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Efficiency of Supercritical CO_2 and Ultrasound-Assisted Extraction Techniques for Isolation of Bioactive Molecules from Sea Buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson) Berry Oils and Cakes

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Abstract: This study compared the efficiency of advanced supercritical CO_2 extraction (SC-CO_2) and conventional Soxhlet extraction (SE) in the isolation of lipophilic bioactive molecules (BAMs) from the oils of different sea buckthorn (SB) berries (Leikora and Ascola). A SB cake, a valuable by-product of oil extraction, was analyzed for phenolic compounds and antioxidant activity (AA). For this purpose, ultrasound-assisted extraction (UAE) with 70% (*v*/*v*) ethanol was optimized, and the following optimal UAE conditions were determined: ultrasonic power of 60%, temperature of 50 °C, and extraction time of 20 min. The individual BAMs in the oils and cake extracts were analyzed chromatographically, while the AAs were determined using the ORAC method. The oils extracted with SC-CO_2 had a higher content of lipophilic BAMs and a higher AA value than the oils extracted with SE. Palmitic and palmitoleic acids, β-sitosterol, and α-tocopherol dominated the Leikora oil, while a higher content of oleic, linoleic, and α-linolenic acids and carotenoids was found in the Ascola oil. The highest phenolic content was found in the Ascola cake, with the flavonols isorhamnetin-3-hexoside, isorhamnetin-3-rutinoside, and quercetin-3-glucoside being the most abundant. Oil and cake extracts of the Ascola variety also had a higher AA.

Keywords: sea buckthorn lipophilic and hydrophilic bioactive molecules; sea buckthorn berry oil and cake; supercritical CO_2 extraction; Soxhlet extraction; ultrasound-assisted extraction; antioxidant activity

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1. Introduction

Sea buckthorn (SB), lat. *Elaeagnus rhamnoides* (L.) A. Nelson, is a shrubby plant with yellow or orange colored berries, naturally distributed in Asia and Europe [1]. Various parts of the plant, especially the berries, are used in traditional medicine for their health-promoting effects, such as antioxidant, anti-inflammatory, antitumor, immunomodulatory, antimicrobial, and other properties [2–5]. SB berries have a high nutritional and biological value, which is due to the presence of both lipophilic and hydrophilic antioxidants such as carotenoids, tocopherols, fatty acids, sterols, phenolic compounds, and ascorbic acid [4,6–8]. It is known that factors such as variety, time of harvest, climate, growing conditions, ripeness at harvest, storage conditions, and processing technologies have a significant influence on the content and composition of BAMs [9,10]. The oil is the most valuable part of SB berries due to the presence of omega-3 (linolenic acid), omega-6 (linoleic acid) and omega-7 fatty acids (vaccenic acid, palmitooleic acid), α-tocopherol, sterols such as β-sitosterol, campesterol, and stigmasterol, and carotenoids such as zeaxanthin, zeaxanthin

dipalmitate and its esters, β-carotene, lycopene, and β-cryptoxanthin palmitate [7,11–13]. The oil quality is significantly influenced by pre-harvest factors such as the variety and the growing region, as well as by processing factors [14]. For the isolation of targeted lipophilic BAMs, i.e., plant oils, advanced extraction techniques such as supercritical CO_2 extraction (SC-CO_2) as a promising alternative to conventional extraction and mechanical pressing of oils have received considerable attention [15]. SC-CO_2 is an effective solvent for dissolving non-polar or slightly polar compounds, although its dissolving power for hydrophilic substrates can be increased by adding a polar co-solvent such as ethanol [16]. The main advantage of SC-CO_2 is the lower viscosity, better diffusion, and surface tension of CO_2, which allow better penetration into the material from which the target compounds are extracted. In addition, as an environmentally friendly and safe solvent (GRAS), CO_2 can be easily removed from the oil as it is inert and does not interact with the extract, so it serves as a carrier fluid, allowing the extract to be free of contaminants [15]. Most importantly, SC-CO_2 protects the antioxidants during extraction as it is carried out in the absence of oxygen, preventing the formation of oxidation products, which is a very important aspect for the preservation of BAMs such as tocopherols, carotenoids, unsaturated fatty acids, and sterols [17–19]. The extraction of oils generates large quantities of by-products that are currently used inefficiently or even disposed of as waste, but which represent a cheap source of valuable nutrients such as phenolic acids and flavonoids [20,21]. Ultrasound-assisted extraction (UAE) is one of the most commonly used extraction techniques for polyphenols, which allows higher reproducibility in a shorter time and the use of less solvent at lower processing temperatures compared with conventional extraction [22]. The efficiency of UAE depends on its parameters, mainly temperature, extraction time, and ultrasonic power, and it is of great importance to optimize it with regard to the plant material used and the desired target compounds. In addition, studies have shown that the biological properties of extracts obtained under optimal UAE conditions are preserved, which increases their application in various fields [23]. Goldsmith et al. [24] and Nunes et al. [25] reported that UAE is a very effective method for phenolic extraction from olive cake obtained after oil extraction, as the yield of phenolic compounds is high in a short time. Nowadays, there is a great need for the characterization of BAMs isolated from plant material and the evaluation of their antioxidant activity (AA). The most effective techniques for the characterization of polyphenols and pigments are liquid chromatographic techniques (HPLC/UPLC) coupled with diode-array detection (DAD), fluorescence detector (FLD), and/or mass spectrometry (MS), while gas chromatography (GC) coupled with MS or FID is used for sterol and fatty acid identification [26,27]. Due to the presence of different BAMs in SB, a suitable method for determining AA is the oxygen reducing antioxidant capacity assay (ORAC), which can measure both lipophilic and hydrophilic antioxidants.

To our knowledge, data on the efficiency of advanced extraction techniques to isolate lipophilic and hydrophilic BAMs with AA from SB berries is limited. In this study, SB berry oil was produced by conventional Soxhlet extraction (SE) and advanced extraction techniques (SC-CO_2) from different SB berry varieties (Leikora and Ascola). Therefore, the aim of this study was to compare the efficiency of SC-CO_2 and SE in the isolation of different lipophilic BAMs in oils. In addition, a SB cake obtained after oil extraction represents a valuable source of BAMs such as phenolic compounds. For this purpose, UAE was optimized with regard to total phenolic content (TPC) and AA. After determining the optimal UAE conditions, the polyphenolic composition of the extracts obtained from the cake under optimal UAE conditions was evaluated by UPLC/ESI-MS^2. The AA of the oils and the cake extracts were also evaluated using the ORAC method.

2. Materials and Methods

2.1. Plant Material

Different SB berry varieties, Leikora and Ascola, grown in Croatia, near Zagreb (Latitude, 45°58′53.05″ N; Longitude, 15°58′1.90″ E) in 2020, were used for this study. The

botanical identification of the plants was carried out by the experts of the Faculty of Agriculture, University of Zagreb, Croatia.

2.2. Sample Preparation

Samples were freeze-dried (CoolSafe 55-9 PRO, Labogene, Allerød, Denmark) to a dry matter content of 90%, ground in a commercial grinder (GT11, Tefal, Rumilly, France), sieved through a 60-mesh sieve, placed in closed plastic containers, and stored at 4 °C until use.

2.3. Chemicals and Reagents

All solvents used for the extraction and analysis were HPLC grade and were obtained from different manufacturers; ethanol, formic acid and acetonitrile were obtained from BDH (Prolabo, Lutterworth, UK), hexane from J. T. Baker (Phillipsburg, NJ, USA), distilled water from Millipore Corp. (Bedford, NY, USA), anhydrous sodium carbonate (≥99.5%) and sodium phosphate (96%) were obtained from Kemika (Zagreb, Croatia), Folin-Ciocalteu reagent from Merck (Darmstadt, Germany), fluorescein sodium salt from Honeywell Riedel-de-Haën (Bucharest, Romania), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) from Acros Organics (Thermo Fisher Scientific, Geel, Belgium), 2,20-Azobis (2-amidinopropane) hydrochloride and methilated β-cyclodextrine were obtained from Sigma-Aldrich (Steinheim, Germany) and CO_2 (99.97%) (*w*/*w*) was obtained from the Messer (Osijek, Croatia).

2.4. Supercritical CO_2 Extraction

Supercritical CO_2 extraction of oil from SB berries was performed in the SC-CO_2 system at an extraction pressure of 276 Bar, a temperature of 35 °C, and a mass flow rate of 2 kg/h according to the optimal conditions obtained in our previous research by Pavlović et al. [28] with minor modifications. The extraction process took 90 min until all the amount of oil was extracted (each 15 min the amount of obtained extracts were collected in glass tubes weighed beforehand using a balance with a precision of ±0.0001 g).

2.5. Soxhlet Extraction

Soxhlet extraction of oil from SB berries was performed in Soxtherm (Gerhardt, Germany) using *n*-hexane as extraction solvent, according to the study by Harkat et al. [29], with minor modifications. The Soxhlet procedure consisted of placing 10 g of freeze-dried and ground SB berries in a thimble into the Soxhlet extraction system heated with a hotplate, in which 50 mL of *n*-hexane was refluxed over the sample for 90 min at increasing temperatures (up to 180 °C). After extraction, the solvent was removed at 60 °C using a rotary evaporator. The obtained oil was filtered and stored in a colored bottle at −18 °C in a nitrogen gas atmosphere until analysis (no longer than 10 days).

2.6. Ultrasound-Assisted Extraction of by-Products of Oil Extraction

A SB cake of the Leikora variety obtained after SC-CO_2 extraction was used to determine the optimal extraction conditions for phenolic compounds. A mass of SB cake of Leikora variety (1 ± 0.001 g) obtained after SC-CO_2 extraction was mixed with 50 mL of aqueous ethanol solution (70%, *v*/*v*), and extraction was performed in an ultrasonic bath (Elmasonic P, Elma-Hans Schmidbauer GmbH & Co., Ltd., Singen, Germany) with 120 W ultrasonic power. A full factorial design comprising 27 experimental trials was chosen to evaluate the effect of three independent variables—ultrasonic power, temperature, and extraction time. According to the experimental design, ultrasonic power (30, 60, and 90%), temperature (35, 50, and 65 °C), and extraction time (10, 20, and 30 min) were varied in order to find the best extraction conditions with the maximum yield of total phenolic compounds. After the extraction, the obtained mixture was centrifuged at 4500× *g* rpm for 15 min, and the supernatants were filtered (Whatman No. 4) into 50 mL volumetric flasks and made up with extraction solvent. Extraction was performed in duplicate (*n* = 2), and

the extracts were stored at −18 °C in a nitrogen gas atmosphere until analysis (no longer than 10 days).

