BioFlux ProEnvironmen Original Article
ProEnvironment 16/54 (2023) 41 - 50

Green Extraction of Carotenoids from Sea Buckthorn Pomace – the Effect of Solvent and Extraction Method

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Received 20 May 2023; received and revised form 2 June 2023; accepted 15 June 2023; Available online 30 June 2023

Abstract

Sea buckthorn pomace (SBP) is produced in large amounts during the processing of berries for the production of oil and juice. Although it is rarely used, the pomace still contains valuable bioactive compounds, such as carotenoids, tocopherols and phenolic compounds. The aim of our study was to evaluate the efficiency of carotenoid extraction from SBP, using green solvents (ethyl acetate - ETA, ethyl lactate - ETL, cyclopentyl methyl ether - CPME, methyl tetrahydrofuran - MeTHF and cold pressed sunflower oil - SFL), compared with a mix of organic solvents. Moreover, two extraction methods were applied: conventional solid-liquid extraction (maceration) and ultrasound assisted extraction. Total and individual carotenoids in the extracts were determined by HPLC-PDA and the antioxidant activity by DPPH assay. The total carotenoid extracted ranged from 45.32 mg/100 g powder (ETA) to 64.31 mg/100 g powder for OS mixture, when maceration was applied. When UAE method was used, the total carotenoids ranged between 45.75-65.82 mg/100 g powder, with the highest value being obtained for CPME, closely followed by MeTHF. CPME was the most efficient in extracting zeaxanthin esters and carotenes, while ETL was the most efficient for extracting free zeaxanthin. Except for sunflower oil, for all the tested solvents UAE gave better yields than conventional solid-liquid extraction. The extracts obtained with green solvents displayed antioxidant activity and there was a positive correlation between the amount of carotenoids in the extract and the inhibition of DPPH radical.

Keywords: green solvents, extraction, ultrasound assisted, carotenoids, sea buckthorn pomace.

1. Introduction

Food waste or by products are constantly generated by the food industry and represent a global economic and environmental challenge. At the same time, fruit and vegetable by products represent valuable sources of bioactive compounds, like polyphenols, carotenoids, vitamins or lipids, which can be recovered and used as food, feed or cosmetics ingredients [1, 2]. Among them, carotenoids draw the attention by being both natural pigments and compounds associated with numerous health benefits. The presence of carotenoids in the human body and especially their accumulation in plasma/serum, retina and brain has been associated with a lower incidence of cardiovascular diseases, diabetes, Age Related Macular Degeneration, various types of cancer but also with antioxidant and anti-inflammatory activities [3, 4].

Carotenoids are highly hydrophobic compounds and their recovery form food matrices implies the use of organic solvents/extractants. The extraction process depends on several physical and chemical factors such as carotenoid polarity, food matrix composition, mechanical and thermal preparation of the matrix, etc. Because of these barriers, numerous studies aimed to improve the extraction of carotenoids, either by the choice of solvent or by applying different

extraction techniques [5]. The recovery process is highly dependent on the food matrix and on the method parameters, such as the solvent used (non-polar solvents for non-polar carotenoids, and polar solvents for polar carotenoids or a mixture of polar and non-polar solvents to extract both polar and non-polar carotenoids), pressure and temperature [6]. Due to the negative impact that organic solvents have on the environment and on the human health, there is an increased need to develop new extraction methods using green solvents produced from renewable biomass, in conjunction with faster and efficient extraction techniques. For example, in a study regarding the substitution of *n*-hexane with green solvents for the extraction of carotenoids from carrots, Yara-Varón et al. (2016) used five green solvents (namely cyclopentyl methyl ether (CPME), dimethyl carbonate (DMC), ethyl acetate (EA), isopropyl alcohol (IPA), and 2-methyltetrahydrofuran (2-MeTHF)), and the highest yield of extraction was achieved using CPME and 2-MeTHF [7].

