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Supercritical fluid extraction of berry seeds: chemical composition and antioxidant activity

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Abstract

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

Introduction

Berries are common raw material used for processing in the food and juice industry. However, the processing of berries generates large amounts of solid by-products in the form of peel, pulp and seeds, that are mostly underutilized [1]. The wastes are usually burnt or deposited in landfills and hence, cause significant impact on the environment [2]. An alternative to reduce the waste production and promote socio-economic and environmental benefits is the valorisation of by-products by extraction of valuable compounds [3].

Berry seeds are rich in oil and their utilization offers several advantages since their lipids in general have 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 also rich in vitamin E, carotenoids and bioactive compounds with antioxidative activity [5].

Supercritical fluid extraction (SFE) is a green method for extraction of valuable compounds in berry seeds in comparison to most conventional extraction methods, using hazardous organic solvents. The advantages of working with SFE are reduced solvent use, shorter extraction time and energy consumption, compared to the conventional extraction methods such as solvent extraction and cold pressing. SFE also allows the fractionation of compounds by adjusting pressure and temperature based on the solubility of targeted compounds [6]. Moreover, the solvent is easily separated from the extract by depressurization of supercritical fluid, saving time and the no use of organic solvents makes the extract suitable to be used in food industry [7]. The selectivity of SFE to extract 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 on the recovery of antioxidants from berry seeds using SFE.

Plant matrix is an important parameter for extraction [7]. Matrix pre-treatments like milling can increase the extraction efficiency by breaking the cells and hence, increasing the surface area for mass transfer [10]. The oil in the surface and shallow subsurface is quickly extracted while the oil inside the intact cells, in the particle core, has stronger mass transfer resistance [11]. An increased understanding of how the different kind of berry matrices and cell structure morphology are affected by milling is essential to improve extraction efficiency.

The objective of this study was to investigate the yield, chemical composition, and recovery of antioxidant compounds of SFE extracted oil from seeds of cloudberry, black currant and bilberry. The oils were extracted by SFE at different temperatures (50°C and 80°C) and compared with oils obtained from the same seeds using conventional solvent extraction with hexane. In addition the microstructures of seed particles were visualised using light microscopy to comprehend the influence of cell morphology on the extraction.

Materials and Methods

Chemicals

Carbon dioxide (>99.99%), was purchased from AGA Gas AB (Växjö, Sweden). The internal standard heptanoic (margaric) acid C 17:0 (≥99%) and the external standards of FAME mixture GLC 463 and stearidonic acid C 18:4 n-3 were purchased from Nu-Chek prep, Inc., (Elysian, USA). DL α-Tocopherol (≥97%) and 1,1-diphenyl-2-picrylhydrazil (DPPH) were obtained from Alfa Aesar (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 Lab-Scan (Dublin, Ireland). Ethanol absolute (AnalaR NORMAPUR) was from VWR International (Spånga, Sweden).

Berry seeds

The berries were picked in Norrbotten, northern Sweden. Cloudberries (Rubus chamaemorus) and bilberries (Vaccinium myrtillus L.) were grown wild and black currants (Ribes nigrum L.) were cultivated. The seeds were separated from the press cake (obtained after juice removal) in a puree 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, 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 used in the extraction experiments. Immediately before extraction the seeds were ground for 30 s in a coffee mill (2393 OBH Nordica, Stockholm, Sweden).

Solvent extraction

The extractions were performed with a proportion of 1 g of milled seeds to 10 mL of hexane. The mixtures were mixed for 2 h on orbital rotary plate (Heidolph Reax-2, Schwabach, Germany) at 150 rpm. After shaking, the mixtures were centrifuged (Heraeus Multifuge 1s, Kendro, Germany) for 10 min (3000g). The supernatants were collected and the procedure was repeated. The supernatants were pooled and the solvent was evaporated by flushing of samples with N2 at 40°C. The evaporated extracts were stored at -18°C until the analysis.

Supercritical fluid extraction (SFE) of berry seeds

The supercritical fluid extractions were carried out in triplicate, using a lab-scale supercritical fluid system (Waters SFE-500M1-2-C50, Pittsburgh, USA). The unit consisted of a CO2 pump connected to a cooling bath (Julabo F32-HD, Seelbach, Germany); an automated back-pressure regulator; a cylindrical extractor vessel of 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 in 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 temperatures were 50 or 80°C. The extracts were stored at -18°C until the analysis.

Fatty acid analysis by GC

The methylation of fatty acids from bilberry, black currant and cloudberry seed oils was based on the method of Cavonius and coworkers [12]. Toluene (1 mL) was added to extracts (0.025 ± 0.005 g) and 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 g. The supernatant containing the methyl esters of fatty acids (FAMEs) were collected in a new tube and evaporated under N2 at 40°C and re-dissolved in 1 mL of 2,2,4-trimethylpentane. The samples were analysed by GC (7890 A, Agilent Technologies, Santa Clara, USA) with triple-axis mass spectrometric (MS) detector in electron impact mode (5975 C, Agilent Technologies, Santa Clara, USA) and equipped with a J&W DB-wax column (30 m×0.250 mm×0.25 μm). Helium was used as a carrier gas. External standards were used for identification of the different peaks and an internal standard (C 17:0) was used for quantification of fatty acids, calculated as grams of FA per 100 g of oil.

