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Targeted analysis of sea buckthorn oil extracted by accelerated solvent extraction technique using green and conventional solvents

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ABSTRACT

Sea buckthorn oil (SBO) was extracted using accelerated solvent extraction with two green solvents (ethanol and 2-methyltetrahydrofuran), and two petroleum-based solvents (hexane and diethyl ether) at four different temperatures (60, 90, 120 and 150 °C), using one extraction cycle at 103.42 bar, 10 min static time, 5 min preheating and 30% flushing volume. β-carotene and α-tocopherol were quantified jointly using HPLC-UV/VIS and the fatty acid profile was analyzed using GC-MS. Extraction yield was also measured and the data was compared to a conventional extraction using Soxhlet. The extraction with ethanol achieved significantly higher concentrations of β-carotene in the final oil (1.67 mg/g oil) compared to the results of any other solvent (e.g. 1.23 mg/g oil for hexane ASE) or to the conventional extraction (1.27 mg/g oil). However, the use of green solvents led to the dragging of unwanted polar compounds (sugars), leading to lower oil yield values after purification.

1. Introduction

The oil from sea buckthorn (SB) can be extracted from two parts: (1) the seed or (2) the pulp and peel. The nutritional profile of the oil differs depending on the extracted part; the seed oil has greater amounts of tocopherols and oleic acid, and the pulp oil contains greater amounts of carotenoids – specially β-carotene – and palmitoleic acid, among other bioactive lipophilic compounds. The intake of either one or both SBO has beneficial effects on human health, such as improvements in blood lipid profile (Guo, Yang, Cai, & Li, 2017) modulation of hypoxia, cardioprotective properties and other antioxidant properties (Olas, 2018). In addition, its nutritional profile makes it very valuable for the development of food products (Vilas-Franquesa, Saldo, & Juan, 2020).

Several techniques have been exploited over the years for the recovery of oils from vegetable matrices. The most exploited extraction methodologies include cold pressing and solvent extraction (Danlami, Arsad, Zaini, and Sulaiman, 2014). Among the solvent extraction methods, accelerated solvent extraction (ASE) is widely used as it allows for the application of high pressures, which translates into high temperatures and shorter extraction times. In addition, ASE has shown promising results in the extraction of bioactive lipophilic compounds, especially when using green solvents (Castejón, Luna, & Señoráns, 2018; Danh et al., 2013). In fact, green solvents are becoming nowadays more relevant, as the consumer is seeking for sustainable products and production processes. Green solvents generally include but are not limited to ethanol or ethyl acetate, or the combination of water and a water-soluble solvent (Castejón et al., 2018; Danh et al., 2013), and recently terpenes (Kumar et al., 2017). Ethanol is used because of its low cost, and in cases in which the interest remains in obtaining somewhat polar compounds (e.g. extraction of slightly polar molecules such as phospholipids). Another interesting green solvent that has been studied recently is 2-methyltetrahydrofuran (2-MTHF). 2-MTHF is produced out of carbohydrates from lignocellulose biomass, which represent the largest terrestrial biomass resource (Sicaire et al., 2014). Previous works reporting extractions with 2-MTHF had yielded oils with (a) higher monounsaturated fatty acid levels when compared to hexane, obtaining a similar overall yield (such is the case of fennel and anise (Rebey et al., 2019)), (b) a slightly higher polyunsaturated fatty acid concentration in rapeseed oil (Sicaire et al., 2015), and (c) higher overall oil yield in caraway seeds when compared to hexane (Bourgou, Tounsi, Ksouri, Fauconnier, & Sellami, 2019; de Jesus, Ferreira, Fregolente, & Filho, 2018).

The objective of this work was to comparatively evaluate oil yield, the concentration of α-tocopherol and β-carotene (as the most important

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vitamers in SBO), and the fatty acid profile of SBO extracted from SB dried berries with an accelerated solvent extractor using different solvents (hexane, diethyl ether, ethanol and 2-MTHF) at different temperatures (60, 90, 120 and 150 °C). Soxhlet extraction was used as a reference method.

2. Materials and methods

2.1. Extraction solvents and material

Hexane, ethanol, diethyl ether and 2-methyltetrahydrofuran were used for the experiment. *n*-Hexane (with isomers) 99% purity (HPLC grade) was purchased from Labbox Labware, S. L., Catalunya, Spain. 2-Methyltetrahydrofuran (2-MTHF) stabilized with 2,6-di-*tert*-butyl-4-methylphenol was purchased from Merck KGaA, Darmstadt, Germany. Diethyl ether, stabilized with 6 mg/L of BHT and ethanol absolute (99.8%) were purchased from Panreac Química S. A. U., Catalunya, Spain. Polytetrafluoroethylene (PTFE) O-rings, PEEK seals, cell frites, 20 mm cellulose filters and PTFE-lined silicone septa for the ASE were purchased from Restek Corporation, Pennsylvania, U.S.