Another green alternative for organic solvents are vegetable oils (sunflower oil, soy oil), which have multiple beneficial effects, such as the reduction of energy consumption, obtaining an uncontaminated extract, delaying the oxidation time and the degradation rate of carotenoids. However, there are also some negative effects such as the high oil viscosity, which leads to a reduced extraction yield [8]. Extraction with supercritical CO_2 is an effective, tunable and environmentally friendly technique which has successfully been applied to extract the lipids fraction from sea buckthorn pomace. The yield of lipids extracted with supercritical CO_2 was 16.99 %, compared to 20.78 % obtained by Soxhlet extraction using hexane [9].

However, the complexity of the equipment required and its high cost are limiting factors for the industrial use of this extraction method. Several methods have been used for carotenoid extraction and the most common are conventional solid-liquid extraction (maceration), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), and enzyme-assisted extraction (EAE). Compared to traditional extraction methods, the use of ultrasounds favors cell ruptures due to the acoustic cavitation and improves the extraction of the analyzed compounds [5].

Among the plant derived foods rich in bioactive compounds, sea buckthorn is one of the most valuable due to its high bioactive content, which include lipophilic (carotenoids and tocopherols) and hydrophilic antioxidants (flavonoids, tannins, phenolic acids, ascorbic acid). The by-products and residues from sea buckthorn processing are also nutritionally abundant and can be reused to add value to food products or nutraceuticals [10, 11]. Following the concept of the circular economy in food science, our purpose was to valorize the sea buckthorn pomace through the green extraction of its rich carotenoid fraction. The aims of the present work were: (i) to test the efficacy of five green solvents for the extraction of carotenoids from sea buckthorn pomace resulted from juice production; (ii) to test the efficacy of two different methods: solid-liquid conventional extraction (maceration) and ultrasound assisted extraction (UAE) for the extraction of total and individual carotenoids; (iii) to determine the antioxidant capacity of sea buckthorn pomace extracts obtained with different solvents.

2. Material and method

2.1. Sample materials and pomace production

Fresh sea buckthorn berries (wild type) were collected in Hunedoara County. Sea buckthorn fruits were washed and juice was obtained, using a low-speed juicer (Kuvings B1700). The juice was filtered in order to obtain the pomace comprising peel, seeds and residual pulp. The pomace was weighed and dried using a laboratory oven (POL-EKO sp.k, Wodzisław Śląski, Poland) for 48 hours, at a constant temperature of 39°C. Further, the pomace was powdered using a kitchen grinder (Heinner HCG-200DGIX2). The obtained powder was divided into two batches, packed in sealed aluminum coated polyethylene bags and stored in a −20 °C freezer until the time of analysis.

One batch was used for carotenoid extraction, spectrophotometric and chromatographic analyzes, and the second batch was used to obtain the microbeads enriched with carotenoid extracts (data not presented).

2.2. Chemicals and standards

All the chemicals and reagents were o analytical or HPLC grade, purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Steinheim, Germany). Ultrapure water was obtained using a Milli-Q water purification system. Carotenoid standards: β-carotene, γ-carotene, lycopene, zeaxanthin, zeaxanthin dipalmitate and lutein, were purchased from Extrasynthese (Lyon, France).

2.3. Carotenoid extraction by conventional solvent extraction (maceration)

Carotenoid extraction was conducted comparatively using an organic solvent mixture (OS) composed by ethyl acetate, methanol and petroleum ether (1:1:1, v/v/v) and, respectively, green solvents, used individually. The green solvents were: ethyl acetate, ethyl lactate, cyclopentyl methyl ether (CPME), methyl tetrahydrofuran (MeTHF) and cold pressed sunflower oil.