The extraction of the phenolic compounds in all SB cakes obtained after oil extraction was carried out under the determined optimal conditions (60% ultrasonic power, 50 °C, 20 min). Prior to UPLC/ESI-MS^2 analysis the extracts were filtered through a 0.45 μm membrane filter (Macherey-Nagel GmbH, Düren, Germany).

2.7. Characterization of Fatty Acids and Sterols in SB Oils

Gas chromatography according to the ISO 12966-2:2017 method [30] and ISO 12228-1:2014 [31] was used for the characterization of fatty acids and sterols.

Fatty acid methyl esters were separated on a TRACE TR-FAME capillary column (30 m × 0.22 mm × 0.25 μm) using a stationary phase of 70% cyanopropyl polysilphenylene–siloxane (Thermo Scientific, Waltham, MA, USA), and the peaks of fatty acid methyl esters were identified by comparing their retention times with the retention times of the 37 Component FAME Mix and petroselinic acid standard (Supelco, Sigma-Aldrich, St. Louis, MO, USA). Results were expressed as weight percentages of total fatty acids (%).

Sterol content and composition analysis were performed using the Agilent Technologies 6890N Network GC System gas chromatograph (Santa Clara, CA, USA) equipped with a sample injection system, a flame ionization detector, and an Agilent Technologies 5973 inert mass selective detector. Peak identification was performed by comparing retention times and mass spectra with authentic standards. All sterols were quantified using an internal standard method with α-cholestanol (1 mg/mL). Results were expressed in mg per 100 g of oil (mg/100 g).

2.8. Characterization of Carotenoids in Oils by HPLC Analysis

High-performance liquid chromatography (HPLC) was used for the characterization of carotenoids using an Agilent 1260 Infinity quaternary LC system (Agilent Technologies, Santa Clara, CA, USA) coupled with a diode array detector (DAD). The reverse-phase separations were performed on a Develosil RP-Aqueous (C30) reversed-phase column (250 mm × 4.6 mm i.d., 5 μm particle size) (Phenomenex, Torrance, CA, USA). For the determination of carotenoids, 1 mL of oil was diluted in 5 mL of *n*-hexane. The solvent composition and gradient program for the analysis were used as described by Casto Puyana et al. [32], and wavelength detection was at 450 nm. Identification of the carotenoids and their derivatives was based on the DAD absorption spectra and retention times of the authentic standards of lutein, zeaxanthin, and β-carotene and literature data [33], while quantification was based on their calibration curves. All results were expressed in mg per 100 g of oil (mg/100 g).

2.9. Determination of α-Tocopherol

The tocopherol content was determined according to ISO method 9936 [34]. Samples were prepared by dissolving 0.1 g of oil in 10 mL of *n*-hexane and then analyzed using the Agilent 1260 Infinity quaternary LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a fluorescence detector. Separation was performed on a LiChroCART Silica 60 column (250 mm 94.6 mm, 5 L; Merck, Darmstadt, Germany), and a standard calibration curve for α-tocopherol was used for quantification. Results were expressed in mg per 100 g of oil (mg/100 g).

2.10. Determination of Total Phenolic Content

The UV-VIS spectrophotometer (UV-VIS UviLine 9400; Secomam, Ales, France) was used for the determination of total phenol content (TPC) according to the spectrophotometric Folin-Ciocalteu method, as previously described by Čulina et al. [35]. All measurements were performed in duplicate, and the results were expressed as mg gallic acid equivalents (GAE) per 100 g of dry matter (mg/100 g dm).

2.11. Characterization of Phenolic Compounds by UPLC/ESI-MS^2 Analysis

The UPLC/ESI-MS^2 analysis using an Agilent 1290 RRLC instrument (Agilent, Santa Clara, CA, USA) coupled with an Agilent triple quadrupole mass spectrometer (6430 QqQ) with an ESI ion source was used for the characterization of phenolic compounds according to the method previously described by Čulina et al. [32]. The reverse-phase separations were performed on a C18 column of Agilent Zorbax Eclipse Plus (100 × 2.1 mm, 1.8 µm particle size) at 35 °C. Identification of phenolic compounds was performed by comparing the mass spectra and fragmentation patterns of the authentic standards and using previously reported data [35,36]. All measurements were done in duplicate, and the obtained results were expressed in mg per 100 g of dried matter (mg/100 g dm).

2.12. Determination of Antioxidant Activity by the ORAC Method

The ORAC assay was conducted on a 96-well microplate using a fluorescence plate reader (Clariostar BMG LABTECH, Offenburg, Germany) according to the method previously described by Elez Garofulić et al. [37] for hydrophilic ORAC (H-ORAC) and according to the method previously described by Naguib et al. [38] for lipophilic ORAC (L-ORAC). The blank for H-ORAC was sodium phosphate buffer (75 mM, pH 7.4), and for L-ORAC was 7% RMCD solvent (w/v) made in a 50% acetone-water mixture (v/v). Trolox (25 µL) was used as the standard, and results were expressed in µmol of Trolox equivalent per g of dried matter (µmol TE/g dm, $n = 2$).

2.13. Statistical Analysis

For statistical analysis, Statistica ver. 10.0 software (Statsoft Inc., Tulsa, OK, USA) was applied. One-way ANOVA and Tukey's HSD test were performed to compare the mass concentrations of individual fatty acids, sterols, and carotenoids in the SBO of Leikora and Ascola obtained by SC-CO_2 and SE and to evaluate the differences between the SB varieties and the efficiency of SC-CO_2 and SE in terms of total fatty acid content (total saturated, unsaturated, monounsaturated, and polyunsaturated fatty acids), total sterol content, total carotenoid content, α-tocopherol content, and antioxidant activity. All tests were carried out at a significance level of $p \leq 0.05$.

For the optimization of the UAE, a three-level full factorial design comprising 27 experimental trials was chosen to evaluate the effect of three independent variables—temperature, ultrasound power, and extraction time—during the UAE on the total phenolic content of the cake. The Shapiro-Wilk and Levene tests were performed to determine the homoscedasticity and normality of the data. A one-way ANOVA and Tukey's HSD test were performed to compare the mass concentrations of individual and total phenolic compounds between the Leikora and Ascola SB cakes. All tests were carried out with a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Fatty Acid Composition of SB Berry Oil

GC-MS analysis was used to compare the fatty acid profile of sea buckthorn oil (SBO) of the Leikora and Ascola varieties obtained by SC-CO_2 and SE. The results of the individual compounds identified are shown in Table 1, while the total content of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs), consisting of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), is shown in Table 2. A total of 14 compounds were identified, including 7 SFAs and 7 UFAs (5 MUFAs and 2 PUFAs) (Table 1).

According to the results presented in Table 2, the predominant fatty acids in the studied berry oils were UFAs (54.75–54.78% in the SBO of the Leikora variety and 72.22–72.25% in the SBO of the Ascola variety), which were mainly composed of MUFAs (42.93–42.96% in the SBO of the Leikora variety and 55.78–55.80% in the SBO of the Ascola of variety) and PUFAs (11.82% in the SBO of the Leikora variety and 16.44% in the SBO of the Ascola variety). The total content of SFAs was 45.22–45.25% in the SBO of the Leikora variety and 27.75–27.78% in the SBO of the Ascola variety.

Table 1. Fatty acid composition (weight percentages of total fatty acids) of SBO of Leikora and Ascola varieties obtained by different extraction methods.

Fatty Acids	Leikora		Ascola	
	SC-CO_2	SE	SC-CO_2	SE
		SATURATED		
Myristic	0.26 ± 0.01 ^{b}	0.25 ± 0.01 ^{b}	0.15 ± 0.01 ^{a}	0.15 ± 0.01 ^{a}
Pentadecanoic acid	0.06 ± 0.01 ^{a}	0.05 ± 0.01 ^{a}	0.07 ± 0.01 ^{a}	0.08 ± 0.01 ^{a}
Palmitic	42.86 ± 1.01 ^{b}	42.85 ± 1.14 ^{b}	25.44 ± 1.05 ^{a}	25.43 ± 0.05 ^{a}
Heptadecanoic acid	1.04 ± 0.01 ^{b}	1.03 ± 0.01 ^{b}	0.11 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}
Stearic	0.77 ± 0.01 ^{a}	0.77 ± 0.01 ^{a}	1.59 ± 0.02 ^{b}	1.59 ± 0.02 ^{b}
Arachidic acid	0.18 ± 0.01 ^{a}	0.18 ± 0.01 ^{a}	0.28 ± 0.02 ^{b}	0.29 ± 0.01 ^{b}
Docosanoic acid	0.09 ± 0.01 ^{a}	0.09 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}
		UNSATURATED		
		MONOUNSATURATED		
Palmitoleic acid	37.47 ± 1.11 ^{b}	37.46 ± 2.01 ^{b}	31.85 ± 1.15 ^{a}	31.83 ± 2.08 ^{a}
Elaidic acid	0.05 ± 0.01 ^{a,b}	0.07 ± 0.01 ^{b}	0.01 ± 0.01 ^{a}	0.01 ± 0.00 ^{a}
Gondoic acid	0.03 ± 0.01 ^{a}	0.03 ± 0.01 ^{a}	0.13 ± 0.01 ^{b}	0.14 ± 0.00 ^{b}
Oleic acid	5.35 ± 0.02 ^{a}	5.36 ± 0.01 ^{a}	16.38 ± 1.04 ^{b}	16.37 ± 1.24 ^{b}
Vaccenic acid	0.03 ± 0.00 ^{a}	0.04 ± 0.00 ^{a}	7.43 ± 1.03 ^{b}	7.43 ± 1.07 ^{b}
		POLYUNSATURATED		
Linoleic acid	11.16 ± 0.05 ^{a}	11.16 ± 0.05 ^{a}	11.20 ± 0.45 ^{a}	11.19 ± 0.63 ^{a}
α-Linolenic acid	0.66 ± 0.01 ^{a}	0.66 ± 0.01 ^{a}	5.24 ± 0.14 ^{b}	5.25 ± 0.01 ^{b}

Results are expressed as mean ± standard deviation. Values with different letters within a row are significantly different at $p \leq 0.05$. SC-CO_2= supercritical CO_2 extraction; SE= Soxhlet extraction.