2.2. Sample preparation

Sun-dried sea buckthorn berries were purchased from a local harvester from the north-east region of Romania. The berries were categorized as the subspecies *caucasica*. Dried sea buckthorn berries were grinded down with a Thermomix® TM 21 (Vorwerk, Wuppertal, Germany). The particle size distribution of the powder was measured by gravimetry using sieves of different mesh sizes. Particle size distribution was quantified twice.

Approximately 8 g of dried and ground sea buckthorn berries were mixed thoroughly with diatomaceous earth at a ratio of 4:1. An accelerated solvent extractor (ASE) extraction cell of 33 ml total volume was filled with a cellulose membrane and the mix, in that order. The cell was closed and inserted in the ASE cell rack. For Soxhlet extraction, the same amount of sample was wrapped in a medium lab-working filter paper (Letslab delivering solutions S.L.U., Catalunya, Spain). The wrapped sample was placed directly in the sample compartment of the Soxhlet set-up.

2.3. Oil extraction and yield

The oil was extracted from sea buckthorn dried berry powder by using an Accelerated Solvent Extractor 200 (Dionex, Thermo Fisher Scientific, California, U.S). The pressure was set at 103.42 bar, static time at 10 min, preheating at 5 min, flushing volume at 30% of the total cell volume, purging time at 30 s. Only one extraction cycle was used, as preliminary trials showed similar oil recoveries when using more cycles (results not shown). SBO was also extracted from the samples by using hexane in a Soxhlet experimental set-up for 5 h. After extraction, the solvent was evaporated using a rotavapor equipment for 45 min at 45 °C and 150 mbar of pressure. Yield was calculated based on weight difference. The extracted oil was then stored in amber-tinted chromatography vials at −80 °C for further analysis.

Ethanol and 2-MTHF dried extracts were further mixed with water (at room temperature) since during the extraction some polar compounds were dragged out because of the polarity of the solvents used (Table 1). The mixture of SBO extracted with ethanol and water resulted in a clear solubilization and the solution was discarded. The oil was recovered from the walls of the lab flask by adding 20 ml of *n*-hexane. Dissolution was complete and SBO was obtained after solvent evaporation at 45 °C using a rotary evaporator as detailed above. In contrast, the mixture of SBO extracted with 2-MTHF and water resulted in a fuzzy mixture. The content of the lab-flask was poured into a centrifugal vial and centrifuged at 10,000 rpm for 10 min at room temperature. SBO was recovered from the upper phase using a glass Pasteur pipette.

2.4. β-carotene and α-tocopherol analysis

A simultaneous quantification of β-carotene and α-tocopherol was adapted from Gimeno et al. (2000) with some modifications. Briefly, 400 mg of sea buckthorn oil (SBO) were mixed with 0.2 g of L-ascorbic acid (99%, Merck KGaA (Darmstadt, Germany), 15 mL of absolute ethanol and 4 mL of a 76 g/100 ml KOH solution in that order in a centrifugation screw-capped tube. The tubes were then mixed gently and incubated at 70 °C for 30 min with slow constant stirring. After cooling, 5 mL of sodium chloride at 25 g/L were added in each tube and the solution was vigorously mixed for 1 min and left undisturbed. Phase separation occurred after 5 min. The resulting product was extracted two times with 20 mL portions of *n*-hexane (≥95%, HPLC grade, Merck KGaA, Darmstadt, Germany) and ethyl acetate (≥99.5% ACS reagent, Merck KGaA, Darmstadt, Germany) at a ratio of 85:15 (*v*/*v*). The organic phase (supernatant) was recovered and brought to dryness at 40 °C under vacuum conditions. Finally, the residue was resuspended with 3 mL of methanol and passed through a 0.45 μm filter and directly injected to the HPLC.

The HPLC system consisted of a P680 HPLC Pump attached to an ASI-100 Automated Sample Injector and a Thermostatted Column Compartment TCC-100 equipped with a reversed phase SunFire C18 column, 100 Å, 4.6 × 150 mm, 5 μm. The detection of the eluted compounds was performed with a UVD170U detector. All the parts of the HPLC were from Dionex Corporation (California, U. S.). HPLC oven temperature was set at 45 °C, sample volume was 50 μl and injected at 1.325 mL/min. Timespan was efficiently set at 20 min, and a gradient profile was 97:3 of solution A and solution B, respectively, for 6 min, then linear gradient to 100% A in 2 min, to an isocratic step of 100% solution A for 10 min and finally a linear gradient to solution A and B (97:3) in 2 min, where solution A was methanol:butanol (92:8, respectively), and solution B was mili-Q water 100%. The wavelength for detection of α-tocopherol was set at 292 nm and the wavelength detection of β-carotene was set at 450 nm. DL-α-tocopherol acetate (HPLC standard, Merck KGaA, Darmstadt, Germany) was used as an internal standard (1 mg/ml processed sample), giving steady recovery values of 70%. α-tocopherol (synthetic, ≥96%, HPLC standard) and

Table 1
Characteristics of the solvents used in the present study.