The extraction was carried out starting from 2 g of powder which were mixed with 20 ml of extraction solvent (individual green solvents or OS) in glass tubes. The samples were vigorously mixed, vortexed and kept in the dark for 1 hour. Afterward, the tubes were centrifuged (2500 rpm) for 10 min and the liquid phase was separated. The protocol was repeated until the organic phase became colorless (3 to 5 extractions). All the collected organic phases were reunited, filtered through anhydrous sodium sulfate. The extracts obtained with OSM and ethyl acetate were evaporated to dryness, using a rotatory evaporator (Heidolph MR Hei-End, Schwabach, Germany). The residue was dissolved in methyl-*tert*-butyl-ether (MTBE) and filtered through 0.22 µm PTFE filters in amber vials prior spectrophotometric and HPLC-DAD analysis. The extracts obtained with ethyl lactate, CPME and MeTHF were directly filtered (without evaporation) and subjected to spectrophotometric and HPLC-DAD analysis. The extract obtained with sunflower oil was dissolved in MTBE prior HPLC. The extraction was performed at room temperature (25°C) in dimmed light.

2.4. Carotenoid extraction using Ultrasound Assisted Extraction (UAE)

The purpose of the ultrasound assisted extraction method was to enhance carotenoid extraction, due to the increased capacity of the ultrasounds to produce major cell rupture, favoring a better extraction of the compounds of interest. There are two types of ultrasounds assisted extractions that can be used: a direct method, which means direct application of the ultrasonication to the sample through an ultrasonicator probe, and an indirect method, which implies using a water bath to apply ultrasounds through the sample tube [8]. In this study the direct method was applied, using a sonicator (Sonics Vibra Cell VCX 130 PB) with a net power output of 130W, 20 kHz of frequency, equipped with a Ti-6Al-4V standard probe (6mm). The used amplitude level was 30%, the pulse duration was 6 seconds on and 2 seconds off, and the extraction time was 10 minutes per cycle, with three repetitions for each sample, starting from 2 g of sea buckthorn pomace powder and 20 ml of solvent. The extracts were analyzed as described below.

2.5. Quantitative determination of carotenoids using spectrophotometric analysis

The spectrophotometric analysis was used in order to determine the total carotenoid content from all samples, following the method described by Britton (1995) [12]. An UV-VIS Spectrophotometer (Jasco V-530) was used and the total carotenoid content was expressed as mg carotenoids/100g powder or microbeads, applying the following Equation (1):

$$C = \frac{AbsxVxDx1000}{A^{1\%}_{1\ cm}x1x100} \quad (1)$$

Where Abs means the absorbance read at 450 nm, V is the sample volume expressed in mL, A1% (2500) represents the absorption coefficient (1cm), defined as the theoretical absorbance of a solution of 1% (w/v) concentration in a cuvette with 1 cm path length.

2.6. Identification and quantification of carotenoids by HPLC-DAD

The main carotenoids from the analyzed samples were identified using an HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a SPDM20A diode array detector and a YMC C30 reversed-phase column (250 × 4.6 mm i.d., 5 µm particle size). The protocol for carotenoid separation used a gradient elution, with methanol/methyl-tert-butyl-ether/water (83:15:2, v/v/v) as solvent A, and methyl-tert-butyl-ether/methanol/water (90:8:2, v/v/v) as solvent B. The gradient was as following: min 0 – 0 % solvent B, min 20 – 0 % B; min 140 – 100 % B; min 145 – 0% B în A, followed by column equilibration, for 30 min. The flow rate was 0.8 mL/min and the injection volume was 20 µl. The carotenoids were identified based on their retention times, absorption spectra and fine structure (III/II ratio) which were compared with those of available standards and literature data. Quantification of carotenoids was performed by external calibration method, as described previously [13].

Hansen Solubility Parameters (HSPs) and Conductor-like Screening Model for Realistic Solvation (COSMO-RS) to understand the dissolving mechanism of carotenoids in the selected solvents and to predict their extraction capacity. Interestingly, the COSMO-RS analysis was in agreement with the experimental values and indicated that CPME and 2-MeTHF were superior to *n*-hexane in terms of total carotenoid yield.