Determination of tocopherols and tocotrienols by HPLC

Methanol (2 mL) was added to 0.04 ± 0.005 g of extracts, and vortexed. The samples were sonicated for 15 s. An aliquot of 1 mL of the upper phase was collected (1 mL) and transferred to a vial. The samples were analysed by HPLC coupled with on-line fluorescence detector (RF-551; Shimadzu, Kyoto, Japan). A reverse-phase C18 Kromasil column (2.1 mm, i.d. 150 mm, particle size 5 μm) was used to separate tocopherols and tocotrienols. The mobile phase was composed of methanol:water (95:5 v/v). Tocopherols and tocotrienols were

detected at 295 nm excitation and 330 nm emission wavelengths. The peaks were identified by standards and quantified by calibration curves. The concentrations were confirmed spectrophotometrically. The results were expressed as mg vitamin E/ 100 g bilberry seed oil.

Determination of carotenoids by HPLC

Tert-butyl methyl ether, MTBE (1 mL) was added to 0.03 ± 0.005 g of extracts, and vortexed. The samples were transferred to amber vials, and analysed by HPLC equipped with UV-visible photodiode array detector (996, Waters, Millipore, MA, USA). The carotenoids were separated using a reverse phase elution on a C30 column (5 μm, 250 × 4.6 mm i.d., YMC Europe GMBH, Schermbeck, Germany). The absorption spectra were measured from 250 to 550 nm. The mobile phase consisted of methanol and MTBE. The following gradient mixture was used for black currant and bilberry seed oils: an isocratic elution of 85% (v/v) MeOH and 15% (v/v) MTBE was kept for 2 min; the gradient built up over 9 min with a composition of 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 85% MeOH and 15% MTBE, followed for isocratic elution for final 4 min. For cloudberry seed oil, the gradient mixture was: the initial conditions of 85% MeOH and 15% MTBE was built over 6 min to 75% MeOH and 25% MTBE, to 60 MeOH and 40% MTBE over 1 min and 10 MeOH and 90% MTBE for 16 min; the gradient was reset to initial conditions over 3 min and equilibrated for an additional 6 min. Lutein and β-carotene were identified on the basis of the retention times and spectral characteristics of pure standards as described by Svelander et al [13]. Quantifications were made based on standard curves with 5 points. The concentration of the standard solutions was previously determined spectrophotometrically. The results were expressed as μg of carotenoids/100 g of bilberry seed oil.

Free radical scavenging activity (DPPH)

The antioxidant activity was evaluated by the 1,1-diphenyl-2-picrylhydrazil (DPPH) method, according to the procedure of Brand-Williams and co-workers [14]. All solutions were prepared in ethanol. The samples were diluted to obtain 5 different concentrations to determine the efficient concentration (EC_{50}). An aliquot of 1 mL of each dilution was mixed with 1 mL of freshly prepared DPPH-solution. Solutions of α-tocopherol were used as positive control [15]. The mixtures were incubated in the dark at room temperature. After 1 h, decrease of absorbance after the reaction of radical scavengers with DPPH was measured at 517 nm in a spectrophotometer (Ultrospec 1000, Pharmacia Biotech (Biochrom) Ltd, Cambridge, England).

Light microscope (LM) analysis

The milled seeds were smeared on the surface of microscope slides and a droplet of water was added to disperse the fragments of seeds. The microstructures of the berry seeds were examined with a Microphot FXA microscope (Nikon, Tokyo, Japan). Images were taken with an Altra 20 camera (Olympus, Tokyo, Japan) and the objective lenses x10 and x20 were used.

Statistical analysis

The analyses were carried out in triplicates. 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) were calculated using the Pearson's correlation coefficient (r) (level of significance of $p = 0.01$).

Results and Discussion

The effect of extraction temperature on yield

The yield and chemical composition of cloudberry, black currant and bilberry seed oils were assessed after SFE extraction at 50 and 80°C or by hexane extraction (which was performed at room temperature and evaporated at 40°C). **Fig. 1** presents the yield % (w/w) of 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 obtain higher extraction yields with conventional extractions [16, 17]. However, hexane is hazardous to workers and the environment and therefore more gentle extractions are needed [18]. In addition, a higher yield may not reflect the quality of the extracts. The highest yields were obtained from bilberry seeds. The measured yields ranged from 2.0% to 18.8% for black currant seeds extracted by SFE at 50°C and bilberry seed extracted by hexane, respectively. For SFE, 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 [19]. 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, improving the solubility of the oil during supercritical CO_2. The effect of the increase in temperature depends on which one of the two forces is predominant. In this study, the vapour pressure was predominant during SFE of black currant and cloudberry seeds at 350 bar.

Effect on fatty acid profile

Table 1 shows the fatty acid composition of cloudberry, black currant and bilberry seed oil extracted by SFE at different temperatures and hexane extraction. The major fatty acids were linolenic (18:2 n-6) and α-linoleic acid (18:3 n-3). Only bilberry seed oil had ω6/ω3 ratios ≤ 1. The sum of polyunsaturated fatty acids (PUFA) ranged from 66.8 to 75.9% of the total fatty acids for bilberry seeds extracted by hexane and black currant seeds extracted by SFE at 50 °C, respectively. There were small but significant differences in the fatty acid composition of the oils obtained by either SFE extraction or hexane extraction. The largest relative compositional difference measured was the concentration α-linoleic acid (18:3 n-3) which was significantly lower in black currant seed oils extracted by hexane extraction.