According to the results presented in Table 2, SBO had a higher content of UFAs than SFAs. A similar trend was observed in studies by Dulf [39], Barkhuu et al. [40], Teleszko et al. [41], Xu et al. [42], and Xu et al. [43]. The fatty acid profile of the Ascola variety is consistent with the study by Vaitkevičiene et al. [44], in which the proportion of total fatty acids was as follows: SFAs (25.01–25.39%), UFAs (74.43–74.70%), MUFAs (46.34–51.27%), and PUFAs (9.37–11.06%). Similar results were obtained by Dulf et al. [39] and Barkhuu et al. [40]. On the other hand, Telesko et al. [41] reported a higher proportion of SFAs (35.05–41.05%), which is consistent with the proportion of SFAs in the Leikora variety.

As shown in Table 1, 14 fatty acids were identified in the SBO samples: myristic, pentadecanoic, palmitic, heptadecanoic, stearic, arachidic, docosanoic, palmitoleic, elaidic, oleic, linolelaidic, linoleic, α-linolenic, and gondoic acid. The most abundant fatty acid in SBO of the Leikora variety was palmitic (42.85–42.86%), followed by palmitoleic (37.46–37.47%), linoleic (11.16%), and oleic (5.36–5.36%) fatty acids. On the other hand, the most abundant fatty acid in SBO of the Ascola variety was palmitoleic (31.83–31.85%), followed by palmitic (25.43–25.44%), oleic (16.37–16.38%), linoleic (11.19–11.20%), vaccenic (7.43%), and α-linolenic acid (5.24–5.25%). The fatty acids determined are consistent with previously reported data [43–45], although in varying proportions, reflecting differences between varieties, geographical origin, fruit development stages, etc. Xu et al. [43] found that the most abundant fatty acids in SBO obtained with SC-CO_2 and SE were palmitoleic (38.8–39.3%), palmitic (28.8–29.4%), and oleic acid (16.4–16.5%), which is consistent with our results. In the study by Yang and Kallio (2001) [45], the following fatty acids were identified in SB berries of different origins: linoleic acid (10.2–33.9), palmitic acid (14.1–29.2%), palmitoleic acid (8.9–31.0%), oleic acid (12.6–24.8%), α-linolenic acid (6.5–18.1%), vaccenic acid (5.1–8.5%), and stearic acid (1–2%). According to Vaitkevičienė et al. [44], the most common fatty acids in SB berries were oleic acid (25.5%), palmitic acid (22.72%), palmitoleic acid (18.5%), linoleic acid (12.7%), and α-linolenic acid (10%). In our study, the SBOs of the Leikora variety had a higher content of palmitic and palmitoleic acid and a lower content of oleic acid than the reported data [44,45]. In addition, significantly lower levels of α-linolenic acid and oleic acid were found.

Table 2. Total fatty acid composition (%) of Leikora and Ascola SBO obtained by different extraction methods.

Source of Variation:		SUM SFAs:			SUM UFAs:			SUM MUFAs:			SUM PUFAs:	
		$SC\text{-}CO_2$	SE		$SC\text{-}CO_2$	SE		$SC\text{-}CO_2$	SE		$SC\text{-}CO_2$	SE
		$p < 0.001$	$p < 0.001$		$p < 0.001$	$p < 0.001$		$p < 0.05$	$p < 0.05$		$p < 0.05$	$p < 0.05$
Leikora	$p = 0.90$	$45.25 \pm 0.02^{b,A}$	$45.22 \pm 0.21^{b,A}$	$p = 0.92$	$54.75 \pm 0.03^{a,A}$	$54.78 \pm 0.26^{a,A}$	$p = 0.95$	$42.93 \pm 0.37^{a,A}$	$42.96 \pm 0.24^{a,A}$	$p = 1.00$	$11.82 \pm 0.17^{a,A}$	$11.82 \pm 0.30^{a,A}$
Ascola	$p = 0.93$	$27.75 \pm 0.20^{a,A}$	$27.78 \pm 0.26^{a,A}$	$p = 0.85$	$72.25 \pm 0.10^{b,A}$	$72.22 \pm 0.09^{b,A}$	$p = 0.99$	$55.80 \pm 0.82^{b,A}$	$55.78 \pm 0.66^{b,A}$	$p = 1.00$	$16.44 \pm 0.27^{b,A}$	$16.44 \pm 0.47^{b,A}$

Results are expressed as mean ± standard error. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between SBO samples within each extraction technique (column), while the same uppercase letters indicate no statistically significant differences ($p > 0.05$) between extraction techniques for individual SBO samples within each fatty acid (row). $SC\text{-}CO_2$ = supercritical CO_2 extraction; SE = Soxhlet extraction.

To evaluate the differences between the SB varieties and the efficiency of SC-CO_2 and SE in terms of individual and total fatty acid content, the mean values were compared (Tables 1 and 2). A statistically significant difference was found in the fatty acid distribution of Leikora and Ascola SBO ($p < 0.05$). SBO of the Ascola variety had a statistically significantly higher total content of MUFAs and PUFAs than Leikora SBO (Table 2). The MUFAs were mainly represented by palmitoleic acid and oleic acid. The content of palmitoleic acid was higher in the SBO of the Leicora variety than in the SBO of the Ascola variety, while higher levels of oleic acid were found in the SBO of the Ascola variety. Other MUFAs, such as elaidic acid and gondoic acid, were detected in traces (<0.15%). Regarding PUFAs, no difference was found in linoleic acid between the two varieties, while a higher proportion of linoleic and α-linolenic acid was found in the Ascola variety, i.e., traces of linoleic and α-linolenic acid were found in Leikora SBO (0.03–0.04 and 0.66%). The total content of SFAs was significantly higher in Leikora SBO, and the most represented fatty acid was palmitic acid. Other SFAs found in trace levels (<1.6%), such as pentadecanoic acid, stearic, arachidic, and docosanoic acid, were found in higher proportions in Ascola SBO, while myristic acid and heptadecanoic acid were more abundant in Leikora SBO. The differences between the varieties can be attributed to morphological characteristics (seed, pulp, and peel content), as the pulp is characterized by palmitic and palmitoleic acids [46], while the seeds contain linoleic, linolenic, and oleic acids [45]. According to Mate et al. [47], the berry and seed weight of SB berries are between 0.25 and 0.64 g and 0.019 and 0.036 g, respectively, depending on the variety. The Leikora variety is characterized by larger berries, i.e., a higher content of pulp and skin in relation to the seeds, which could be one of the reasons for the different fatty acid composition, i.e., a higher proportion of palmitic acids. The studies by Kuhkhei et al. [48], Yang and Kallio [45], Vaitkevičiene et al. [44], and Teleszko et al. [41] also confirmed the differences in fatty acid content between different varieties. On the other hand, the extraction method used had no influence ($p < 0.05$) on the proportion of identified fatty acids (Table 2). Despite the fact that a significantly higher temperature was used in the SE than in the SC-CO_2 extraction, the fatty acid content did not change significantly. The possible reason could be the presence of a high proportion of palmitic acid in both varieties of SB berries, which is known to be less susceptible to oxidation than UFAs. A similar trend was observed in the study by Pavlović et al. [28], Xu et al. [42], and Xu et al. [43] when comparing the influence of SC-CO_2 and SE on the fatty acid profile of SBO.

3.2. Sterol Composition of SB Berry Oil

GC-MS analysis was used to compare the sterol profiles of the SBO of Leikora and Ascola varieties obtained by SC-CO_2 and SE. The mass concentration of individual identified compounds and the total sterol content are shown in Tables 3 and 4.

A total of 16 compounds were identified, including desmosterol, kampesterol, stigmasterol, 5-α-colestanol, sitosterol, stigmast-8-en-3β-ol, 24(Z)-stigmasta-5,24(241)-dien-3β-ol, obtusifoliol, Δ5-avenasterol, stigmas-ta-7,24(28)-dien-3-ol, anosta-7,9(11)-dien-3-ol, uvaol, eritrodiol, citrostadienol, Δ7-avenasterol, and ursolic aldehyde (Table 3). The statistical analysis of the individual sterols revealed a statistically significant difference ($p \leq 0.05$) between the oils of the different varieties obtained by different extraction methods (Table 3). The most abundant sterol in SBO was β-sitosterol which ranged from 473.85 to 589.96 mg/100 g in Leikora SBO and from 352.56 to 433.82 mg/100 g in Ascola SBO. In various studies, the content of β-sitosterol has been reported in the range of 320–1377 mg/100 g, depending on the varieties, the plant part of the plant, the geographical origin, the fruit development stage, the extraction method used, etc. [13,49–51]. According to Yang et al. [13], β-sitosterol as the main sterol compound accounts for 57–83% of total sterols and 576.9 mg/100 g of oil, which is consistent with our results. Similar levels of β-sitosterol in the pulp oil (504–557 mg/100 g dm) were found in the study by Zheng et al. [49], while a significantly higher level of β-sitosterol (990–1031 mg/100 g dm) was found in the seed oil. SBO contains a higher content of β-sitosterol compared to

sunflower and virgin olive oil [1,49]. Overall ranges of campesterol (22.31–32.3 mg/100 g), uvaol (19.01–22.02 mg/100 g), and obtusifoliol (12.30–13.31 mg/100 g) were determined in the Leikora SBO, and 24(Z)-stigmasta-5,24(241)-dien-3β-ol (7.81–8.83 mg/100 g), uvaol (19.01–22.02 mg/100 g), obtusifoliol (12.30–13.31 mg/100 g), Δ5-avenasterol (4.10–5.09 mg/100 g) and campesterol (22.31–32.3 mg/100 g) were more abundant in the Ascola SBO. For the other sterols identified, the order of decreasing concentrations was as follows: 24(Z)-stigmasta-5,24(241)-dien-3β-ol > stigmasta-7,24(28)-dien-3-ol > cirtostadienol > stigmast-8-en-3β-ol > Δ5-avenasterol > eritrodiol > Δ5-avenasterol > stigmasterol > ursolic aldehyde > lanosta-7,9(11)-dien-3-ol > 5-α-colestanol > demosterol in the variety Lekora and stigmasta-7,24(28)-dien-3-ol > stigmast-8-en-3β-ol > eritrodiol > citrostadienol > Δ5-avenasterol > ursolic aldehyde > demosterol > lanosta-7,9(11)-dien-3-ol > 5-α-colestanol in the variety Ascola. Sterols determined in our study are consistent with previously reported data [12,41,52–54]. The most abundant sterols found in the seeds were β-sitosterol, Δ5-avenasterol, and obtusifoliol (approx. 15–17% of total sterols), which, together with stigmasta-8-en-3β-ol, constitute approximately 5–6% and 8–10% of the total sterols found in soft tissues and whole fruits, respectively [1,49].