Solvent	Density (g/cm3)	Molecular weight (g/mol)	Purity (%)	Boiling point (°C)	Polarity[a]	Molecule scheme
Diethyl ether	0.71	74.12	≥99.5	34.60	5.77	
Hexane	0.66	86.18	≥99	68.00	2.56	
2-MTHF	0.85	86.13	≥99	80.20	6.99[b]	
Ethanol	0.79	46.07	≥99.5	78.37	8.05	

[a] Polarity according to the Spectral Polarity Index developed by Freed, Biesecker, & Middleton (1990).
[b] Polarity value from tetrahydrofuran. According to Aycock (2007), solvent polarity and Lewis base strength properties of MeTHF is somewhere between THF and diethyl ether.

β-carotene (synthetic, ≥93%, analytical standard) from Merck KGaA (Darmstadt, Germany) were used as standard (stock solutions were used at concentrations of 1 mg of α-tocopherol/ml of methanol ($y = 0.0045x + 0.0007$, $R^2 = 0.99$, concentrations from 0.6 to 0.08 mg/ml) and 0.5 mg β-carotene/ml of *n*-hexane ($y = 0.0011x + 0.0046$, $R^2 = 0.96$, concentrations from 0.15 to 0.02 mg/ml)).

2.5. Fatty acid profile

The fatty acid composition of the extracted oils were determined by derivatizing the fatty acids to fatty acid methyl esters (FAME) using the previously reported method by Lamba, Modak, and Madras (2017) with minor changes. Briefly, 0.15 g of extracted SBO was poured into a 15 mL screw-capped test tube and mixed with 2 mL of *n*-hexane (≥95%, HPLC grade, Merck KGaA, Germany) and 1 mL of 2 mol/L methanolic KOH solution (methanol GC/HPLC GGR from Labbox Labware S. L. (Catalunya, Spain); KOH pellets ACS reagent ≥85% from Merck KGaA (Darmstadt, Germany)). The tubes were vigorously shaken for 30 s and incubated in a water bath previously heated at 70 °C for 2 min. The tubes were then taken out of the bath and cooled at room temperature for 2 min, and immediately after 1.2 mL of HCl 1 mol/L (Panreac Química S. L. U., Catalunya, Spain) were added to the tube. Gently stirring was applied for 10 min and then the mix was left undisturbed. Separation in two phases occurred after 15 min. An aliquot of the upper phase was directly injected into the gas chromatograph for analysis.

A capillary column VF-5 ms, 30 m × 0.25 mm with 0.25 μm film thickness containing 5% phenyl-methylpolysiloxane attached to a gas chromatograph 6890 (Agilent Technologies, California, U. S.) was used for the chromatographic analysis. Helium was used as the carrier gas at 1.7 mL/min, and oxygen and hydrogen served as fuel gases. The oven temperature was raised from 75 to 240 °C at 5 °C/min and held at 240 °C for 20 min. The split value was 1:40 and isopropanol (≥99.5 ACS reagent, Merck KGaA, Darmstadt, Germany) was used as a rinsing agent.

Peak identification was performed using different techniques. First, a standard mix of alkanes (Standard Connecticut ETPH Calibration Mixture (15 Components, C9 to C36 at concentration rate of 1000 μg/mL dissolved in methylene chloride), Restek, Bellefonte, U.S.) was used to selectively isolate and identify the hydrocarbons of the sample with the GC 6890 system. Secondly, the retention time of each fatty acid of interest appearing in the chromatogram was compared with the known Kovats retention index. Finally, peak identification was performed with a GC System 7890A attached to an MS triple-axis detector 5975C (Agilent Technologies, California, U. S.) with the Wiley library and by comparison of the retention time of bought standards of methylated fatty acids (Supelco 37 Component FAME Mix, Merck KGaA, Darmstadt, Germany).

Every sample analysis procedure was performed thrice, and measurements by the appropriate equipment were performed in duplicate.

2.6. Statistical analysis

All statistical analysis was performed with the software R-4.0. Assumptions were checked by first visually interpreting the Q-Q and boxplots from all analyses. Normality was double-checked by the Shapiro-Wilk test, which gave non-significant values for all the analysis, therefore proving the normality of the whole data. Subsequently, the statistical analysis of the data was performed. A two-way ANOVA was first run to understand the importance of the interaction between the temperature and the solvent on the final extraction yield, and concentration of α-tocopherol and β-carotene as well as the fatty acid profile. Further analysis involved the use of Tukey's *post hoc* tests to understand possible significant differences within variables. The statistical significance of the results was set at $p < .05$.

