Journal of Thermal Analysis and Calorimetry (2020) 142:735–747
https://doi.org/10.1007/s10973-019-08875-5

Edible vegetable oils enriched with carotenoids extracted from by-products of sea buckthorn (*Hippophae rhamnoides* ssp. *sinensis*): the investigation of some characteristic properties, oxidative stability and the effect on thermal behaviour

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Received: 3 June 2019 / Accepted: 28 September 2019 / Published online: 12 October 2019

Abstract

In the recent years, it has been granted a growing interest to the substitution of synthetic food antioxidants by natural ones, while a special attention was directed to their extraction from the by-products of the food industry. Sea buckthorn (*Hippophae rhamnoides*) by-products are promising sources of bioactive compounds that could be used for their favourable nutritional and functional properties. In this study, ultrasound-assisted extraction and maceration have been used for the direct enrichment of three edible oils (refined sunflower oil, cold-pressed sunflower oil and extra virgin olive oil) with carotenoids from dried sea buckthorn by-products. Total carotenoids content and ABTS free radical scavenger activity of both enriched and commercial oils were determined by spectrophotometric methods, and the colour was evaluated according to the CIELab colour space. The oxidative stability of vegetable oils containing the extracted carotenoids was assessed in terms of peroxide value, while the thermal stability of the oils was evaluated by thermogravimetry and by differential scanning calorimetry. It was shown that the ultrasound-assisted extraction was more effective than the maceration for the extraction of carotenoids from dried sea buckthorn by-products. ABTS radical scavenging activity has been slightly improved for all the oils studied after carotenoid enrichment, while the oxidative stability increased in extra virgin olive oil but decreased in unrefined and refined sunflower oils. Extraction of sea buckthorn by-products significantly ($P < 0.05$) reduced lightness and increased redness and yellowness of the oils.

Keywords Antioxidant activity · Carotenoids · Extraction · Oxidative stability · Thermal stability

Introduction

Vegetable oils hold an important place in human nutrition since most of the time they are edible, therefore added in salads or used for enhancing some thermal treatments such

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10973-019-08875-5) contains supplementary material, which is available to authorized users.

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as cooking and frying [1–3]. Many times they are enriched with natural dyes such as the carotenoids for their colour since this is an important factor for the appeal, and enhances the acceptability and valorisation of the product for being sold [4]. Unfortunately, edible oils are highly susceptible to oxidation, leading to rancid odours, unpleasant flavours and discoloration; thus, this results in the modification of sensorial and nutritional properties, while their shelf life is diminished [5]. When exposed to increased temperatures, they undergo accelerated oxidation, thermal degradation or polymerisation, which usually leads to the accumulation of several residual products with harmful effects for the human health [6, 7].

In order to increase the stability of edible oils, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) have been used as food additives; however, recent reports revealed that these compounds may have harmful side effects [8]. Hence, there is an increased interest to enrich oils with natural antioxidants of vegetal origin in order to retard or prevent oxidative deterioration [5, 9]. Spices, herbs, teas, oils, seeds, cereals, cocoa shell, grains, fruits, vegetables, enzymes and proteins have been used as sources of natural antioxidants [10, 11]. Special attention was focussed on their extraction from inexpensive or residual sources from the agricultural industries [12, 13].

Tomato by-products [4, 14], fruits by-products [15], fresh carrots [16], potato peels, sugar beet pulp and sesame cake [7], olive leaves [2] and olive waste cake [17] are some of the agricultural and industrial by-products that have been used as sources of potentially safe natural antioxidants in various oils.

Carotenoids are one of the most important groups of natural pigments in fruits and vegetables, well known for their ability to scavenge reactive oxygen species and for their role in photosynthesis and photoprotection [16, 18]. They present physiological and biological function in the human body as they are important antioxidants and precursors of vitamin A. Several epidemiological studies linked a high carotenoid intake with a lower risk of chronic degenerative diseases such as cardiovascular diseases, several types of cancer or neurological, and photosensitive or eye-related disorders [19]. Carotenoids are often used as colourants that are added directly to various food products such as butter, popcorn, salad dressings and beverages [20].