The differences obtained using these green solvents could be associated with different polarities of the solvents and carotenoids, and/or carotenoid solubility, but they do not depend entirely on this aspect.

It must be taken into consideration that, even though the total carotenoid content was higher for one type of solvent, the extraction yields for the specific carotenoids were different, also depending on their specific polarities and physical properties.

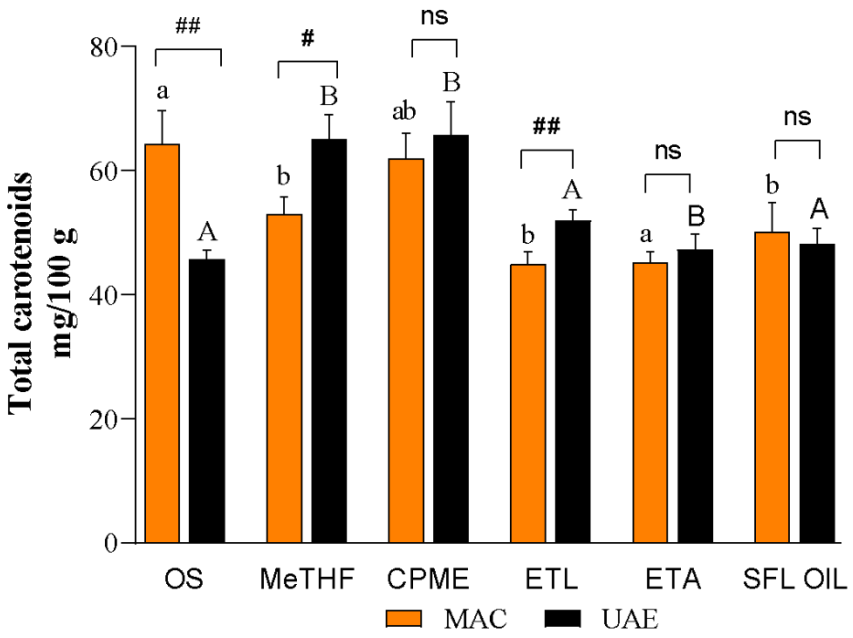


Figure 1. Total carotenoids extracted from sea buckthorn powder using different types of solvents and two types of extraction. OS-organic solvents, MeTHF-methyl tetrahydrofuran, CPME-cyclopentyl methyl ether, ETL-ethyl lactate, ETA- ethyl acetate, SFL OIL-sunflower oil. Different symbols indicate a significant difference between all the analyzed solvents, by two types of extractions, lowercase letters (a-b) represent the differences between the solvents using maceration (MAC) and capital letters (A-B) represent the differences between the solvents using ultrasound assisted extraction (UAE), determined by Ordinary one-way ANOVA Multiple Tukey comparison test. The differences between the samples extracted with the same solvent but subjected to different extraction procedure were determined using Unpaired t test (the symbols represent: extremely significant ###, p = 0.0001 to 0.001; very significant ## p = 0.001 to 0.01; significant #; p = 0.01 to 0.05; non-significant – ns, p ≥ 0.05.

In order to better understand these interaction, we used C30-HPLC-PDA method to determine the impact of the solvent and that of the extraction method on the extraction yield of major carotenoids from sea buckthorn pomace (Fig. 2). The quantitative data regarding the major carotenoid composition of the sea buckthorn pomace extracts are presented in Table 1 and Table 2. Previous studies performed in our group showed that sea buckthorn berries grown in Romania are characterized by a high content of zeaxanthin (mainly in esterified form) and carotenes, such as β-carotene, γ-carotene and lycopene [16, 17, 18]. However, a variability, especially in terms of total carotenoid content was observed, depending on the cultivar [17]. We found a similar composition in the extracts obtained from sea buckthorn pomace, where zeaxanthin dipalmitate was the major compound, accounting for 34 %, followed by β-carotene – 26 % and zeaxanthin-myristate-palmitate – 14 %. Free zeaxanthin, lycopene and γ-carotene were identified in significantly lower amounts. Some other carotenoids were also present, like zeaxanthin esters with various fatty acids, β-cryptoxanthin and its esters, lutein and its esters,