Table 3. Sterol content (mg/100 g) of Leikora and Ascola SBO obtained by SC-CO_2 and SE.

Compound	Leikora		Ascola	
	SC-CO_2	SE	SC-CO_2	SE
Desmosterol	0.53 ± 0.01 ^{a}	0.31 ± 0.01 ^{a}	2.68 ± 0.01 ^{b}	2.20 ± 0.01 ^{b}
Campesterol	32.30 ± 0.15 ^{d}	22.31 ± 0.14 ^{c}	16.91 ± 0.12 ^{a,b}	11.89 ± 0.11 ^{a}
Stigmasterol	3.08 ± 0.01 ^{d}	1.11 ± 0.05 ^{a,b}	1.43 ± 0.01 ^{c}	0.45 ± 0.01 ^{a}
5-α-kolestanol	0.76 ± 0.01 ^{b}	0.22 ± 0.00 ^{a}	1.36 ± 0.01 ^{c}	0.85 ± 0.01 ^{b}
β-Sitosterol	589.96 ± 2.45 ^{c}	473.85 ± 2.14 ^{b}	433.82 ± 2.11 ^{b}	352.56 ± 2.41 ^{a}
Stigmast-8-en-3β-ol	6.51 ± 0.08 ^{c}	3.45 ± 0.02 ^{a}	7.28 ± 0.07 ^{c}	5.26 ± 0.02 ^{b}
24(Z)-stigmasta-5,24(241)-dien-3β-ol	8.83 ± 0.05 ^{a}	7.81 ± 0.05 a	30.26 ± 0.45 ^{b}	23.23 ± 0.52 ^{b}
Obtusifoliol	13.31 ± 0.12 ^{a}	12.30 ± 0.05 ^{a}	24.27 ± 0.25 ^{c}	18.51 ± 0.12 ^{b}
Δ5-avenasterol	5.09 ± 0.01 ^{a}	4.10 ± 0.01 ^{a}	17.67 ± 0.23 ^{b}	15.21 ± 0.11 ^{b}
Stigmasta-7,24(28)-dien-3-ol	7.50 ± 0.02 ^{b}	3.10 ± 0.01 ^{a}	9.85 ± 0.05 ^{c}	6.86 ± 0.05 ^{b}
Lanosta-7,9(11)-dien-3-ol	1.92 ± 0.01 ^{c}	0.58 ± 0.00 ^{a}	1.43 ± 0.00 ^{a,b}	1.12 ± 0.01 ^{a,b}
Uvaol	22.02 ± 0.05 ^{a}	19.01 ± 0.04 ^{a}	26.23 ± 0.11 ^{b}	20.25 ± 0.06 ^{a}
Eritrodiol	5.12 ± 0.03 ^{b}	3.02 ± 0.01 ^{a}	6.84 ± 0.01 ^{c}	5.85 ± 0.00 ^{b}
Citrostadienol	7.05 ± 0.02 ^{b}	6.01 ± 0.01 ^{a,b}	5.91 ± 0.01 ^{a,b}	4.78 ± 0.01 ^{a}
Δ7-avenasterol	3.35 ± 0.01 ^{a,b}	3.14 ± 0.01 ^{a}	4.36 ± 0.01 ^{c}	3.95 ± 0.00 ^{b,c}
Ursolic aldehyde	1.99 ± 0.04 ^{a,b}	1.01 ± 0.01 ^{a}	3.04 ± 0.01 ^{b}	2.52 ± 0.00 ^{b}

Results are expressed as mean ± standard deviation. Values with different letters within a row are significantly different at $p \leq 0.05$. SC-CO_2 = supercritical CO_2 extraction; SE = Soxhlet extraction.

Table 4. Total sterol content (mg/100 g) of Leikora and Ascola SBO obtained by SC-CO_2 and SE.

Source of Variation:	Total Sterol Content (mg/100 g)		
		SC-CO_2	SE
		$p < 0.05$	$p < 0.05$
Leikora	$p < 0.05$	709.32 ± 4.20 ^{b,B}	561.33 ± 3.10 ^{b,A}
Ascola	$p < 0.05$	593.34 ± 4.18 ^{a,B}	475.49 ± 4.20 ^{a,A}

Results are expressed as mean ± standard error. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between SBO samples within each extraction technique (column), while different uppercase letters indicate statistically significant differences ($p < 0.05$) between extraction techniques for individual SBO samples within each fatty acid (row). SC-CO_2 = supercritical CO_2 extraction; SE = Soxhlet extraction.

As shown in Table 4, the total content of sterols in the SBOs ranged from 561.33 to 709.32 mg/100 g in Leikora and from 475.49 to 593.34 mg/100 g in Ascola SBO. Our results are consistent with previously reported data [49,51,55]. In the study by Yang et al. [55], the total sterol content in the seed oil, soft parts, and whole fruit was 12–23 g/kg, 10–29 g/kg, and 13–33 g/kg, respectively. Zheng et al. [49] reported a total sterol content in the range of

778–1847 mg/100 g, depending on the parts of the berry (pulp/seed) and the extraction method used. A similar range of total sterols (440.3–1688.7 mg/100 g) in oil obtained from different parts of the berries and by different extraction methods was reported in the study by Arimboor et al. [51].

To evaluate the differences between the SB varieties and the efficiency of SC-CO_2 and SE in terms of total sterol content, the mean values were compared (Table 4). A statistically significant difference was found in the total sterol content of Leikora and Ascola SBO ($p < 0.05$) within each extraction method. SBO of the Leikora variety had a statistically significantly higher total sterol content than Ascola SBO. This is due to a significantly higher content of β-sitosterol, while the other identified sterols were mainly present in higher concentrations in the Ascola variety. Teleszko et al. [41] also reported a statistically significant difference in the sterol content in the berries of eight Russian SB cultivars. The extraction method used also had a statistically significant effect ($p < 0.05$) on the total sterol content within each SB variety. A similar trend was observed in the study by Sajfrtova et al. [56] when the content of β-sitosterol was compared in extracts obtained with SC-CO_2 and SE. SC-CO_2 resulted in up to five-fold higher concentrations of β-sitosterol in the extract (0.50% w/w) than the conventional SE with hexane (0.10% w/w). The lower selectivity of hexane and the high extraction temperature, in combination with the influence of atmospheric oxygen, probably led to a partial degradation of β-sitosterol. Li et al. [12] reported that SC-CO_2 extraction was better than extraction with hexane and cold pressing in terms of total and individual sterol content in SBO. Arimboor et al. [51] also reported a higher content of sterols in extracts obtained by SC-CO_2. Yang et al. [57] pointed out that the supercritical fluid (CO_2) has a high penetrating and dissolving capacity and thus offers advantages in the extraction of lipids and other bioactive substances from various types of raw materials. As it is an environmentally friendly process, supercritical solvent extraction is usually carried out at low temperatures and under vacuum, which protects the BAMs from thermal and oxidative degradation [58].

3.3. Carotenoid Content of Leikora and Ascola SBO Obtained by SC-CO_2 and SE

The carotenoid profiles of the SBO of Leikora and Ascola varieties obtained by SC-CO_2 and SE were analyzed by HPLC-DAD and compared. The mass concentration of the individual compounds identified and the total carotenoid content are shown in Tables 5 and 6.

Table 5. Carotenoid content of Leikora and Ascola SBO obtained by SC-CO_2 and SE.

Carotenoids:	Mass Concentration (mg/100 g dm)			
	Leikora		Ascola	
	SC-CO_2	SE	SC-CO_2	SE
Zeaxanthin	8.50 ± 0.15 ^{a}	5.42 ± 0.16 ^{a}	8.77 ± 0.01 ^{a}	8.45 ± 0.05 ^{a}
Lutein	3.38 ± 0.05 ^{a}	4.33 ± 0.01 ^{b}	nd	nd
β-cryptoxanthin	5.21 ± 0.06 ^{b}	3.76 ± 0.01 ^{a}	6.85 ± 0.02 ^{c}	5.75 ± 0.01 ^{b}
γ-carotene	0.07 ± 0.00 ^{a}	0.05 ± 0.00 ^{a}	0.18 ± 0.00 ^{b}	0.09 ± 0.00 ^{a}
cis γ-carotene	0.44 ± 0.01 ^{b}	0.19 ± 0.00 ^{a}	1.18 ± 0.01 ^{d}	0.99 ± 0.01 ^{c}
β-carotene	2.09 ± 0.01 ^{b}	1.73 ± 0.00 ^{a}	2.09 ± 0.01 ^{b}	1.95 ± 0.15 ^{a,b}
β-cryptoxanthin palmitate	0.73 ± 0.02 ^{b}	0.78 ± 0.01 ^{b}	0.14 ± 0.00 ^{a}	nd
Zeaxanthin-myristate	26.66 ± 0.15 ^{a}	24.76 ± 0.25 ^{a}	28.65 ± 0.75 ^{a}	27.52 ± 0.25 ^{a}
Lutein-palmitate	5.85 ± 0.01 ^{b}	3.07 ± 0.01 ^{a}	3.30 ± 0.00 ^{a}	2.95 ± 0.00 ^{a}
Zeaxanthin-pamitate	10.86 ± 0.14 ^{a}	8.18 ± 0.15 ^{a}	31.01 ± 0.20 ^{b}	30.82 ± 0.45 ^{b}
Lutein di-myristate	nd	nd	10.39 ± 0.15 ^{b}	9.12 ± 0.12 ^{a}
Zeaxanthin-palmitate-myristate	19.97 ± 0.25 ^{b}	12.87 ± 0.16 ^{a}	36.22 ± 1.05 ^{c}	35.98 ± 1.21 ^{c}
Lutein di-palmitate	6.44 ± 0.05 ^{a}	5.40 ± 0.05 ^{a}	24.17 ± 0.95 ^{b}	23.25 ± 0.85 ^{b}
Zeaxanthin-di-palmitate	56.73 ± 1.12 ^{b}	43.38 ± 1.25 ^{a}	58.08 ± 1.75 ^{b}	57.05 ± 1.44 ^{b}
Lutein-palmitate-stearate	35.05 ± 1.15 ^{c}	26.94 ± 0.45 ^{b}	4.37 ± 0.00 ^{a}	3.75 ± 0.00 ^{a}

Results are expressed as mean ± standard deviation. Values with different letters within a row are significantly different at $p \leq 0.05$. SC-CO_2 = supercritical CO_2 extraction; SE = Soxhlet extraction; nd-not detected.