3. Results and discussion

3.1. Extraction yield of SBO extracted with different solvents

The SBO yield using hexane, ethanol, diethyl ether and 2-MTHF were affected differently by temperature. Thus the most efficient temperature for extracting SBO was different depending on the solvent used, being 120 °C for hexane and diethyl ether (23.25% and 24.98% yield, respectively), 90 °C for ethanol (21.75% yield), and 150 °C for 2-MTHF extraction (14.65% yield). The efficiency of extraction seemed to be greater by increasing the extraction temperature when using 2-MTHF, which was partly true when using diethyl ether as an extraction solvent. However, the latter achieved the poorest extraction yield at 150 °C, making evident the different behaviour of each solvent across different temperatures. Interestingly, the pattern clearly pointed out that the more polar the solvent was, the more was it affected by changes in temperature. It is important to note that the solvent 2-MTHF was strongly and significantly influenced by changes in temperature when compared to the rest of the solvents. Results from the extraction yield reported by other authors indicated that the greater the temperature of the extraction, the greater the extraction yield (Castejón et al., 2018), differing from what was observed in the present experiment. Castejón et al. (2018) found that at temperatures of 150 °C, the greatest yield was obtained when using ethanol or hexane in echium seed oil extraction when compared to lower temperatures.

Interestingly, using the Soxhlet technique (control) instead of ASE (using hexane as solvent) yielded significantly more oil. Nevertheless, this difference translated only into 0.65% more oil extracted by the former technique, which was in line with what previous authors found using hexane on flaxseeds (42.40% using Soxhlet and 41.90% using ASE (Khattab & Zeitoun, 2013)) or echium seeds (31.3% using Soxhlet and 31.2% using ASE (Castejón et al., 2018)). One of the possible reasons behind this could be the longer time the sample is left during Soxhlet extraction, which may help to extract more oil when compared to the usually short static time employed in ASE extractions.

When comparing the same technique (ASE), ethyl ether was the solvent achieving significantly greater extraction yield when compared to ethanol, hexane or 2-MTHF (Fig. 1). The results indicated that SBO may contain mostly non-polar compounds that can be extracted by using a non-polar solvent such as hexane. However, there may also be other polar compounds found at lower concentrations that would benefit from the extraction with diethyl ether. Similar results were reported using Soxhlet extraction with diethyl ether on peach kernels, which achieved significantly greater oil extraction yield when compared to all other solvents, including hexane (Wu et al., 2011), or on potato peel oil

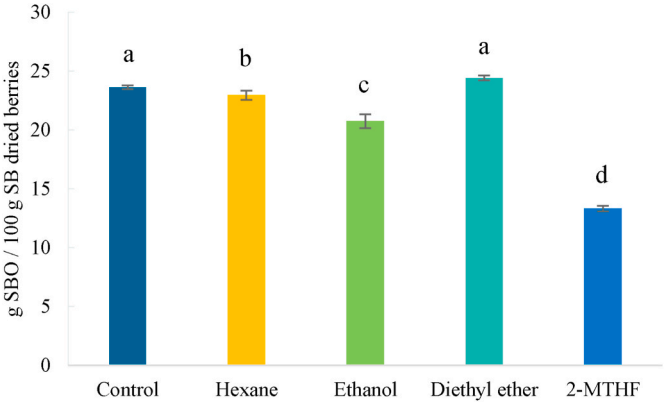

Fig. 1. Extraction yield of SBO by ASE using different solvents at high pressure compared to Soxhlet used as a conventional method (Control). Bars display the average value of the resulting extractions at different temperatures (60, 90, 120 and 150 °C). Different letters mean significant differences at $p < .05$.

extracted with different organic solvents, including ethanol, hexane and diethyl ether (Zia-ur-Rehman, Habib, & Shah, 2004).

The extractions using 2-MTHF and ethanol yielded significantly lower oil yield when compared to the "non-green" solvents. Due to the relatively high polarity of both green solvents (Table 1), the extraction yield was affected greatly. Ethanol yielded less oil than hexane using pressurized liquid extractions at all temperatures (Fig. 1), similar to what was found in echium seeds at different temperatures (Castejón et al., 2018), probably due to the lipophilic nature of the extract. Ethanol can extract the part of lipids that have a slightly polar behaviour, such as phospholipids or waxes, or even proteins (Dunford & Zhang, 2003). In the case of SBO, proteins or phospholipids are not expected to interfere greatly. Nevertheless, ethanol could also extract other water-miscible components present in dried berries. An important group of hydrophilic molecules in dried SB berries is carbohydrates, specially sugars. SB sugars could be mostly extracted when using ethanol, yielding up to 75% of the initial sugar content (Baümler, Carrín, & Carelli, 2016). The applied further treatment allowed the confirmation of the theoretical reasoning. Great values of extraction yield (around 50 g of oil/100 g of dried berries) were achieved during the first exploration with ethanol (results not shown), but when water was added to rinse the outcoming oil, the water drained most of the hydrophilic extracted compounds, including sugars, obtaining the final values represented in Fig. 1.