All the oil analysed had high percentages of PUFA and the major fatty acids were linoleic and α-linolenic acid. Additionally, black currant seed oil had high 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 reduction of inflammation [20, 21]. Similar results of fatty acid profile were reported in previous work on SFE of cloudberry, black currant and bilberry seed oils [4, 22-24]. Triacylglycerols are the major constituents of berry seed oils and their solubilities are different for those of the corresponding pure compounds and hence, their solubility behaviours are complex [25, 26].

Effect on recovery of antioxidants and antioxidant activity

Table 2 displays the content of tocopherols and tocotrienols in the berry seed oils. The major vitamin E compounds detected in seed oils from cloudberry, black currant, and bilberry were γ-tocopherol, α-tocopherol and γ-tocotrienol, respectively. The highest vitamin E concentration was obtained in bilberry seed oil extracted by SFE at 50°C, while black currant seed oil extracted by hexane extraction had the lowest vitamin E content. For cloudberry

seeds, hexane extraction resulted in highest recovery of vitamin E. The increase in temperature in SFE enhanced the extraction of vitamin E for cloudberry and bilberry seeds, but for black currant seeds an increase in temperature was found to decrease the extraction yield. In general, oils with lower yields contained higher concentration of vitamin E. A high negative correlation between the oil yield and the concentration of vitamin E was found ($r = -0.846$, $p = 0.01$), indicating that most of vitamin E is extracted in the beginning of the extraction process. Shen and coworkers also reported a higher extraction rate in the beginning of extraction for α-tocopherol at similar pressure (310 bar) and low temperature (40°C). [27]. Oil samples with high yields had lower vitamin E concentration due to a diluting effect from the other compounds recovered during the final part of the extraction.

The use of SFE at 350 bar and 50°C for extraction of black currant seed oil was advantageous for the recovery of α-tocopherol (122 mg/100 g oil), while for bilberry seeds, extraction at 80°C was more efficient. Temperature is an important parameter in extraction, as it influences the solubility as well as the cellular matrix containing the compound to be extracted. In plant matrixes, the vitamin E is located in the cell membranes and forms complexes with lysophospholipids and free fatty acids [28]. As vitamin E is fat-soluble, it is highly distributed in domains rich in lipids, to protect the lipids from oxidation [29]. In plants seeds, the lipids (predominantly triacylglycerols) are also stored in organelles called oil bodies [30]. When the seeds are dried and ground, the cell membranes and walls are broken and the oil bodies are easily accessed by the SC-CO_2 or the hexane, increasing the extractability.

Analyses of vitamin E in bilberry seed oil extracted by SFE (59 to 69 mg/100 g oil) and black currant seed oil (242 to 113 mg/100 g oil) showed that the concentrations were higher than reported in literature (40 and 110 mg/100 g oil, respectively) [4]. In contrast, the concentration of vitamin E in cloudberry seed oil (111 to 132 mg/100 g oil) was considerably lower than described in literature (260 to 270 mg/100 g oil) [4, 9]. The variability in the concentration of vitamin E compared to other studies can be due to extraction efficiency differences between different extraction methods, differences in drying and milling of the seeds before extraction, and also by environmental and physical factors such as weather conditions, region, ripeness and type of variety, known to affect the chemical composition in the raw material [31, 32].

Cloudberry seed oil extracted by hexane had the highest recovery of vitamin E, when analysing the normalized data, expressed as mg of vitamin E/100 g of dry seeds (**Table 2**). Black currant seed oil extracted by SFE at 50°C, which had the highest recovery of vitamin E per oil, had comparatively low normalized concentration. The low yield obtained by this oil, kept the extracted vitamin E concentrated in the oil.

Table 3 provides the contents of carotenoids in the berry seed oils. Lutein and β-carotene were identified in all berry seed oils. Significantly higher recoveries (16 to 57 mg/100 g oil) of carotenoids were obtained from hexane extraction than SFE for all three berry seeds. This was also the case when analysing the normalized data, expressed as mg of carotenoids/100 g of dry seeds. Lutein was the major carotenoid in black currant and bilberry seed oils. Cloudberry seed oils had the highest concentrations of β-carotene and carotene equivalents, which contributed to an intense orange colour in the extracted oils. The concentration of carotenoids were slightly lower than the value reported by Manninen and co-workers for 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 had a high recovery of carotenoids, similar to cloudberry seed oils extracted by SFE.

The EC_{50} is the concentration at which an extract reduces the DPPH absorbance to 50 %. The lower the value is for an extract, the stronger is its antioxidant activity. As can be observed from **Fig. 2**, bilberry seed oils had the highest EC_{50} values, and hence, the lowest antioxidant activity compared to the oils extracted from cloudberry and black currant seeds. This is probably explained by its low vitamin E and carotenoid content. Significant negative correlation was found among EC_{50} values and vitamin E content ($r = -0.763$, $p = 0.01$). The lowest EC_{50} values were obtained by black currant seed oils extracted by SFE (at 50 and 80°C), which also had highest vitamin E content. The correlation among EC_{50} values and carotenoid content was also negative, but not significant ($r = -0.377$), indicating only a trend. This is probably explained by that the antioxidant activity of carotenoids is not only restricted to scavenging activity, since these compounds also quench singlet oxygen [33]. However, when the values of 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 continue acting as an antioxidant [34]. Previous work has also reported a synergistic contribution of vitamin E and carotenoids for antioxidant activities of lentils [35].