but they could not be quantified due to the low amount or the lack of commercial standards. Carotenes (hydrocarbons) are non-polar compounds, thus susceptible to be soluble in non-polar (e.g. hexane) or weakly polar solvents. Similarly, zeaxanthin diesters are large and hydrophobic molecules, due to the presence of long aliphatic non-polar fatty acids chain attached to the hydroxyl group of zeaxanthin.

On the other side, zeaxanthin is a xanthophyll and the presence of the hydroxyl group increases the polarity of the molecule. In the case of zeaxanthin, the range of polarity of the solvents which can be used for extraction is larger and a slight polarity of the solvents should improve the extractability.

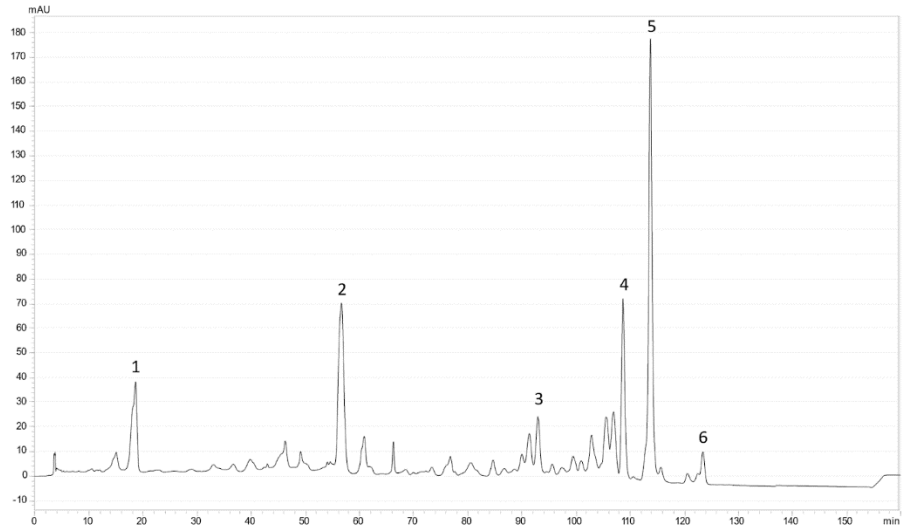


Figure 2. C30-HPLC-PDA chromatogram (λ=450 nm) of carotenoids extracted from seabuckthorn pomace with ethyl acetate by conventional extraction (maceration). Peak identification: 1 – Zeaxanthin; 2 - β-carotene; 3 - γ-carotene; 4 – Zeaxanthin-myristate-palmitate; 5 - Zeaxanthin dipalmitate; 6 - Lycopene

Table 1. Major carotenoids yield (mg/100 dry powder) obtained using conventional extraction (maceration) from sea buckthorn pomace with different solvents

Carotenoid	OS	MeTHF	CPME	ETL	ETA	SFL oil
Zeaxanthin	6.96±0.8^{a}	2.18±0.3^{b}	2.19±0.3^{b}	7.63±0.8^{a}	5.16±0.45^{c}	2.68±0.2^{b}
ZMP	9.13±0.8^{a}	8.34±1^{a}	9.81±1.2^{a}	6.24±0.55^{ab}	6.93±0.85^{ac}	3.26±0.3^{bd}
ZDP	22.23±1.7^{a}	22.41±4^{a}	25.35±4.6^{a}	9.62±1.8^{b}	18.4±1.4^{a}	18.53±2.8^{a}
β-carotene	16.79±1.3^{a}	13.69±2^{a}	16.24±1.5^{a}	14.17±1.8^{a}	10.76±0.95^{ab}	15.82±2.2^{a}
γ-carotene	5.24±0.6^{a}	4.52±0.4^{a}	4.14±0.6^{a}	5.98±0.5^{ac}	2.49±0.3^{b}	5.11±0.6^{a}
Lycopene	3.97±0.5^{a}	1.86±0.1^{b}	3.28±0.3^{a}	1.4±0.1^{b}	1.59±0.4^{b}	3.72±0.45^{a}