Table 6. Total carotenoids of Leikora and Ascola SBO obtained by SC-CO_2 and SE.

Source of Variation:		Total Carotenoid Content (mg/100 g)	
		SC-CO_2	SE
		$p < 0.05$	$p < 0.001$
Leikora	$p < 0.05$	181.98 ± 1.73 ^{a,B}	140.86 ± 1.50 ^{a,A}
Ascola	$p = 0.09$	215.4 ± 2.18 ^{b,A}	207.67 ± 1.11 ^{b,A}

Results are expressed as mean ± standard error. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between SBO samples within each extraction technique (column), while different uppercase letters indicate statistically significant differences ($p < 0.05$) between extraction techniques for individual SBO samples within total carotenoid content (row). SC-CO_2 = supercritical CO_2 extraction; SE = Soxhlet extraction.

The total carotenoid content of SBO was relatively high, ranging from 140.88–187.99 mg/100 g for the Leikora variety and 217.93–226.84 mg/100 g for the Ascola variety, depending on the extraction method (Table 6). The high concentration of pigments in SBO was also confirmed in previous studies [59,60]. As shown in Table 5, a total of 16 compounds were identified, including free form carotenoids (zeaxanthin, lutein, β-cryptoxanthin, γ-carotene, cis-γ-carotene, and β-carotene) and the esterified forms of zeaxanthin, lutein, and β-cryptoxanthin. Esterified forms (with one or two fatty acids) of zeaxanthin and lutein corresponded to 71% of the total identified carotenoids [33]. The presence of individual carotenoids differs between the SB varieties (Table 5). In SBO of Leikora variety, zeaxanthin-di-palmitate (43.38–56.73 mg/100 g) was the most abundant, followed by lutein-palmitate-stearate (26.94–35.05 mg/100 g) and zeaxanthin myristate (24.76–26.66 mg/100 g), while zeaxanthin-di-palmitate (57.05–58.08 mg/100 g) was the most abundant in SBO of Ascola variety, followed by zeaxanthin-palmitate-myristate (35.98–36.22 mg/100 g), zeaxanthin-palmitate (30.82–31.01 mg/100 g), zeaxanthin-myristate (27.52–28.65 mg/100 g) and lutein-dipalmitate. Tudor et al. [59] also reported that the most common compounds in SBO are esterified forms of zeaxanthin, with zeaxanthin-di-palmitate being the most abundant. Pop et al. [33] also found zeaxanthin di-palmitate in the highest amount in SB berries. In our study, β-Cryptoxanthin palmitate was the only β-cryptoxanthin ester detected in significantly lower concentrations than zeaxanthin and lutein esters (0.14–1.12 mg/100 g). The zeaxanthin content was between 5.42 and 8.50 mg/100 g for the Leikora variety and between 8.45 and 8.77 mg/100 g for the Ascola variety. Lutein was only detected in the extracts of the Leikora variety (3.38–4.33 mg/100 g). The lower content of carotenoids in free form could be due to the fact that xanthophylls such as zeaxanthin are converted to esterified forms during ripening, which are more stable forms of carotenoid storage in fruits [61]. In contrast, β-cryptoxanthin was present at a higher concentration than its ester, ranging from 3.76 to 5.21 mg/100 g in SBO of the Leikora variety and from 5.75 to 6.85 in SBO of the Ascola variety. Among the carotenes, β-, γ-, and cis-γ carotene were detected in SBOs in a range of 0.99–4.15 mg/100 g, 0.05–0.21 mg/100 g and 0.07–1.52 mg/100 g, respectively, depending on the variety and extraction method.

To evaluate the differences between the SB varieties and the efficiency of SC-CO_2 and SE in terms of total carotenoid content, the mean values were compared (Table 6). A statistically significant difference was found in the total carotenoid content of Leikora and Ascola SBO ($p < 0.05$) within each extraction method. SBO of the Ascola variety had a statistically significantly higher total carotenoid content than SBO of the Leikora variety. Various studies have found considerable differences in carotenoid composition and content depending on variety, geographical location, growing conditions, harvest maturity, storage conditions, and analytical methods [33,41,62–64]. As SB is a wind-pollinated species, a high degree of genetic variation is also observed [64]. The extraction method also had a statistically significant effect ($p < 0.05$) on the total carotenoid content of the SBO of the Leikora variety, and a higher content of carotenoids was found when SC-CO_2 was used. The high temperatures and long extraction time used in SE lead to thermal degradation and cis to trans isomerization of carotenoids, while the higher diffusivity and lower viscosity of supercritical fluids, shorter extraction time, and lower temperatures used in SC-CO_2 prevent the degradation of BAMs such as the carotenoids [58]. Arimboor et al. [51] also

reported that SC-CO_2 contributed to the higher carotenoid content in SBO than solvent extraction with hexane.

3.4. α-Tocopherol Content in SB Berry Oil

The α-tocopherol content of SBO of the Leikora and Ascola varieties obtained with SC-CO_2 and SE was determined by HPLC-FID and compared in Table 7.

Table 7. Mass concentration of α-tocopherol (mg/100 g) determined in Leikora and Ascola SBO obtained by different extraction methods.

Source of Variation:		α-Tocopherol (mg/100 g dm)	
		SC-CO_2	SE
		$p = 0.33$	$p = 0.14$
Leikora	$p < 0.05$	106.86 ± 2.61 ^{a,B}	84.23 ± 1.00 ^{a,A}
Ascola	$p < 0.05$	102.45 ± 2.33 ^{a,B}	81.06 ± 0.85 ^{a,A}

Results are expressed as mean ± standard error. The same lowercase letters indicate no statistically significant differences ($p > 0.05$) between SBO samples within each extraction technique (column), while different uppercase letters indicate statistically significant differences ($p < 0.05$) between extraction techniques for individual SBO samples within each fatty acid (row). SC-CO_2 = supercritical CO_2 extraction; SE = Soxhlet extraction.

As shown in Table 7, the α-tocopherol content in SBO of the Leikora variety ranged from 84.23 to 106.86 mg/100 g and in SBO of the Ascola variety from 81.06 to 102.45 mg/100 g. Our results are in agreement with the studies of Zadernowski et al. [65] and Bal et al. [66]. On the other hand, Otgonbayar et al. [67] reported a lower content of α-tocopherol in seeds (34.57 mg/100 g oil) and pulp oil (59.02 mg/100 g oil). Similar results were obtained in the study by Andersson et al. [10]. Tocopherol content depends on the extraction and analysis methods used, the part of the berry, genetic variation, climate, growing conditions, annual variation, ripeness at harvest, storage conditions, and harvesting method [9,10].

To evaluate the differences between the SB varieties and the efficiency of SC-CO_2 and SE in terms of α-tocopherol content, the mean values were compared (Table 7). A statistically significant difference ($p < 0.05$) in α-tocopherol content was found between the two extraction methods used. In addition, more tocopherols were found in oil extracted with SC-CO_2. Xu et al. [43] and Arimboor et al. [51] also reported a higher α-tocopherol content in oils obtained using SC-CO_2. Several studies have confirmed that the oil obtained by SC-CO_2 from different matrices has a higher tocopherol content than the oil obtained by conventional extraction with hexane [68–71]. Although it is stable at higher temperatures, the oxidation rate of α-tocopherol increases at high temperatures and in the presence of oxygen [72,73]. This explains the higher content of α-tocopherol in SC-CO_2, which is carried out under vacuum and at significantly lower temperatures compared to SE.

3.5. Antioxidant Activity of SB Berry Oil

The lypophilic ORAC method was used to evaluate the AA of the oils obtained by SC-CO_2 and SE (Table 8).

Table 8. Antioxidant activity of SBOs obtained by SC-CO_2 and SE.

Source of Variation:		L-ORAC (μmolTE/100 g dm)	
		SC-CO_2	SE
		$p = 0.20$	$p = 0.55$
Leikora	$p < 0.05$	1537.10 ± 4.19 ^{a,B}	1356.58 ± 19.62 ^{a,A}
Ascola	$p < 0.05$	1558.50 ± 10.34 ^{a,B}	1372.63 ± 10.47 ^{a,A}

Results are expressed as mean ± standard error. The same lowercase letters indicate no statistically significant differences ($p < 0.05$) between SBO samples within each extraction technique (column), while different uppercase letters indicate statistically significant differences ($p < 0.05$) between extraction techniques for individual SBO samples within the ORAC value (row). SC-CO_2 = supercritical CO_2 extraction; SE = Soxhlet extraction.