The reason for the variations in extraction yields and recovery of antioxidants obtained with hexane extraction and as well as for SFE at the two different temperatures might be related to structural differences and composition between the three berry seeds. Therefore, the SFE conditions need to be optimized for each individual raw material. Additionally, pre-treatments (e.g. drying and milling) of the seeds should be taken into account. Milling seeds with different sizes and structural features lead to different particles sizes and surface area for mass transfer thus influencing the yield and composition of the oil.

Light microscopy analysis of berry seed particles

The extraction process is controlled by two different extraction periods; the first period is characterized by a quick extraction of surface and shallow subsurface oil, and the second period is slower and controlled by diffusion of the fluid into matrix [11]. **Fig. 3a-c** shows the LM images of the dry and ground seeds of cloudberry (**a**) black currant (**b**) and bilberry (**c**) before extraction. The pre-treatments disrupted the seeds revealing the interior part divided by the lamellar structure. The milling step broke several of the cells releasing immediately part of oil from the cells, which can be easily extracted. The oil droplets spread around the seed particles were dispersed from the particle surface by addition of a water droplet in the objective glass. In **Fig. 3a**, the presence of a big oil droplet is shown (indicated by a white arrow). The milling step disrupted each berry seed in different ways, depending on the size of the seeds s and the cell shapes. . The seeds of cloudberry are larger and with higher density compared to bilberry and black currant seeds, which might have influenced the milling step [25]. The disrupted cells are indicated by arrows (black) in **Fig. 3a-c**. Cloudberry seeds have the largest cells among the three berries while bilberry seeds have the longest and most narrow cells (**Fig. 3a and c**), which possibly could have facilitated the extraction from the inner part of the broken bilberry seed cells. Black currant seeds (**Fig. 3b**) have the smallest cells and particles. Longer milling times could break the cloudberry and bilberry seeds in smaller particles with larger surface, resulting in more efficient extractions. Further investigations on the effects of pre-treatments in the investigated berries are necessary to optimize the extraction.

The small cells and seed particles expose a higher membrane surface, facilitating the extraction of non-polar compounds from the membranes e.g. of vitamin E, which is located in the cell membranes [28]. Milling of the seeds broke the cell membranes and released the oil

containing vitamin E to the surface. This could explain why a major part of vitamin E was extracted during the first part of the extraction, followed by a decrease in the extraction rate due to mass transfer resistance from cell membrane. As can be seen in the **Fig. 3a-c**, the particle cores have many intact cells which may have decreased the extraction rate of other compounds present in the oil.

Conclusions

This study investigated the yield, chemical composition and recovery of antioxidant compounds of seed oils extracted from cloudberry, bilberry and black currant. The results suggest that berry seed oil from cloudberry, bilberry and black currant can be excellent sources of PUFAs. The recovery of antioxidants was more influenced by the extraction solvent than the SC-CO_2 temperature. In this study, SC-CO_2 was more efficient for extraction of vitamin E but for carotenoids, hexane extraction produced higher yields. The highest antioxidant activity, expressed by low EC_{50} values, and the highest concentrations of vitamin E and carotenoids were obtained for black currant seed oil extracted by SC-CO_2. The seed cells differed in size and shape and the milling step led to differences in the number of broken cells and particle size, probably influencing the diffusion of the solvents and the extractability. These aspects need further investigations for optimisation of pre-treatments in order to increase the total surface area of cell wall particles and the number of broken cells.

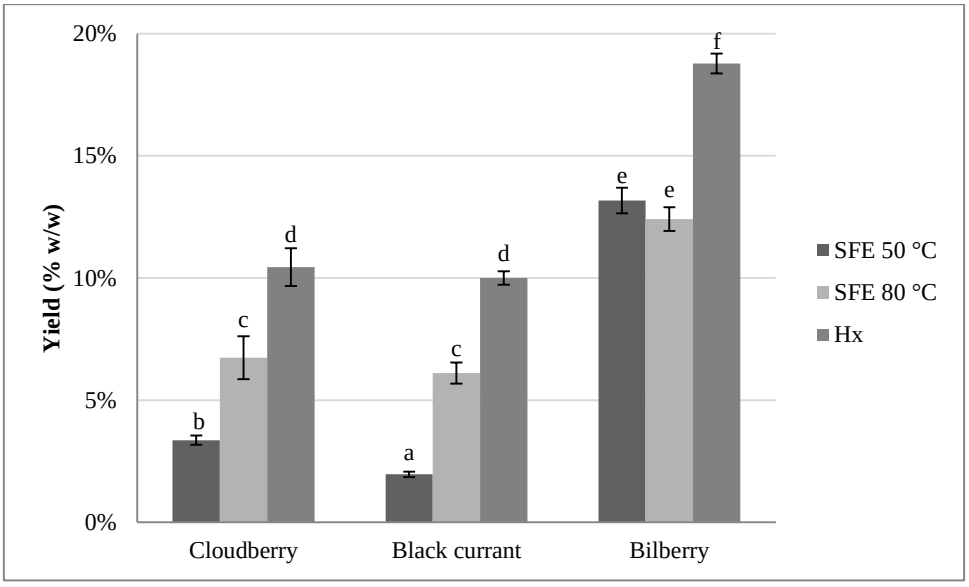


Figure 1: Extraction yield (% w/w) of cloudberry, black currant and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction. Results are mean values ± standard deviation of triplicate extractions. Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