Finally, the lowest yield was recorded for the extraction with 2-MTHF, roughly achieving an extraction yield of 13% (Fig. 1). A lower extraction yield using 2-MTHF was recorded previously by other authors on Tunisian date palm seeds when compared to other solvents (Ben-Youssef, Fakhfakh, Breil, Abert-Vian, & Chemat, 2017), but higher extraction yields were recorded for anise seeds when using 2-MTHF (Rebey et al., 2019). Consonant to what resulted by using ethanol, 2-MTHF effectively achieved a preliminary extraction of higher yield from SB dried berries. The original extraction yield (around 32%) dropped to 13% after applying the water rinsing process. Thus, it was considered that most of the 'extra' yield was derived from the extraction of sugars or other water-soluble compounds. The difference between the final yield of 2-MTHF and ethanol could be because of two main reasons. First, it could be a consequence from the polarity difference (Table 1); ethanol then extracted a greater polar fraction than 2-MTHF, and part of it could remain in the final oil yield (after rinsing it with water) due to its great amount. Second, it could be a consequence of distinct further processing. While in ethanol a normal rinse would suffice, with 2-MTHF a centrifugation was needed to allow phase separation, leading to possible residues of oil left in the centrifuge vial.

3.2. Concentration of α-tocopherol in SBO

There was a significant main effect of the solvent used in the extraction on the concentration of α-tocopherol in the extracted oil (Fig. 2). The concentration of α-tocopherol in extracted SBO was greater in the oil extracted with ethanol than in the oil extracted with hexane, and both were significantly greater than the oil extracted by Soxhlet (control). The lowest concentration of α-tocopherol was observed in SBO extracted with diethyl ether or 2-MTHF. A matrix of methanol/chloroform also extracted higher amounts of different tocopherols when extracting oil from almond, Brazil nuts, hazelnuts or pecan nuts when compared to other less polar compounds (Miraliakbari & Shahidi, 2008). In addition, other authors reported a higher extraction of tocopherols – including α-tocopherol – when using ethanol as compared to the extraction with hexane (Baümler et al., 2016). The results could be attributed to differences in solvent polarities (Table 1). Overall concentrations of extracted α-tocopherol ranged from 0.6 to 1.0 mg/g SBO, values similar to what previous authors found in SB (Kallio, Yang, & Peippo, 2002). It is important to mention that the values of the present research correspond to SB dried berries instead of different fractions from the fruit (i.e. peel and seeds), differing from values found by other authors.

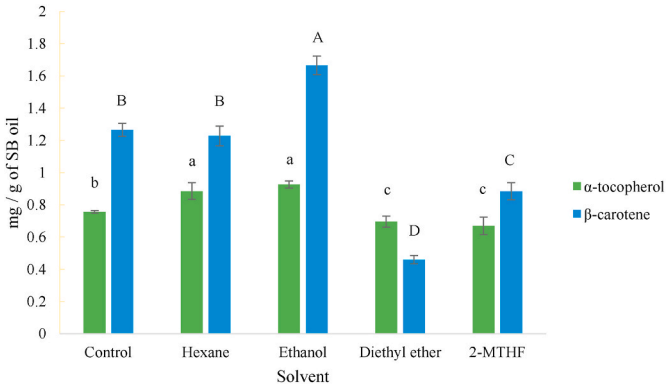

Fig. 2. Average concentration of α-tocopherol and β-carotene in SBO extracted with different solvents. Error bar show SD. Bars display the average value of the resulting extractions at different temperatures (60, 90, 120 and 150 °C). Different letters mean significant differences at $p < .05$. Comparisons are not made across upper- and lower-case letters (i.e. across vitamers).

The concentration of α-tocopherol in SBO was greater in the samples extracted by ASE (hexane) when compared to the same solvent and Soxhlet technique (control). The longer extraction times in Soxhlet extraction may trigger the destabilization of α-tocopherol to a relevant extent in which differences between these two groups become significant. In contrast, the concentration of α-tocopherol in SBO extracted with diethyl ether or 2-MTHF was significantly lower than the concentration in control samples (Fig. 2). No significant differences were observed in the concentration of α-tocopherol in oils extracted with diethyl ether or 2-MTHF. This was interesting since previous authors found greater saponification values from peach kernel oil extracted with ethyl ether rather than oil extracted with hexane, translating into a greater extraction of unsaponifiable compounds, including tocopherols and carotenoids (Wu et al., 2011).