According to the results presented in Table 8, a higher AA (1558.50 µmolTE/100 g) was observed in the SBO of the Leikora variety obtained with SC-CO_2 than in the SBO of the Ascola variety (1537.10 µmolTE/100 g), although no significant difference (p = 0.20) was observed. On the other hand, the lower AA was determined in the SBO of Ascola (1356.58 µmolTE/100 g) and Leikora (1372.63 µmolTE/100 g) obtained by SE. The high AA of the oils could be related to the high content of antioxidants such as α-tocopherol and carotenoids in both varieties. Andrei et al. [74] have shown that carotenoids from SB fruits have a good antioxidant effect and protect vegetable oils from peroxidation processes induced in the presence of AAPH. In addition, vitamin E is one of the most important antioxidants in SB berries [10]. The statistical analysis (Table 8) showed that the oils obtained by SC-CO_2 had a significantly ($p < 0.05$) higher AA, which correlates with the higher content of BAMS in the oils obtained by SC-CO_2.

3.6. Polyphenolic Compounds in SB Cake Obtained after Oil Extraction

SB cake of the Leikora variety was used to determine the optimal UAE conditions, i.e., temperature (35, 50, and 65 °C), ultrasound power (30, 60, and 90%), and extraction time (10, 20 and 30 min).

The results of TPC and AA in SB cake extracts produced under different UAE conditions are shown in Table 9. To determine the optimal UAE conditions, an ANOVA statistical analysis of the effects of ultrasound power, temperature, and extraction time was performed (Table 10). The extraction of phenolic compounds from the cakes of Leikora and Ascola SB obtained after SC-CO_2 and SE was carried out under the determined optimal extraction conditions, and the extracts were analyzed by the UPLC/ESI-MS^2 method (Table 11). The AA of SB cake extracts obtained at optimal UAE conditions is shown in Figure 1.

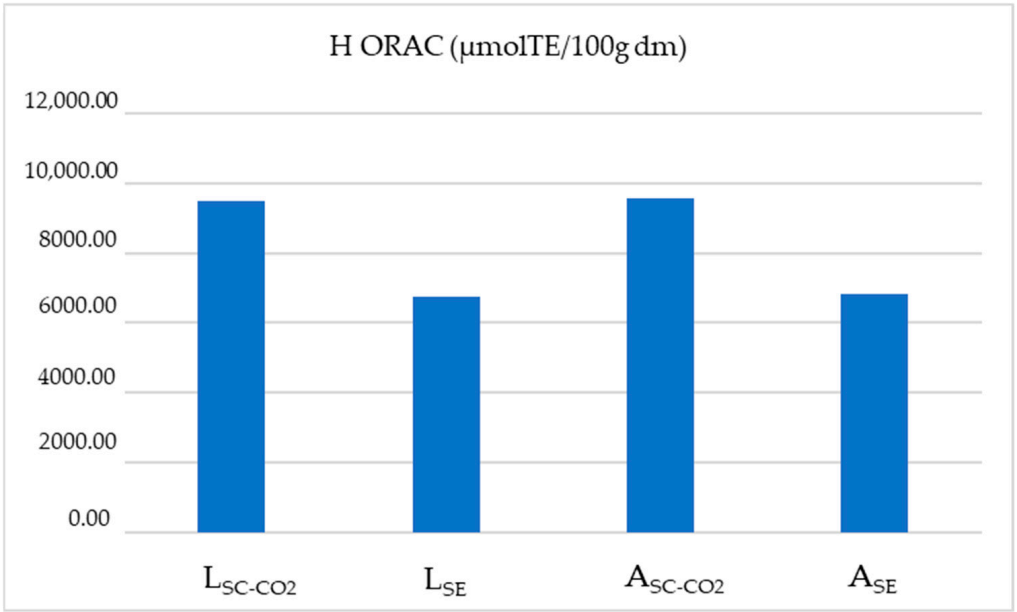

Figure 1. AA of SB cake extracts obtained under optimal UAE. $L_{SC\text{-}CO2}$ = cake of Leikora variety obtained after supercritical CO_2 extraction, L_{SE} = cake of Leikora variety obtained after Soxhlet extraction, $A_{SC\text{-}CO2}$ = cake of Ascola variety obtained after supercritical CO_2 extraction, A_{SE} = cake of Ascola variety obtained after Soxhlet extraction.

Table 9. Total phenolic content of SB cake extracts obtained using different UAE conditions.

Source of Variation		Ultrasound Power (%)					
		30	60	90	30	60	90
Temperature (°C)	Time (min)	TPC mg/100 g dm			AA µmol TE/100 g dm		
35	10	1123.25 ± 25.02	1254.77 ± 22.15	1242.25 ± 24.81	6871.34 ± 121.45	7524.12 ± 65.74	7192.18 ± 132.14
	20	1256.36 ± 31.45	1596.75 ± 18.45	1591.90 ± 35.62	7322.90 ± 147.23	8194.90 ± 111.41	8280.29 ± 101.81
	30	1499.83 ± 33.15	1366.05 ± 21.75	1437.08 ± 85.41	8759.13 ± 150.74	8113.61 ± 121.71	8189.16 ± 108.45
50	10	1572.21 ± 45.18	1851.72 ± 75.41	1749.01 ± 58.41	8983.91 ± 101.41	8756.20 ± 125.41	8751.92 ± 102.71
	20	1795.40 ± 52.41	1998.98 ± 33.45	1792.11 ± 62.35	8700.93 ± 121.74	9504.85 ± 125.36	8795.53 ± 125.85
	30	1735.74 ± 65.85	1965.86 ± 25.41	1846.55 ± 55.24	8440.58 ± 122.52	9448.77 ± 145.87	8931.60 ± 123.52
65	10	1303.05 ± 25.42	1381.00 ± 25.74	1281.03 ± 55.24	8887.88 ± 95.41	8631.72 ± 155.71	8427.25 ± 185.41
	20	1753.91 ± 75.41	1853.88 ± 65.41	1829.31 ± 52.36	8994.28 ± 127.34	9332.67 ± 191.74	9485.58 ± 155.24
	30	1808.65 ± 48.26	1793.14 ± 75.61	1761.33 ± 75.14	8931.72 ± 154.25	8912.14 ± 185.74	9104.90 ± 151.74

Results are expressed as mean ± standard deviation. TPC = total phenolic content, AA = antioxidant activity.

Table 10. Influence of UEA parameters on TPC of SB cake extracts.

Source of Variation	TPC mg/100 g dm	AA µmol TE/100 g dm
Ultrasound power (%)	$p = 0.29$	$p = 0.39$
30	1538.71 ± 59.40 ^{a}	8432.52 ± 179.75 ^{a}
60	1673.57 ± 64.51 ^{a}	8735.44 ± 161.66 ^{a}
90	1614.51 ± 54.90 ^{a}	8706,49 ± 167.81 ^{a}
Temperature (°C)	$p \leq 0.001$	$p \leq 0.001$
35	1374.25 ± 38.75 ^{a}	7827.51 ± 142.20 ^{a}
50	1811.95 ± 30.04 ^{c}	8946.03 ± 90.92 ^{b}
65	1640.59 ± 55.80 ^{b}	9100.90 ± 67.87 ^{b}
Time (min)	$p \leq 0.001$	$p = 0.11$
10	1417.59 ± 57.62 ^{a}	8336.28 ± 205.74 ^{a}
20	1718.73 ± 49.37 ^{b}	8756.88 ± 170.40 ^{a}
30	1690.47 ± 47.21 ^{b}	8781.29 ± 105.64 ^{a}

Results are expressed as mean value ± standard error. Means with different letters within the column are significantly different at $p \leq 0.05$. TPC = total phenolic content; AA = antioxidant activity.

Table 11. Mass spectrometric data and identification of phenolic compounds in SB cake extracts obtained using optimized UAE conditions.

Phenolic Compounds	Precursor Ion (*m*/*z*)	Fragment Ions (*m*/*z*)	Ionization Mode	Mass Concentration (mg/100 g dm)			
				$L_{SC\text{-}CO2}$	L_{SE}	$A_{SC\text{-}CO2}$	A_{SE}
FLAVONOLS							
Isorhamnetin	317	201	positive	0.45 ± 0.01 ^{a}	0.93 ± 0.02 ^{b}	1.05 ± 0.01 ^{b}	0.98 ± 0.01 ^{b}
Isorhamnetin-3-sinapoyglucose-glucoside-7-rhamnoside	993	463, 317	positive	1.71 ± 0.05 ^{b}	0.62 ± 0.01 ^{a}	1.80 ± 0.02 ^{b}	0.53 ± 0.00 ^{a}
Ishorhamnetin-3-sophoroside-7-rhamnoside	787	463, 317	positive	11.67 ± 0.12 ^{c}	6.79 ± 0.01 ^{b}	8.17 ± 0.02 ^{b}	3.20 ± 0.01 ^{a}
Isorhamnetin-3-rutinoside-7-glucoside	787	479, 317	positive	1.98 ± 0.05 ^{b,c}	1.46 ± 0.01 ^{b}	2.25 ± 0.01 ^{c}	0.33 ± 0.00 ^{a}
Isorhamnetin-3-hexoside	479	317	positive	65.37 ± 2.14 ^{c}	35.90 ± 1.45 ^{a}	79.44 ± 2.74 ^{d}	56.54 ± 1.85 ^{b}
Isorhamnetin-3-rhamnoside	463	317	positive	2.20 ± 0.00 ^{a}	4.01 ± 0.01 ^{b}	4.67 ± 0.01 ^{b}	2.68 ± 0.00 ^{a}
Isorhamnetin-3.7-dihexoside	641	479, 317	positive	0.55 ± 0.00 ^{a,b}	1.25 ± 0.01 ^{b}	0.51 ± 0.00 ^{a}	2.91 ± 0.01 ^{c}
Isorhamnetin-3-rutinoside	625	479, 317	positive	16.01 ± 0.54 ^{b}	9.64 ± 0.65 ^{a}	30.88 ± 0.17 ^{c}	15.87 ± 0.28 ^{b}
Kaempferol	287	145	positive	7.56 ± 0.01 ^{b}	3.53 ± 0.00 ^{a}	8.53 ± 0.01 ^{b}	2.97 ± 0.00 ^{a}
Kaempferol-3-O-sophorose-7-O-rhamnoside	757	287	positive	1.76 ± 0.01 ^{a}	1.71 ± 0.01 ^{a}	1.36 ± 0.01 ^{a}	3.98 ± 0.01 ^{b}
Kaemferol-3-O-glucoside-7-O-rhamnoside	595	433, 287	positive	15.71 ± 0.54 ^{b}	12.38 ± 0.25 ^{a}	18.72 ± 0.23 ^{c}	13.89 ± 0.08 ^{a,b}
Kaempferol-3-rutinoside	595	287	positive	1.97 ± 0.01 ^{a}	0.42 ± 0.02 ^{a}	2.03 ± 0.02 ^{a}	1.22 ± 0.02 ^{a}
Kaempferol-rhamnoside	433	287	positive	3.32 ± 0.01 ^{b}	3.12 ± 0.01 ^{b}	9.39 ± 0.05 ^{c}	1.27 ± 0.05 ^{a}
Quercetin-3-sophoroside-7-rhamnoside	773	611, 303	positive	2.32 ± 0.00 ^{b}	0.20 ± 0.00 ^{a}	2.11 ± 0.01 ^{b}	0.33 ± 0.00 ^{a}
Quercetin-3-rhamnosylglucoside-7-rhamnoside	757	303	positive	1.97 ± 0.02 ^{b}	1.65 ± 0.01 ^{b}	0.75 ± 0.02 ^{a}	0.55 ± 0.01 ^{a}
Quercetin-3-rutinoside (rutin)	611	303	positive	47.14 ± 2.45 ^{b}	32.49 ± 1.24 ^{a}	41.74 ± 0.98 ^{b}	33.27 ± 0.98 ^{a}
Quercetin-3-glucoside	465	303	positive	20.93 ± 0.04 ^{b}	13.46 ± 0.02 ^{a}	48.16 ± 0.51 ^{c}	16.43 ± 0.09 ^{a}
Quercetin-3-rhamnoside (quercitrin)	449	303	positive	1.70 ± 0.01 ^{b}	1.60 ± 0.01 ^{b}	0.95 ± 0.00 ^{a}	2.95 ± 0.01 ^{c}
Quercetin-3-pentoside	435	303	positive	0.81 ± 0.00 ^{b}	0.50 ± 0.00 ^{a}	0.54 ± 0.00 ^{a}	0.38 ± 0.00 ^{a}
SUM:				205.13 ± 6.01 ^{c}	131.66 ± 3.52 ^{a}	263.05 ± 4.81 ^{d}	160.28 ± 4.92 ^{b}