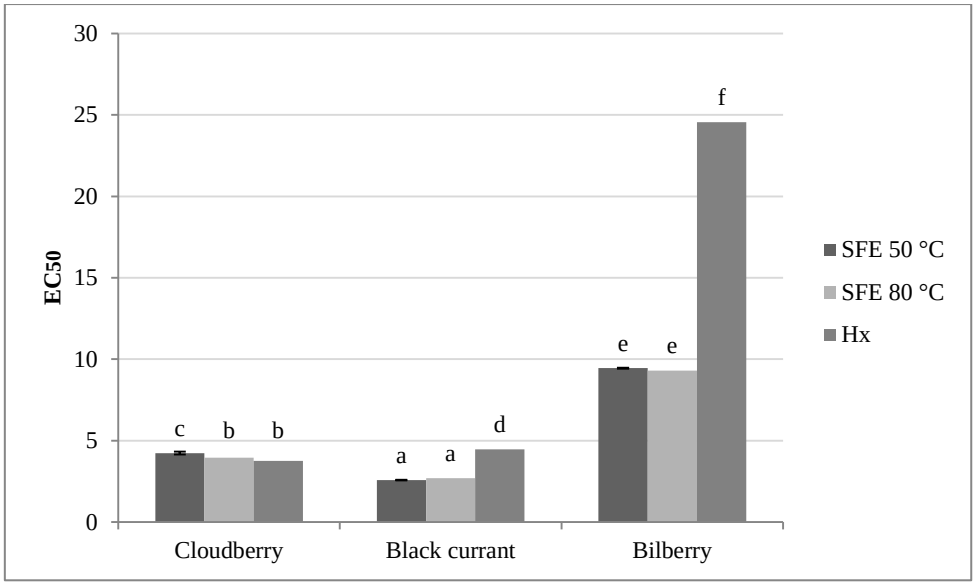
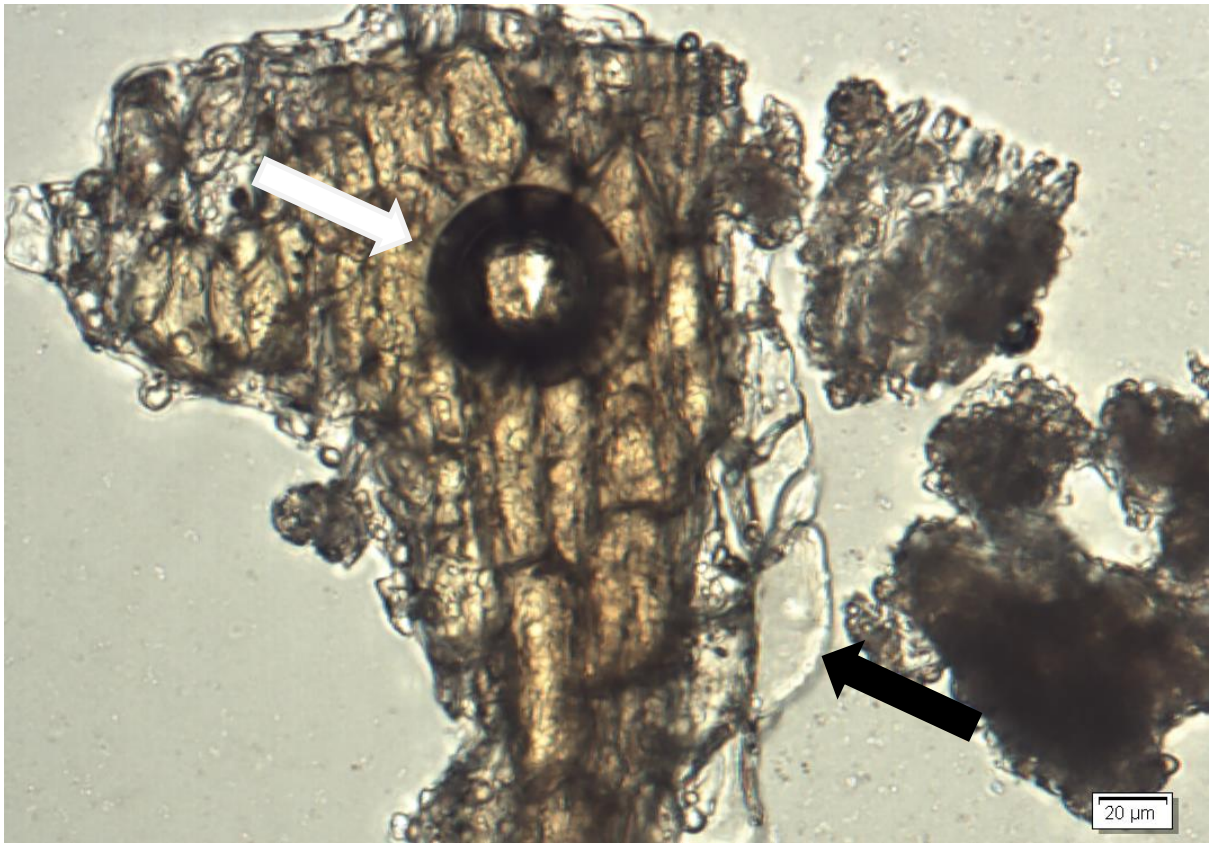