Sea buckthorn (*Hippophae rhamnoides*) is a bush from the family *Elaeagnaceae* with yellow or orange berries that grows in various regions of Asia, Europe and North America. The leaves, berries and seeds of sea buckthorn are known to be used in traditional medicine and to have antimicrobial, dermatological and antioxidant effects [21]. Sea buckthorn berries are a rich source of carotenoids, vitamins, phenolic compounds, tocopherols, health beneficial fatty acids, phytosterols, terpenes, tannins and minerals [22, 23]. These berries have been used for the production of jams, syrups, liquors, oil and lately as viable candidates for functional food ingredients [24].

Juice extraction from sea buckthorn berries leads to a residual pomace consisting of pulp, seed and skin, which is rich in biologically active compounds with antioxidant properties. In order to produce high-value natural food additives and supplements, and to reduce the waste, exploitation of these by-products for the extraction of beneficial antioxidants has gained increasing interest over the recent period of years [25]. Sea buckthorn pomace has a high content of moisture that makes it susceptible to microbial proliferation; therefore, it must be preserved by drying and then used for the extraction of carotenoids. Based on the lipophilic properties of carotenoids, they may be extracted from dried sea buckthorn pomace directly in edible oils, in order to improve the quality and functionality of the oils and to increase the dietary carotenoid intake. In addition, the direct extraction in oil offers the advantage of avoiding the drawbacks associated with the use of solvents.

In this study, dried sea buckthorn by-products have been used as a source of natural carotenoids for the enrichment of vegetable oils. The aim of this present work consists in enriching edible oils by using the lipophilic properties of the oil itself to extract directly residual carotenoids still present in the by-products of sea buckthorn extraction. Here, we show the application of a direct extraction of sea buckthorn by-products in edible oils without solvent and by eliminating evaporation and extraction unit operations. This process is not efficient using normal maceration, but the transfer of carotenoids into the oil can be accelerated significantly with the use of ultrasounds; therefore, maceration and ultrasound-assisted extraction procedures were applied separately to extract carotenoids directly in the oils (here: refined sunflower oil, cold-pressed sunflower oil and extra virgin olive oil). The total phenolic content, total and individual carotenoids content and antioxidant activity were assessed for the dried sea buckthorn by-products. The resulted oils were analysed for their total carotenoid content, ABTS antioxidant activity and colour. The oxidative stability of the oils was evaluated by using the UV accelerated method in terms of peroxide value, while the thermal stability was investigated by thermogravimetric analysis (TG) and by differential scanning calorimetry (DSC).

Materials and methods

Materials and reagents

The sea buckthorn by-products were obtained from Biocat Prod S.R.L., a local sea buckthorn producer and processor from Grădina, Constanţa County, located 44°33′N, 28°26′E in Southeast Romania. The sea buckthorn materials were dehydrated in a hot air industrial dryer (Blue Spark Systems S.R.L., Romania) at 60 °C, then ground to a powder to pass through a 0.70-mm sieve, packed in aluminium coated polyethylene bags and stored in ambient conditions for further analysis and use. Total carotenoids, total phenolics and the ABTS antioxidant activity of the buckthorn by-products powder were determined in triplicate.

Extra virgin sunflower oil "VirginOil" (Virginoil S.R.L., Bacau, Romania), extra virgin olive oil "Campo Dorato" (Spoleto, Italy) and refined sunflower oil "Surasul soarelui" (Prutul S.A., Galati, Romania) were purchased from local supermarkets.

For carotenoid identification and quantification, pure commercial standards (Sigma-Aldrich, Germany) of lutein, zeaxanthin, canthaxanthin, astaxanthin, lycopene, β-carotene and trans-β-apo-8′-carotenal were used. Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid), butylated hydroxytoluene (BHT), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and Folin–Ciocalteu reagent were obtained from Sigma-Aldrich (Germany). All other reagents were of analytical grade and purchased from Merck (Germany). Double distilled water was used throughout the experiments.