Table 11. *Cont.*

Phenolic Compounds	Precursor Ion (*m*/*z*)	Fragment Ions (*m*/*z*)	Ionization Mode	Mass Concentration (mg/100 g dm)			
				L_{SC-CO2}	L_{SE}	A_{SC-CO2}	A_{SE}
FLAVAN-3-OLS							
Catechin	291	139	positive	2.15 ± 0.00 ^{b}	0.64 ± 0.00 ^{a}	3.16 ± 0.00 ^{c}	2.10 ± 0.00 ^{b}
Epicatechin	291	165	positive	0.99 ± 0.00 ^{a}	0.51 ± 0.00 ^{a}	3.82 ± 0.00 ^{c}	2.50 ± 0.00 ^{b}
SUM:				3.14 ± 0.01 ^{b}	1.15 ± 0.00 ^{a}	6.98 ± 0.01 ^{c}	4.6 ± 0.01 ^{b}
PHENOLIC ACIDS							
Caffeic acid	179	135	negative	23.14 ± 1.41 ^{a}	21.01 ± 0.95 ^{a}	23.03 ± 1.01 ^{a}	22.05 ± 1.11 ^{a}
Chorogenic acid	353	191	negative	2.33 ± 0.01 ^{a}	14.35 ± 0.85 ^{b}	3.46 ± 0.01 ^{a}	17.63 ± 1.14 ^{c}
Gallic acid	169	125	negative	23.92 ± 1.05 ^{a}	21.82 ± 0.98 ^{a}	21.30 ± 1.75 ^{a}	20.71 ± 1.14 ^{a}
p-hydroxybenzoic acid	137	93	negative	31.49 ± 2.14 ^{b}	18.75 ± 0.85 ^{a}	38.13 ± 1.74 ^{c}	29.72 ± 1.12 ^{b}
p-coumaric acid	163	119	negative	14.67 ± 0.85 ^{b}	11.51 ± 0.08 ^{b}	13.13 ± 0.75 ^{b}	7.55 ± 0.05 ^{a}
Protocatechuic acid	153	109	negative	38.08 ± 1.74 ^{c}	9.95 ± 0.83 ^{a}	29.53 ± 1.41 ^{b}	9.55 ± 0.81 ^{a}
Vanillic acid	169	125	positive	46.80 ± 1.28 ^{a}	42.63 ± 1.95 ^{a}	60.46 ± 2.53 ^{b}	48.32 ± 1.75 ^{a}
SUM:				180.43 ± 8.48 ^{b}	140.02 ± 6.49 ^{a}	189.04 ± 8.94 ^{b}	155.53 ± 6.21 ^{a,b}

Results are expressed as mean ± standard deviation. Values with different letters within a row are significantly different at $p \leq 0.05$. L_{SC-CO2} = cake of Leikora variety obtained after supercritical CO_2 extraction, L_{SE} = cake of Leikora variety obtained after Soxhlet extraction, A_{SC-CO2} = cake of Ascola variety obtained after supercritical CO_2 extraction, A_{SE} = cake of Ascola variety obtained after Soxhlet extraction.

The TPC of the SB cake extracts ranged from 1123.25 to 1998.98 mg/100 g dm (Table 9). The lower TPC content in SB cake defatted with SC-CO_2 (4.71–7.87 mg/g dm) was reported in the study by Dienaite et al. [20]. On the other hand, our results of TPC were similar with studies on cakes obtained after oil extraction from other raw materials such as sunflower (751.00–1851.00 mg/100 g dm), hemp (911.30–1542.03 mg/100 g), and flax (873.97–1257.35), confirming that the cake obtained after oil extraction is a valuable source of BAMs such as phenolic compounds [21,75]. In our previous study [36], a significantly lower TPC was found in freeze-dried SB berries compared to the TPC of the cake obtained after oil extraction. However, the content and type of phenols depend on the extraction methods used, their chemical nature, the particle size, the presence of interfering compounds, and the storage conditions [76]. SB berries are rich in fats and pigments, which can impair the extraction of phenolic compounds. Therefore, the removal of fats and pigments after oil extraction probably led to an increase in the yield of TPC in cake extracts. In addition, the cavitation effect of UAE promotes the damage of cell walls, which facilitates the release of phenolic compounds into the extraction solvent as done by Rodriguez et al. [77].

The complete recovery of antioxidants and other BAMs is very important for the development of effective processes for the utilization of by-products [20]. Therefore, the ORAC method was used to determine the effect of ultrasound on the AA of the extracted phenolic compounds. The AA of the SB cake extracts ranged from 6871.34 to 9504.85 µmol TE/100 g dm (Table 9). The ORAC values were significantly higher than the ORAC values determined in our previous study Čulina et al. [35] on freeze-dried SB berries, which is consistent with the higher TPC content in SB cake extracts. Also, lower ORAC values in SB cake obtained after SC-CO_2 (15.84–35.26 µmolTE/g dm) were reported in the study by Dienaite et al. [20].

3.6.1. Optimization of the UAE of Polyphenols from SB Cake

To achieve a high extraction yield, the variables that influence UAE, such as ultrasonic power, extraction time, and temperature, must be optimized. According to the results of the statistical analysis in Table 10, temperature and extraction time had a statistically significant effect ($p < 0.001$) on the TPC, while temperature had a statistically significant effect on the AA of the SB cake extracts.

Ultrasonic power is considered one of the critical parameters that need to be optimized, as the use of high values usually improves the extraction yield due to the generation of strong shear forces. In addition, a higher ultrasonic power shortens the extraction time [78]. As shown in Table 10, increasing the ultrasonic power from 30 to 60% increased the TPC and AA of SB cake extracts. The ultrasonic waves cause cavitation and thermal and mechanical phenomena leading to cell wall disruption, particle size reduction, and a higher

reaction rate due to mass transfer through the cell wall, which improves solvent penetration and accelerates the extraction of BAMs. A similar trend of increase in TPC and AA, with an increase in ultrasound power from 20 to 60%, was observed in the study by Sharayei et al. [79] on pomegranate peels. On the other hand, increasing the ultrasonic power from 60 to 90% led to a decrease in TPC and AA in SB cake extracts. At higher ultrasound power, cavitation phenomena can initiate new reaction mechanisms, i.e., bubble destruction, degassing, and free radical formation, and cause changes in temperature, pressure, and physical interaction between solid and liquid interfaces that favor the degradation of BAMs [80]. Borrás-Enríquez et al. [81] and Pedisić et al. [82] found that the optimal ultrasonic power for obtaining extracts with high TPC and AA in mango residues and monofloral honeys is 60%, which is consistent with our study. In addition, temperature can affect the integrity of BAMs, as most of them are thermolabile. Considering that high ultrasonic power combined with long extraction times can lead to sample degradation, temperature control is essential for the proper design of the cooling reactor and the optimization of the UAE. Increasing the temperature from 35 to 50 °C led to an increase in the TPC and AA in the SB cake extracts (Table 10). The application of high temperatures leads to a softening of the plant tissue and a weakening of the interactions between phenolic compounds and proteins, which ultimately leads to a better migration of phenolic compounds into the extraction solvent [83]. Although a higher temperature leads to a higher yield, above a certain temperature, the TPC decreases due to a lower effect of cavitation, i.e., a large number of bubbles implode with less intensity, causing less damage to the plant material [84]. Also, excessively high temperatures can impair the propagation of ultrasound in the medium [85]. Thus, increasing the temperature to 65 °C led to a decrease in the TPC (Table 10). The study by Setyaningsih et al. [86] reported that many phenolic compounds were stable at an extraction temperature of 70 °C. In contrast, some phenolic compounds started to degrade at 50 °C. To achieve a yield of more than 90%, it is recommended to perform ultrasonic extraction at temperatures between 10 °C, and 50 °C. Increasing the temperature from 50 °C to 65 °C resulted in a higher AA, but no significant difference was found between the values obtained. The study by Shehata et al. [87] has shown that 50 °C is the optimal temperature to achieve the highest TPC and AA, which is consistent with our study. UAE enables a good extraction yield with relatively short extraction times (maximum 60 min), as longer times can lead to undesirable changes in the extracted compounds. The optimized extraction time is usually between 20 and 60 min, which minimizes energy consumption and reduces the exposure of the compounds to the process [88]. TPC and AA in SB cake extracts increased with prolongation of the extraction time from 10 to 20 min. However, excessively long extraction times can lead to a decrease in yield due to BAM degradation [84]. Thus, increasing the extraction time to 30 min resulted in a decrease in TPC and an increase in AA; however, no statistically significant difference was observed between the values obtained (Table 10). Our results are in accordance with the studies by Teh and Birch [21], Dobrinčić et al. [89], Galviz-Quezada et al. [90], and Borrás-Enríquez et al. [81], in which an extraction time of 20 min proved to be the most suitable to obtain extracts with high TPC and AA. The results of the statistical analysis show that the optimal UAE conditions for obtaining SB cake extracts with high TPC and AA were: an ultrasonic amplitude of 60%, a temperature of 50 °C, and a sonication time of 20 min.