Figure 2: Values of EC50 of cloudberry, black currant and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction. Results are mean values ± standard deviation of triplicates. Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

3a



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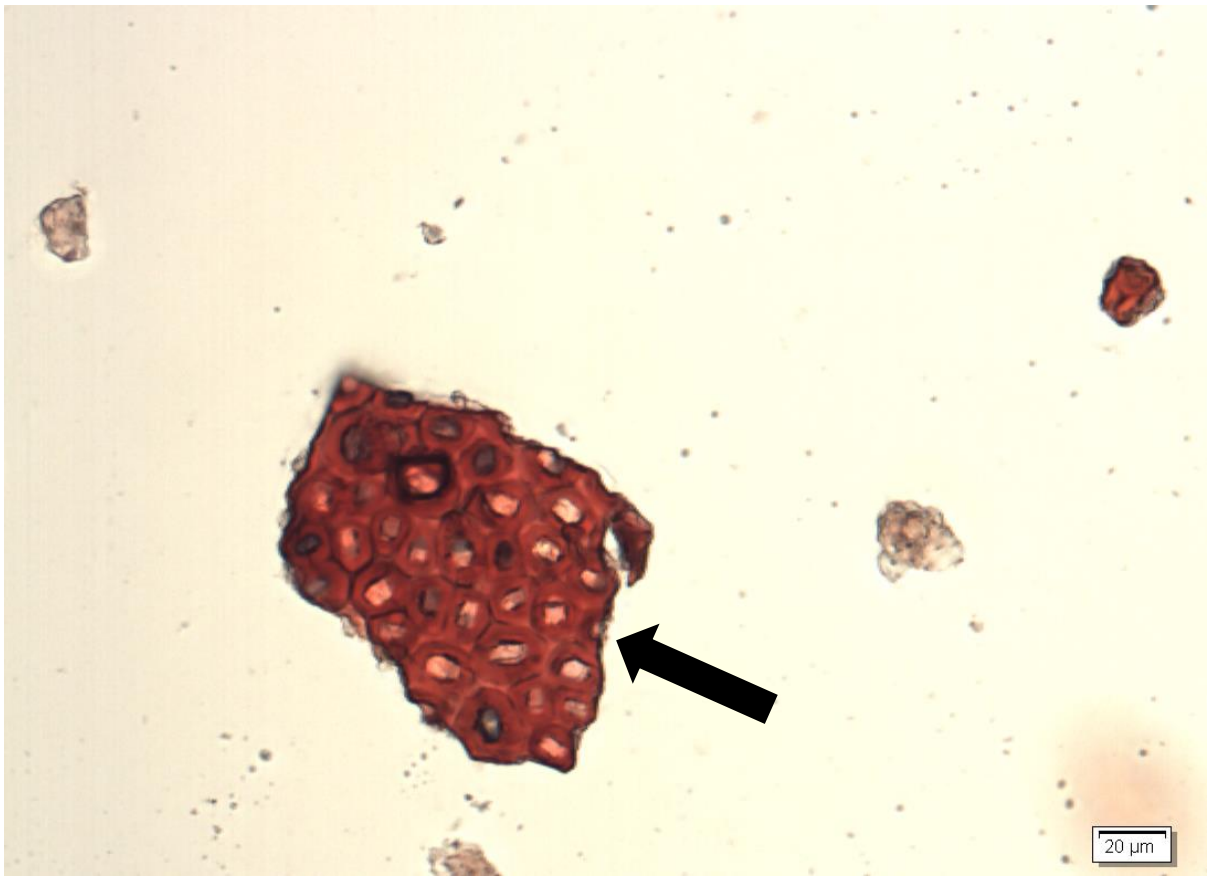