Data represents the arithmetic mean and standard deviation (SD) of 3 different experiments. Statistically significant differences were determined using Ordinary one-way ANOVA (Tukey's Multiple Comparison Test; GraphPad Prism Version 9.3). Different superscript letters (a–d) in the same row indicate a significant difference between the solvents used for conventional extraction (maceration).

Analyzing the data in Table 1, it can be observed that in the case of zeaxanthin, the only green solvent which gave better yield (106 %) compared to OS was ethyl lactate (although not significant), while for all the other solvents the amount extracted was significantly lower. This can be explained by the fact that ethyl lactate is a polar protic solvent (logP 0.17), able to form not only intra- and inter- molecular hydrogen bonds with polar molecules, but also Van der Waals interactions with non-polar molecules, which make it capable of extracting molecules with a wide range of polarity [19, 20]. When UAE was applied, the effect was even more important, ethyl lactate significantly improved the extraction of zeaxanthin (192 %) (Table 2). Significantly higher extraction yield was obtained for zeaxanthin, when extracted with ethyl acetate and UAE, compared to OS.

Zeaxanthin esters (ZMP, ZDP) were better extracted with CPME (logP 1.41) and MeTHF (logP 0.82), especially when UAE was applied. CPME,

which is a diprotic dipolar solvent, according to Moity at al. (2014), determined a slight increase for zeaxanthin myristate-palmitate (107.45±16.2%) and zeaxanthin dipalmitate (114.04±12.9%), in comparison with the OS, but the results were not significantly different [21].

At the same time, the use of MeTHF, ethyl acetate (logP 0.71) and sunflower oil, as extraction solvents, did not lead to notable improvements in the extraction process for these compounds and the values obtained did not exceed the yield of the OS samples. Zeaxanthin dipalmitate registered a significant decrease (to 43.3±4.9%) of the extraction yield when ethyl lactate was used as solvent and the conventional extraction was applied.

Regarding the carotenes, the extraction yield for β-carotene was the highest for OS and the lowest for ETA, but CPME and sunflower oil gave very close values to OS for conventional extraction. When UAE was used, both MeTHF and CPME gave good yields, even better than OS mixture. It is important to note that sunflower oil extracted 94 % (by CE) and 97 % (UAE) of the β-carotene extracted with OS. In the case of γ-carotene, CPME with UAE were the optimal extraction conditions. Lycopene was the most efficiently extracted with CPME (by CE) and MeTHF (by UAE).

Similar with the other carotenes, the extraction yield with sunflower oil was comparable with the OS, with 94 % of the value when conventional extraction was applied. The lowest yields for lycopene were obtained with ETL and ETA, regardless the extraction technique. CPME, MeTHF and ethyl acetate also showed good results and high extraction yields when used to extract carotenoids from carrot waste, in the following order: CPME > MeTHF > ethyl acetate, with 95.1±7.3% > 80±5.9% > 64.4±10%. However, when analyzing these values, it must be taken into consideration that the control solvent was n-hexane, the extraction was conducted at 65°C, and the food matrix was different (carrot waste). For CPME and MeTHF, the yield for β-carotene were lower than our values, respectively 66±1.7% and 65.3±1.5%, but for ethyl acetate the value was approximately the same, 65.5±0.5% [7]. Another study reported a good extraction yield for total carotenoids from tomato waste using ethyl lactate, from 83.4%, at 25°C, to 85.5% at 50°C and 100% at 70°C [22].