Significant differences were also observed in the concentration of α-tocopherol between the control group and the extraction at 120 and 150 °C, being higher in the latter two. The greater the temperature, the greater the recovery of α-tocopherol, which achieved a plateau after the extraction temperature was set at 120 °C (Fig. 3). This was in line with what other authors observed, although using only one solvent (hexane) and observing the steady recovery of tocopherols above 100 °C (Sanagi, See, Ibrahim, & Naim, 2005). Greater temperatures generally allow getting better mass transfer rates between the solvent and the sample matrices, which directly increase the capacity of the solvent to solubilize analytes (Sanagi et al., 2005). Lower recoveries of α-tocopherol were

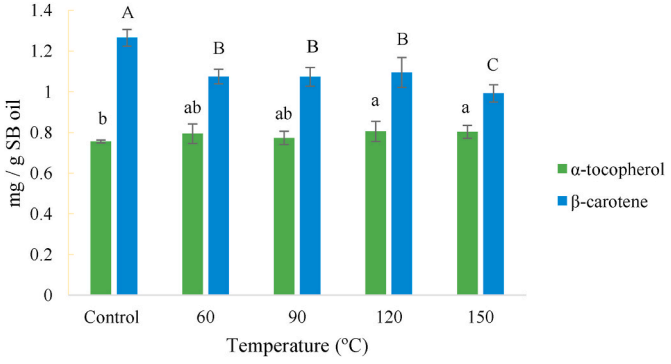

Fig. 3. Average concentration of α-tocopherol and β-carotene in SBO extracted at different temperatures. Error bars show SD. Bars display the average value of the resulting extractions with different solvents (hexane, diethyl ether, ethanol and 2-MTHF). Different letters mean significant differences at $p < .05$. Comparisons are not made across upper- and lower-case letters (i.e. across vitamers).

observed in the control extraction, which may come from the longer extraction times and the subsequent oxidation of the analytes.

3.3. Concentration of β-carotene in SBO

The recovery of β-carotene from sea buckthorn oil differed significantly between all solvents, except the oil extracted with hexane using the technique ASE when compared to the same solvent using the Soxhlet methodology (control, Fig. 2). The use of different solvents was more relevant than the use of different techniques. However, more solvents should be tried in the Soxhlet extraction in order to get more reliable results to support this conclusion.

The lowest concentration of β-carotene was found in oil extracted with diethyl ether and the greatest concentration was found in oil extracted with ethanol. Carotenoids had also been extracted in significantly greater amounts when using ethanol as the extraction solvent instead of other conventional solvents such as diethyl ether (Lichtenthaler & Wellburn, 1983) or acetone (Marsili & Callahan, 1993). Ethanol was also found to be the most efficient solvent for the extraction of β-carotene when compared to other green alternatives, such as ethyl acetate and ethyl lactate (Ishida & Chapman, 2009). Thus, ethanol comes out as a promising solvent for the extraction of β-carotene from sea buckthorn dried berries. Like what was observed for α-tocopherol, the concentration of β-carotene in extracted SBO was significantly greater in the control samples when compared to the samples extracted with diethyl ether or 2-MTHF (Fig. 2). However, in this case, differences were not observed between the two extractions with hexane (control samples and ASE extraction), contrarily to what had been reported by Saini and Keum (2018).

Wu et al. (2011) found greater antioxidant capacity (in terms of DPPH and Trolox equivalent antioxidant capacity assay (TEAC)) in the peach kernel oil extracted with hexane when compared to the oil extracted with ethyl ether. Greater antioxidant capacity does not imply greater vitamin concentration, yet vitamins could contribute significantly to this attribute. Hexane could then extract oil with greater antioxidant activity, and thus probably with greater vitamin concentration, as observed in the present experiment. Nevertheless, different vitamins could be present in the matrix, as it was observed in peach kernel (tocopherols, carotenoids (Wu et al., 2011)), and it is thereupon difficult to assume whether the greater antioxidant capacity could come from one vitamin or another.

The concentration of β-carotene extracted with diethyl ether was significantly lower compared to the values obtained by 2-MTHF extraction. Diethyl ether is a less polar solvent when compared to 2-MTHF, closer to the polarity of hexane (Table 1). Rebey et al. (2019) reported higher antioxidant values of fennel and anise oils extracted with hexane compared to oils extracted with 2-MTHF. β-carotene, together with lycopene, are highly lipophilic non-polar carotenoids mainly because of the lack of a functional polar groups in their structure (Saini & Keum, 2018), therefore making them theoretically chemically more prone to their extraction with non-polar solvents.

Significant differences were also observed in the concentration of β-carotene between the control group and the extraction at any other temperature, being higher in the former (Fig. 3). It seemed that the degradation of β-carotene during extraction was slightly more influenced by the temperature rather than by the exposure of the compound to the light and oxygen during the extraction (control extraction). This was clearly in contrast with what was observed during the extraction of α-tocopherol (Fig. 3). The concentration of β-carotene was significantly lower in the oil extracted at 150 °C when compared to all other temperatures. Other authors already reported losses of antioxidant capacity of carotenoids extracts – from *Haematococcus pluvialis* microalga – when increasing the extraction temperature (Jaime et al., 2010). The loss of antioxidant capacity was attributed to the loss of important carotenoid fractions, being severe above 100 °C. The present work adds evidence to this fact in a new matrix, with a stable extraction of β-carotene over temperatures up to 120 °C, and significantly diminishing the concentration of the analyte in the oil extracted at 150 °C (Fig. 3).