3c

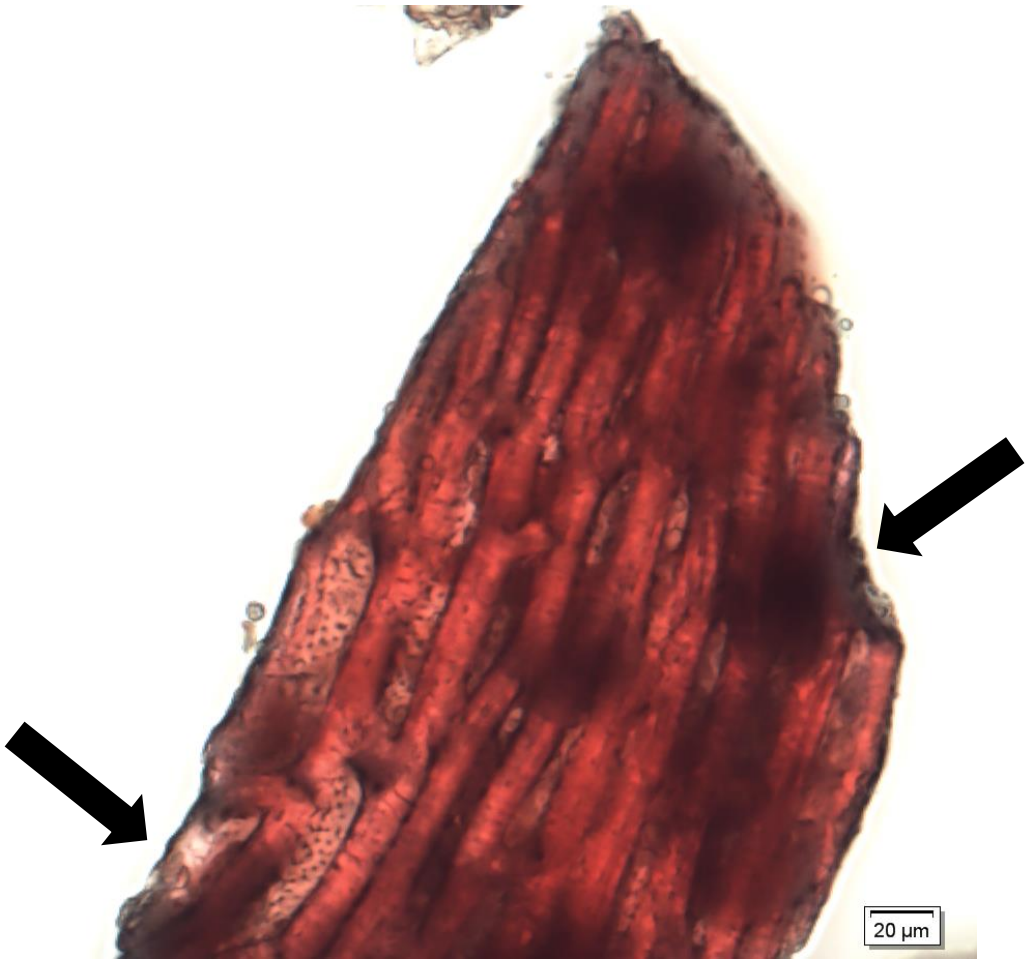

Figure 3: Seeds of cloudberry (a), black currant (b) and bilberry (c) dried and milled for 30 s. White arrow indicates oil droplet released after milling. Black arrows indicate disrupted cells.

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)										ω6/ω3 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	
Cloudberry 50°C	3.7 ± 0.1^{a}	1.8 ± 0.0^{ab}	16.0 ± 0.1^{b}	1.0 ± 0.0^{a}	41.6 ± 0.2^{bc}	32.4 ± 0.1^{d}	1.5 ± 0.0^{a}		1.1 ± 0.0^{a}	0.9 ± 0.0^{c}	1.3 ± 0.0^{b}
Cloudberry 80°C	3.4 ± 0.0^{a}	1.8 ± 0.0^{ab}	16.6 ± 0.0^{b}	1.0 ± 0.0^{a}	41.3 ± 0.1^{bc}	33.8 ± 0.0^{ef}			1.2 ± 0.0^{b}	0.9 ± 0.0^{c}	1.2 ± 0.0^{b}
Cloudberry Hx	3.4 ± 0.0^{a}	1.9 ± 0.0^{abc}	16.6 ± 0.0^{b}	1.0 ± 0.0^{a}	40.8 ± 0.0^{b}	34.1 ± 0.1^{ef}			1.2 ± 0.0^{b}	1.0 ± 0.0^{d}	1.2 ± 0.0^{b}
Black currant 50°C	7.9 ± 0.0^{c}	2.1 ± 0.0^{bc}	12.3 ± 0.0^{a}	1.0 ± 0.0^{a}	42.5 ± 0.0^{cd}	16.4 ± 0.0^{b}	13.4 ± 0.0^{c}	3.5 ± 0.1^{a}		0.8 ± 0.0^{b}	2.8 ± 0.0^{c}
Black currant 80°C	7.7 ± 0.1^{c}	2.1 ± 0.1^{bc}	13.0 ± 0.1^{a}	1.1 ± 0.0^{a}	42.1 ± 0.4^{bc}	17.5 ± 0.1^{c}	12.7 ± 0.0^{b}	3.2 ± 0.2^{a}		0.7 ± 0.0^{a}	2.7 ± 0.1^{c}
Black currant Hx	7.7 ± 0.6^{c}	2.4 ± 0.0^{c}	12.9 ± 0.1^{a}	1.1 ± 0.1^{a}	44.1 ± 0.1^{d}	14.5 ± 0.6^{a}	12.8 ± 0.3^{b}	3.6 ± 0.4^{a}		0.8 ± 0.0^{b}	3.2 ± 0.2^{d}
Bilberry 50°C	5.9 ± 0.1^{b}	1.6 ± 0.0^{a}	24.2 ± 0.9^{c}	1.0 ± 0.1^{a}	32.6 ± 1.6^{a}	34.7 ± 0.6^{f}					0.9 ± 0.1^{a}
Bilberry 80°C	6.1 ± 0.5^{b}	1.8 ± 0.5^{ab}	23.9 ± 0.0^{c}	1.1 ± 0.1^{a}	33.4 ± 0.5^{a}	33.7 ± 0.4^{e}					1.0 ± 0.0^{a}
Bilberry Hx	6.1 ± 0.2^{b}	1.7 ± 0.1^{ab}	24.3 ± 0.1^{c}	1.1 ± 0.0^{a}	32.9 ± 0.6^{a}	34.0 ± 0.2^{ef}					1.0 ± 0.0^{a}