Beside the toxicity aspects, another issue related to the use of solvents for extraction of carotenoids is the amount of energy consumed for evaporation and the amount of CO_2 generated by the solvent.

Yara-Varon et al. (2016) analyzed these aspects and concluded that the energetic impact of CMPE, MeTHF and ETA compared to *n*-hexane is higher (with 9 %, 4 % and 5 %) but it is compensated by the superior extraction yield (especially for CPME and MeTHF) and by their lower toxicity [7]. On the other hand, ETL is a 100 % biodegradable solvent, permitted in food applications but has a very high boiling point (154°C) which makes difficult the removal by conventional techniques [20].

Table 2. Major carotenoids yield (mg/100 dry powder) obtained using ultrasound-assisted extraction from sea buckthorn pomace powder with different solvents

Carotenoid	OS	MeTHF	CPME	ETL	ETA	SFL oil
Zeaxanthin	3.76±0.4^{a}	2.55±0.2^{a}	4.69±0.35^{ac}	7.23 ±0.75^{b}	6.29±0.85^{b}	4.62±0.5^{abc}
ZMP	8.13±0.6^{a}	9.84±1.6^{a}	9.2±2.1^{a}	8.1±1.1^{a}	7.02±1.2^{a}	7.23±0.6^{a}
ZDP	20.97±1.9^{a}	26.76±3.5^{a}	24.49±3.8^{a}	18.24±2.7^{ab}	18.63±2.1^{ab}	16.17±1.4^{ab}
β-carotene	14.83±1.4^{a}	16.65±3.2^{a}	15.45±1.8^{a}	13.69±2.45^{a}	11.1±1.7^{a}	14.42±1^{a}
γ-carotene	3.52±0.5^{a}	5.82±0.9^{a}	8.41±2.3^{b}	3.24±0.4^{a}	2.78±0.3^{ac}	3.2±0.2^{a}
Lycopene	3.94±0.4^{a}	3.54±0.6^{a}	2.58±0.25^{ab}	1.54±0.3^{b}	1.63±0.4^{b}	2.77±0.3^{ab}

Data represents the arithmetic mean and standard deviation (SD) of 3 different experiments. Statistically significant differences were determined using Ordinary one-way ANOVA (Tukey's Multiple Comparison Test; GraphPad Prism Version 9.3). Different superscript letters (a–d) in the same row indicate a significant difference between the solvents used for ultrasound assisted extraction (UAE).

DPPH· scavenging assay is probably the simplest antioxidant method, based on the property of DPPH· stable radical to react with both electron and hydrogen donors, reaction which is accompanied by the discoloration of the initial deep purple colour (515 nm). Radical scavenging activity (DPPH·) of the carotenoid extracts are presented in Table 3.

The inhibition of DPPH· ranged between 27.2-45.1 % for extracts obtained by maceration, and from 18.3-48.2% for extracts obtained by UAE. Carotenoids are known to possess radical

scavenging activity, which was proved by several antioxidant methods.

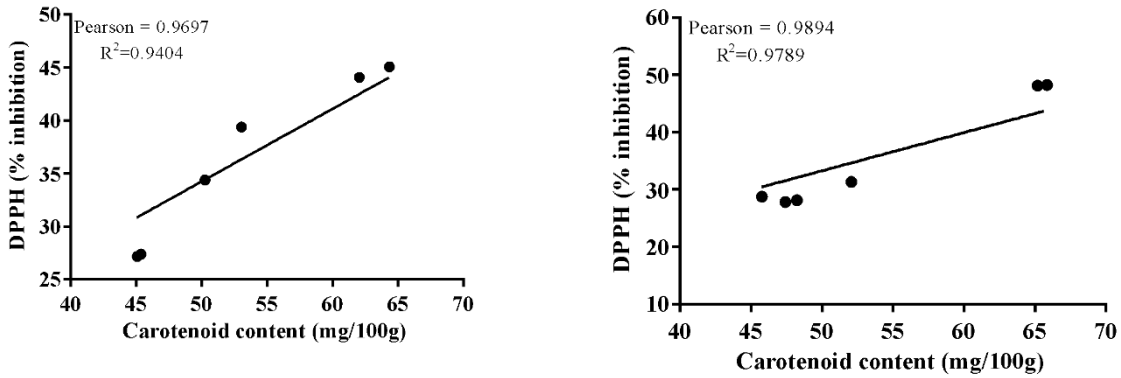
Assessing the antioxidant capacity of a lipophilic extract from sea buckthorn berries, Vişan et al. (2023) found an inhibition of DPPH·ranging from 5.68–68.65%, depending on the concentration used, with a IC50 = 32.14 %, for the concentration of 261.12 µg/mL [18].