3.4. Concentration of monounsaturated (MUFA), polyunsaturated (PUFA) and saturated (SFA) fatty acids in SBO

Considering the extraction with ASE – and excluding Soxhlet extraction (Control) –, using less polar solvents yielded less SFA than using more polar solvents (Fig. 4). Due to the saturation degree of the SFA and the triglycerides in which they are packed, the less polar solvents should have been the solvents extracting more quantity (Tir, Dutta, & Badjah-Hadj-Ahmed, 2012). Obtaining almost 10% more SFA by Soxhlet extraction when compared to ASE extraction using hexane may serve as a proof that time could be an important factor when considering the extraction of SFA from SB dried berries. At the extraction conditions used in the present research, it could be concluded that ethanol and 2-MTHF are suitable for the extraction of SFA from SBO, yielding significantly greater concentrations than hexane or diethyl ether, two solvents widely used for that purpose (Fig. 4).

The greatest concentration of MUFA in SBO was observed in the samples extracted with hexane – both extracted with ASE and Soxhlet –, followed by all other extractions (Fig. 4). Soxhlet extraction had been also previously reported to extract greater amounts of MUFA than other techniques, including supercritical fluid extraction and ASE, probably due to the longer extraction time (Castejón et al., 2018; Rebey et al., 2019; Reddy, Moodley, & Jonnalagadda, 2012). The higher extraction yield using hexane as compared with other solvents was in line with what other authors observed (Bourgou et al., 2019). According to Mezzomo, Mileo, Friedrich, Martínez, and Ferreira (2010), the reason for greater extraction may rely on the polarity index of the solvent, which is lower in apolar solvents when extracting long-chain fatty acids. The fact that diethyl ether did not achieve similar extraction results for MUFA when compared to hexane extraction may be due to its higher polarity index (4.4, compared to hexane (0) and ethanol (5.2) (Mezzomo et al., 2010)).

All solvents achieved significantly different concentrations of PUFA in the resulting SBO (Fig. 4). Control samples (Soxhlet) extracted significantly lower amounts of PUFA when compared to any ASE extraction. Other authors also reported lower amounts of PUFA in oil from caraway (*Carum carvi*) seeds extracted by using the Soxhlet technique (and hexane as solvent) when compared to other techniques and greener solvents (Bourgou et al., 2019). The longer extraction time when using Soxhlet is a great barrier to the efficient recovery of PUFA in SBO. The long extraction time implies prolonged contact with air and prolonged temperature exposure of the sample, two critical factors when extracting polyunsaturated molecules, as they may lead to degradation

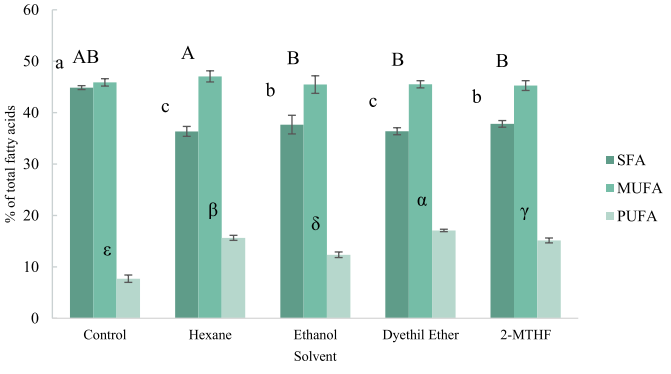

Fig. 4. Average concentration of SFA, MUFA and PUFA in SBO extracted using different solvents. Error bars show SD. Bars display the average value of the resulting extractions at different temperatures (60, 90, 120 and 150 °C). Different letters - upper-, lower-case and Greek letters - mean significant differences at $p < .05$.

of the most unsaturated fatty acids. The results from the ASE extraction indicated that the less polar solvents achieved greater amounts of PUFA in the extracted SBO when compared to greener solvents (i.e. ethanol, 2-MTHF). The greater extraction of more polar lipids with apolar solvents may be due to the form in which these are found in the original matrix; they may be bond to a triglyceride molecule (more apolar than the fatty acid itself) and thus more difficult for them to be extracted using polar solvents. The extract using diethyl ether yielded more quantity of PUFA when compared to its non-polar counterpart hexane, probably due to its relatively higher polarity (Mezzomo et al., 2010; Tir et al., 2012). Nevertheless, other authors have found hexane to yield higher amounts of PUFA in extracted oils when compared to other non-polar solvents (Wu et al., 2011), although the extraction was performed using a Soxhlet apparatus, differing from what had been used in the present experiment. Extracting SBO with 2-MTHF yielded significantly higher PUFA when compared to ethanol. Ethanol is stated to be slightly more polar than 2-MTHF, which in turn could influence the efficiency in recovery of fatty acids, especially PUFA (Fig. 4).