Sample preparation

Samples of sea buckthorn by-products powder were subjected to ultrasound-assisted extraction and to maceration in extra virgin sunflower oil (EVS), extra virgin olive oil (EVO) and refined sunflower oil (RS) at different concentrations (2.5, 5 and 10%, w/v). Maceration was carried out at 20 °C over 10 days, while ultrasound-assisted extraction (UAE) was performed for 50 min in a Bandelin Sonorex Digital 10P ultrasound bath (Bandelin Electronic GmbH, Berlin, Germany) operating at 35 kHz with 480 W power. The bath temperature was maintained at 20 °C using cold water. Each experiment was conducted three times. All the extractions were followed by filtration through Whatman No.1 filter paper, and the resulting oils were collected in screw-capped dark plastic containers completely filled with oil and stored at 4 °C until they were analysed. The commercial oils were also stored under the same conditions as the enriched oils to be used as blanks for all the tests. The oils were sampled for each measurement from separate bottles. Total carotenoid content, peroxide value, ABTS antioxidant activity and CIELab colour parameters were determined in all oil samples.

Determination of total carotenoid content

Total carotenoid content was determined spectrophotometrically as described by Szydłowska-Czerniak et al. [26]. The oil samples (1 g) were dissolved in 50 mL of *n*-hexane, and the absorbance at 450 nm was measured against *n*-hexane using a Varian Cary 50 UV spectrophotometer (Varian Co., USA) in a 1-cm quartz cell. A calibration curve of β-carotene standard solutions in *n*-hexane (0.1–7.0 mg L^{-1}) was used to determine the carotenoid content of oil samples. The final results were expressed as mg of β-carotene per kg of oil.

Determination of ABTS antioxidant activity

Antioxidant activity in oil samples was determined using an ABTS (2,29-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) procedure described by Re et al. [27]. The ABTS cation radical solution (ABTS+) was prepared by mixing 5 mL of a 7.0 mM ABTS solution and 88 μL of a 145 mM potassium persulfate solution. After 16 h of incubation at room temperature in a dark place, the ABTS+ solution was diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. Twelve mL of ABTS+ solution was added to 120 μL of sample extract and vigorously mixed in a vortex. After 6 min, the absorbance at 734 nm was read using ethanol as blank. The calibration curve was constructed using ethanol solutions with known concentrations of Trolox (100–2000 μM Trolox L^{-1}), and the results were expressed in mmoles of Trolox per kg of oil.

Determination of peroxide value

The oxidative stability of vegetable oils was assessed using the UV accelerated method as described by Lalas and Tsaknis [28]. Ten grams of each oil sample was accurately weighed into a glass Petri dish (87 mm i.d. and 15 mm high). The dishes were irradiated for 12 h with UV light from an UV lamp (36 W) situated 50 cm above. The extent of oil oxidation was evaluated from peroxide value (PV) according to the method described by the American Oil Chemists' Society [29]. The method is based on iodometric titration, which measures the iodine produced from potassium iodide by the peroxides present in the oil. Peroxide value was expressed as milliequivalents (meq) of peroxides per kg of oil.

Colour analysis

The colour of oils was evaluated by measuring the L^*, a^* and b^* values of the CIELab system with a Thermo Scientific Evolution 600 UV/VIS spectrophotometer, using quartz cuvettes of 1 mm. The L^* value indicates lightness, a^* value indicates chromaticity on a green (–) to red (+) axis, and b^* value represents chromaticity on a blue (–) to yellow (+) axis. All measurements were conducted in triplicate. Mean values per sample were used for statistical analysis.