In another study, an inhibition of 61 % was reported for the aqueous extract obtained from sea buckthorn berries, while for the extract obtained with acetic acid, acetone and water the inhibition was 92 % (303 mg/100 g dry weight) [23]. However, an earlier study found no inhibition of DPPH· by individual carotenoid (xanthophylls and hydrocarbons), although they showed antioxidant activity by other methods (FRAP, TEAC, LPSC) [24]. A correlation analysis was performed to a ssess a potential relationship between carotenoid content and DPPH scavenging radical (Fig. 3).

Table 3. DPPH· scavenging activity of extracts obtained from sea buckthorn pomace with different solvents and extraction technique

Solvent	DPPH (% inhibition)	
	Maceration	UAE
OS	45.1 ± 0.39	28.8 ± 0.24
MeTHF	39.4 ± 0.36	48.2 ± 0.39
CPME	44.1 ± 042	18.3 ± 0.20
ETL	27.2 ± 0.21	31.4 ± 0.28
ETA	27.4 ± 0.25	27.9 ± 0.24
SFL	34.4 ± 0.36	28.2 ± 0.22

A good correlation was observed between the total carotenoid content and DPPH scavenging activity (Pearson's correlation coefficient of 0.9697, respectively 0.9894) in both extraction procedures used, maceration and UAE.



Maceration UAE

Figure 3. Pearson's correlation test between carotenoid content (mg/100 g DW) and antioxidant activity measured with DPPH assay (% of inhibition)

Our study was focused on carotenoids, but it possible that other molecules with antioxidant properties, like tocopherols and phenolic compounds, were also present in the extract and contributed to DPPH· scavenging activity.

4. Conclusions

Sea buckthorn pomace resulted from juice production is a valuable source of carotenoids (66 mg/100 g dry pomace), containing as major compounds zeaxanthin esters (dipalmitate, myristate-palmitate) and β-carotene. Among the green solvents tested, CPME (for conventional extraction) and CPME and MeTHF (for UAE) where the most efficient, while ETA lead to lower extraction yields (73 %). Although sunflower oil had a lower extraction yield compared to OS or other solvents (78 %), it can be used for the extraction of carotenoids due to its lower cost and to the possibility to further use the extracts without the need of evaporation. Differences in the carotenoid composition of the extracts were observed depending on the solvent used: CPME was the most efficient in extracting zeaxanthin esters and carotenes, while ETL was the most efficient for extracting free zeaxanthin. Except the sunflower oil, for all the tested solvents, UAE gave better yields than conventional solid-liquid extraction (maceration). The extracts obtained with green solvents displayed antioxidant activity and there was a positive correlation

between the amount of carotenoids extracted and the inhibition of DPPH radical.

Acknowledgement: This work benefited from financial support through the project "Development of the skills of advanced and applied research in STEAM+ Health logic", POCU/993/6/13/153310, project co-financed from the European Social Fund through the Human Capital Operational Program 2014-2020" and from PN-III-P4-ID-PCE-2020-1172 grant, number 243/2021.

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