The results showed significant differences between the tested temperatures on the recovery of SFA, MUFA and PUFA from SBO. SFA concentration in control samples was greater when compared to all other samples (Fig. 5). When it comes to ASE extractions, temperatures of 120 and 150 °C achieved statistically significantly lower concentrations of SFA when compared to temperatures of 60 and 90 °C (Fig. 5). In the case of polar solvents, such as ethanol and 2-MTHF, polarizability may be negatively affected – so the polarity of the solvent is reduced – at higher temperatures (Lu, Boughner, Liotta, & Eckert, 2002), which could then trigger a greater extraction of SFA at higher temperatures. Results herein show otherwise, indicating a drop in the concentration of SFA at higher temperatures (Fig. 5). There may be the need of rising more the temperature in order to see improvements in the extraction of SFA from SBO. Even though it may have an influence, it is also noteworthy that the results show the effect on the overall extraction of SFA, not only the effect of the extraction using the most polar solvents.

In contrast, extractions at higher temperatures (120 and 150 °C) yielded a greater concentration of MUFA when compared to extractions at lower temperatures (60 and 90 °C; Fig. 5). The fact that higher temperatures may decrease the polarity of polar solvents may be the reason that could explain why at higher temperatures the extraction of MUFA was higher when compared to lower temperatures. However, all solvents may influence the results in the temperature, making it closer to a conjecture than to a conclusion.

At last, the concentration of PUFA in the SBO from control samples was significantly lower than any other temperature (Fig. 5). PUFAs are the most oxidizable fatty acid molecules herein explored and are especially vulnerable at high temperatures, which makes them prone to degradation. The use of longer processing times in more cycles when using the Soxhlet extraction technique and the high extraction temperatures employed in this technique could explain the lower concentration obtained in the resulting oil. In fact, other authors already observed lower recoveries of PUFA from Soxhlet when compared to ASE extractions (Castejón et al., 2018). From all other temperatures, the recovery of PUFA seemed steady, except for 90 °C, in which the recovery of PUFA was significantly greater compared to 60 or 120 °C. Interestingly, the concentration of PUFA in SBO extracted at 150 °C was non-significantly different from that obtained at 90 °C (Fig. 5). This seemed to indicate that there could exist an ideal temperature value for the extraction of PUFA from SBO.

4. Conclusions

The present research shows that SBO extraction with the conventional method Soxhlet yields more oil when compared to the ASE technique using the same solvent (hexane). Nevertheless, the latter oil has greater concentration of α-tocopherol, PUFA and MUFA when compared to the former, probably deriving from the greater extraction time when using Soxhlet (5h) when compared to the extraction time of ASE (10 min).

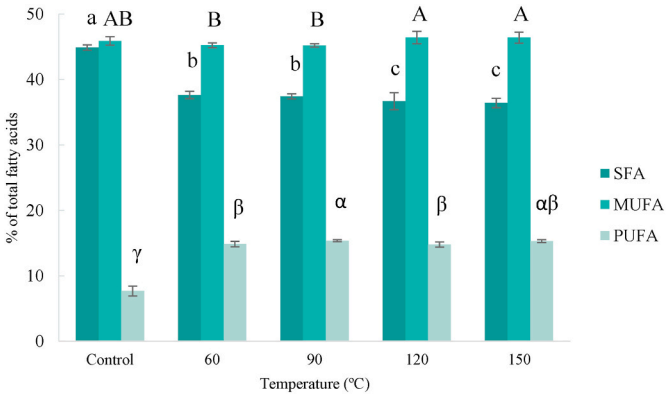

Fig. 5. Average concentration of SFA, MUFA and PUFA in SBO extracted using different temperatures. Error bars show SD. Bars display the average value of the resulting extractions with different solvents (hexane, diethyl ether, ethanol and 2-MTHF). Different letters mean significant differences at $p < .05$. Comparisons are not made across upper-, lower-case and Greek letters (i.e. across groups).

The extraction using green solvents achieves nutritionally good values when compared to the petroleum-based solvents, especially when using ethanol. The values of β-carotene concentration are far better in SBO after ethanol extraction when compared to all other solvents. For that reason, ethanol should be considered positively when evaluating the extraction and isolation of β-carotene from sea buckthorn pulp, even when considering that the extraction with ethanol intrinsically carries the application of an extra processing step to wash the dragged polar compounds that influence the yield. In contrast, the fatty acid profile of SBO oil extracted with green solvents is worse than petroleum-based solvents, with the noteworthy greater extraction of SFA.

CRediT authorship contribution statement

Arnau Vilas-Franquesa: Investigation, Conceptualization, Methodology, Data curation, Writting and Editing. **Bibiana Juan:** Validation, Reviewing and Editing. **Jordi Saldo:** Validation, Reviewing and Editing.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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