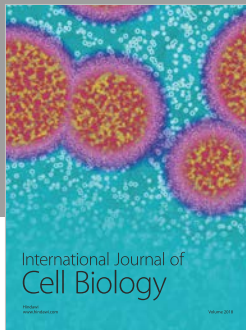
Acknowledgments

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