Results are mean values ± standard deviation of triplicates and different letters within the same raw indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

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 ± 1.4^{d}	67.1 ± 3.0^{b}	4.9 ± 0.0^{b}		0.8 ± 0.0^{a}	111.0 ± 5.4^{c}	3.7 ± 0.2^{a}
Cloudberry 80°C	40.2 ± 1.3^{d}	74.9 ± 1.9^{bc}	4.9 ± 0.0^{b}	0.2 ± 0.0^{a}	1.1 ± 0.0^{a}	121.3 ± 4.7^{cd}	8.2 ± 0.3^{c}
Cloudberry Hx	40.3 ± 0.1^{d}	84.6 ± 1.5^{c}	5.5 ± 0.0^{b}		1.0 ± 0.1^{a}	131.5 ± 2.4^{d}	13.7 ± 0.2^{e}
Black currant 50°C	122.1 ± 1.2^{f}	102.7 ± 6.1^{d}	16.0 ± 0.7^{d}	0.3 ± 0.0^{b}	0.7 ± 0.0^{a}	241.8 ± 9.6^{f}	4.7 ± 0.2^{b}
Black currant 80°C	93.2 ± 1.2^{e}	87.4 ± 0.0^{c}	13.7 ± 0.1^{c}	0.3 ± 0.0^{b}	2.4 ± 0.0^{a}	197.0 ± 1.6^{e}	12.0 ± 0.1^{d}
Black currant Hx	30.7 ± 1.1^{c}	69.7 ± 1.8^{b}	12.5 ± 0.2^{c}		0.1 ± 0.0^{a}	113.0 ± 4.3^{cd}	11.3 ± 0.4^{d}
Bilberry 50°C	18.1 ± 0.1^{b}	3.6 ± 0.2^{a}	0.3 ± 0.0^{a}	0.6 ± 0.0^{c}	36.4 ± 2.2^{c}	59.1 ± 3.1^{b}	7.8 ± 0.4^{c}
Bilberry 80°C	25.8 ± 0.3^{c}	4.8 ± 0.0^{a}	0.5 ± 0.0^{a}	0.7 ± 0.0^{d}	37.4 ± 1.8^{c}	69.3 ± 3.1^{b}	8.6 ± 0.4^{c}
Bilberry Hx	4.7 ± 0.2^{a}	1.6 ± 0.0^{a}	0.2 ± 0.0^{a}		10.7 ± 0.4^{b}	17.2 ± 0.8^{a}	3.2 ± 0.2^{a}

Results are mean values ± standard deviation of triplicates and different letters within the same raw indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

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 ± 0.0^{a}	4.3 ± 0.0^{g}	31.3 ± 0.0^{f}	36.5 ± 0.2^{f}	1.2 ± 0.0^{d}
Cloudberry 80°C	0.2 ± 0.0^{a}	2.6 ± 0.0^{e}	34.9 ± 0.0^{g}	37.7 ± 0.2^{g}	2.5 ± 0.0^{e}
Cloudberry Hx		3.4 ± 0.0^{f}	53.5 ± 0.0^{h}	57.0 ± 0.1^{h}	5.9 ± 0.0^{h}
Black currant 50°C	12.1 ± 0.0^{e}		4.8 ± 0.0^{b}	16.9 ± 0.0^{e}	0.3 ± 0.0^{a}
Black currant 80°C	10.1 ± 0.0^{d}	1.4 ± 0.0^{d}	1.7 ± 0.0^{d}	13.2 ± 0.1^{c}	0.8 ± 0.0^{c}
Black currant Hx	30.8 ± 0.0^{f}	1.5 ± 0.0^{d}	5.6 ± 0.0^{e}	38.0 ± 0.2^{g}	3.8 ± 0.0^{g}
Bilberry 50°C	2.4 ± 0.0^{b}	0.1 ± 0.0^{a}	0.4 ± 0.0^{a}	2.8 ± 0.0^{a}	0.4 ± 0.0^{ab}
Bilberry 80°C	3.2 ± 0.0^{c}	0.3 ± 0.0^{b}	0.6 ± 0.0^{a}	4.1 ± 0.0^{b}	0.5 ± 0.0^{b}
Bilberry Hx	12.1 ± 0.0^{e}	0.9 ± 0.0^{c}	2.6 ± 0.0^{c}	15.6 ± 0.6^{d}	2.9 ± 0.1^{f}

Results are mean values ± standard deviation of triplicates and different letters within the same raw indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

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Conflict of interest statement

The authors declare no conflict of interest.

Data Availability Statement

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.

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