3.6.2. Polyphenolic Characterization of Different SB Cake Extracts

UPLC/ESI-MS^2 was used to compare the polyphenolic profile of SB cake extracts obtained under optimal UAE conditions. A total of 29 compounds were identified, including 19 flavonols, 2 flavan-3-ols, and 7 phenolic acids (Table 11). Spectral mass data for identified compounds have already been reported in our previous study [36].

According to the results presented in Table 11, the most abundant phenolic compounds in both varieties of SB cake were flavonols such as isorhamnetin, kaempferol and its glycosides, and quercetin glycosides. In general, most flavonols in SB are either 3-glycosides or 3,7-diglycosides [91], with large differences in the sugar units within each

aglycone group. The most common sugar residues are glucose and galactose, although rhamnose, xylose, and arabinose have also been found [92]. In the SB cake extracts, 19 flavonols were identified, as shown in Table 11. Isorhamnetin-3-hexoside was determined to have the highest concentration (35.90–79.44 mg/100 g dm), followed by quercetin-3-glucoside (13.46–48.16 mg/100 g dm), quercetin-3-rutinoside (32.49–47.14 mg/100 g dm), isorhamnetin-3-rutinoside (9.66–30.88 mg/100 g dm), kaemferol-3-O-glucoside-7-O-rhamnoside (12.38–18.72 mg/100 g dm), and isorhamnetin-3-sophoroside-7-rhamnoside (3.20–11.67 mg/100 g dm). Similar findings were reported by Rösch et al. [91] and Yang et al. [92], who found that isorhamnetin and quercetin glycosides, such as isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside-7-O-rhamnoside, quercetin-3-O-glucoside, and quercetin-3-O-rutinoside, were the most prevalent flavonol glycosides in SB. Kallio et al. [93] reported that the most abundant phenolic compounds determined in the berries were isorhamnetin-3-sophoroside-7-rhamnoside (32%), followed by quercetin-3-glucoside (15%), quercetin-3-rutinoside (9%), isorhamnetin-3-rutinoside (6%), and isorhamnetin-3-glucoside (4%). Other flavonol glycosides such as isorhamnetin-3-sinapoyglucose-glucoside-7-rhamnoside, isorhamnetin-3-rutinoside-7-glucoside, isorhamnetin-3-rhamnoside, isorhamnetin-3,7-dihexoside, kaempferol-3-O-sophoroside-7-O-rhamnoside, kaempferol-3-rutinoside, kaempferol-rhamnoside, quercetin-3-sophoroside-7-rhamnoside, quercetin-3-rhamnosylglucoside-7-rhamnoside, quercetin-3-rhamnoside (quercitrin), and quercetin-3-pentoside were determined in a significantly lower concentration (less than 10 mg/100 g dm). These compounds were also detected in the study by Rosch et al. [91] and Pop et al. [94]. In the analyzed extracts, the aglycones isorhamnetin and kaempferol were determined in a range of 0.45–0.98 mg/100 g dm and 2.97–8.53 mg/100 g dm. According to Fatima et al. [95] and Guo et al. [96], the most important aglycones in berries are kaempferol (32–72 mg/kg fresh weight (fw)), quercetin (67–175 mg/kg fw), myricetin (36–172 mg/kg fw), and isorhamnetin (45–106 mg/kg fw). The higher content of kaempferol and isorhamnetin compared to our results is probably due to the acid hydrolysis used in the extraction [95]. Among the flavan-3-ols, catechin and epicatechin were detected in the SB cake extracts in a range of 0.64–2.15 mg/100 g dm and 0.51–2.82 mg/100 g dm, respectively (Table 10). The presence of catechin in SB berries was also confirmed by Bittova et al. [97] in a range of 4.1–22.2 mg/kg dm, which is in accordance with our study. Dienaite et al. [20] reported a similar concentration of epicatechin in defatted SB berry cake, while the concentration of catechin was significantly higher than in our study. In the study of Guo et al. [96], the content of catechin and epicatechin in four SB subspecies varied from 0.82 to 4.51 and from 7.60 to 8.99 mg/100 g dm, respectively. In the analyzed SB cake extracts, seven phenolic acids were identified, as shown in Table 11. Vanillic acid was determined to have the highest concentration (42.63–60.46 mg/100 g dm), followed by *p*-hydroxybenzoic (18.75–38.13 mg/100 g dm), protocatechuic (9.55–38.08 mg/100 g dm), gallic (20.71–23.92 mg/100 g dm), caffeic (21.01–23.14 mg/100 g dm), chlorogenic (2.33–17.63 mg/100 g dm), and *p*-coumaric acid (7.55–14.67 mg/100 g dm). The presence of the mentioned phenolic acids in SB berries was confirmed by literature data [35,98], but their contribution differs from our results. In the study of Zadernowski et al. [98], the most represented phenolic acid was salicylic acid, followed by gallic, cinnamic, protocatechuic, *p*-coumaric, and other minor phenolic acids. In contrast, the study by Arimboor et al. (2008) [99] showed that gallic acid was identified as the predominant phenolic acid in both free and bound forms, accounting for 66.0% of the total phenolic acids in SB berry pulp. In addition, considerable amounts of protocatechuic acid (136 mg/kg), ferulic acid (69 mg/kg), salicylic acid (54 mg/kg), *p*-hydroxybenzoic acid (40 mg/kg), and *p*-coumaric acid (37 mg/kg) were determined in the SB berry pulp.

In the analyzed SB cake extracts, the total content of phenolic groups is shown in Table 11. The total content of flavonols ranged from 131.66 to 205.13 mg/100 g dm for the Leikora variety and 160.28 to 263.05 mg/10 g dm for the Ascola variety; the total content of flavan-3-ols ranged from 1.15 to 3.14 mg/100 g dm for the Leikora variety and from 4.60 to 6.98 mg/100 g dm for the Ascola variety; and the total content of phenolic acids ranged from

140.02 to 180.43 mg/100 g dm for the Leikora variety and from 155.53 to 189.04 mg/100 g dm for the Ascola variety. The results presented in Table 11 show that the Ascola variety has a statistically significant ($p \leq 0.05$) higher content of individual and total flavonols and flavan-3-ols than the Leikora variety. A large variability in phenolic composition between different species and varieties has been confirmed by various studies [100–103]. According to earlier studies, SB berries have a high polyphenol content. According to Ercisli et al. [104], the TPC value of SB berries was between 213.1 and 553.8 mg GAE/100 g. In contrast, Korekar et al. [64] reported an 11-fold difference (from 964 to 10704 mg GAE/100 g) in TPC content. The significant difference in the TPC of the cultivated varieties under the same climatic conditions can be clearly explained by the different genetic material [103]. Even the same varieties grown in different areas can have different levels of phenolic compounds. According to Rop et al. [105], Leikora berries collected near Brno in the Czech Republic had more than three-fold higher TPC content than 'Leikora' berries grown under Hungarian growing and climatic conditions. In addition, the cake remaining after SE had a statistically significant ($p \leq 0.05$) lower content of individual and total flavonols and flavan-3-ols. Since the phenols are heat-sensitive and easily oxidizable compounds [106], the SE of the oil had an influence on the degradation of the phenolic compounds in the residual cake due to the high extraction temperature and the long extraction time in the presence of oxygen.

3.7. Antioxidant Activity of SB Cake

The AA of SB cake extracts obtained under optimal UAE conditions was determined using hydrophilic (H) ORAC (Figure 1).

The AA ranged from 6825.15 to 9550.54 μmolTE/100 g dm in Ascola SB cake and from 6750.15 to 9504.85 μmolTE/100 g dm in Leikora SB cake. The cakes produced after SC-CO_2 had a higher AA than cakes produced with SE, which is consistent with the higher mass concentration of phenolic compounds in SC-CO_2 cakes. SE can lead to the degradation of thermolabile bioactive compounds during a long extraction time when heated, thus reducing AA [107]. According to Guo et al. [96], the total phenols and flavonoid aglycones in SB extracts had antioxidant and antiproliferative activities. Previous studies had also shown that the fruits of SB varieties have strong AA, but there were significant differences between varieties [63,64].

4. Conclusions

The method of oil extraction and the variety influenced the content of BAMs and AA in the SB oils and cakes. SC-CO_2 proved to be the better method for obtaining oils and cakes with a higher content of BAMs and AA than SE. The oil of the Leikora variety had a higher content of palmitic and palmitoleic acids, β-sitosterol, and α-tocopherol, while the Ascola variety had a higher content of oleic, linoleic, and α-linolenic acids and carotenoids. In addition, the Ascola cake had the highest content of phenols, with flavonols being the most abundant and isorhamnetin-3-hexoside, isorhamnetin-3-rutinoside, and quercetin-3-glucoside dominating. SB oils and cake extracts showed high AA, but the Ascola variety had higher ORAC values. A combination of SC-CO_2 for oil extraction and UAE for other bioactive compound extraction is a good choice for maximal valorization of the SB berries, which have great potential for the food, nutritional, and cosmetic industries.

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