Carotenoid analysis in dried sea buckthorn pomace

Dried sea buckthorn pomace was subjected to triplicate analyses for carotenoids using high-performance liquid chromatography with diode-array detection at 450 nm, as described by Corbu and Nour [30]. Separation of carotenoids was achieved on a reversed-phase Hypersil Gold C18 column (Thermo) at 20 °C using a Finningan Surveyor Plus system (Thermo Electron Corporation, San Jose, CA). Carotenoids were extracted from 0.5 g sample with 10 mL of petroleum ether/methanol/ethyl acetate (1:1:1, v/v/v) containing 0.1% butylated hydroxytoluene (BHT) by homogenising for 5 min at 2500 rpm using a vortex homogeniser. The sample was centrifuged for 6 min at 6000 rpm, and the supernatant was collected. The residue was extracted following the same procedure until the supernatant was colourless. The combined supernatants were washed by adding 10 mL of 5% NaCl solution, mixing vigorously and incubating for 30 min until two layers were separated. The upper layer was collected, evaporated to dryness under nitrogen and then re-dissolved in 2 mL of acetonitrile/methanol/ethyl acetate (60:20:20, v/v/v) containing BHT (1% w/v). Before HPLC injection, the final solution was filtered through a 0.45 µm syringe filter. The mobile phase comprised acetonitrile/methanol (95:5, v/v) (A), acetonitrile/methanol/ethyl acetate (60:20:20, v/v/v) (B) and water (C). Carotenoids were eluted at a flow rate of 1.5 mL min^{-1} with the following gradient: 96% A and 4% C in the beginning, maintained for 10 min, changed linearly to 100% B in 13 min, maintained 5 min and returned to 96% A and 4% C in 2 min. Quantification was performed using ChromQuest 4.2 software by comparing peak area with those of the known standards.

Thermal analysis and calorimetry study

Thermal analysis is often used for characterisation of natural products, oils and oil systems [14, 31–33]. Here, the thermal behaviour of the samples (three edible oils, the sea buckthorn by-products powder and the three 5% carotenoid-enriched edible oils prepared by ultrasound extraction) was performed with a horizontal DIAMOND TG/DTA thermal analyser from PerkinElmer Instruments, in dynamic air atmosphere (150 cm^3 min^{-1}), under non-isothermal linear regime at constant heating rate of 10 K min^{-1}. The mass changes determined by thermogravimetric analysis (TG curve) and its rate of change by the derivative thermogravimetric analysis (DTG curve), as well as the difference in the temperature between the sample and the reference material (DTA) and the difference in the heat flow change between the sample and the reference material (DSC) were registered simultaneously. The samples were placed in aluminium crucibles, and the thermoanalytical curves were recorded as a function of temperature, from room temperature to 600 °C. The results were processed and graphically represented using the dedicated Pyris software, from PerkinElmer Instruments. The thermal stability was evaluated from the extrapolated onset temperature of the first step of thermal decomposition from respective TG curves.

Statistical analysis

All determinations were made in triplicate, and the results were reported as mean ± standard deviation. The significance of differences was assessed using the Student's t test at a significance level of 0.05. Statistical analysis of the data was performed using Statgraphics Centurion XVI software (StatPoint Technologies, VA, USA).

Results and discussion

Carotenoid content in dried sea buckthorn by-products

The carotenoids present in the dried sea buckthorn by-products (DSB) were quantified by HPLC/DAD analysis. Figure 1 shows a typical HPLC chromatogram from one of the samples. Amounts of carotenoids determined in the samples are shown in Table 1.

Total carotenoid content of dried sea buckthorn by-products was 24.56 ± 1.55 mg 100 g^{-1} as determined by the spectrophotometric method. Total carotenoids acquired by HPLC were only 16.67 mg 100 g^{-1}, but it is generally accepted that some carotenoids with their isomers present in low concentration might not be detected as peaks and are not included in the HPLC method, while in the spectrophotometric analysis, the absorbance is highly increased by other than carotenoid compounds dissolved in lipids also active in carotenoids spectral range [34].

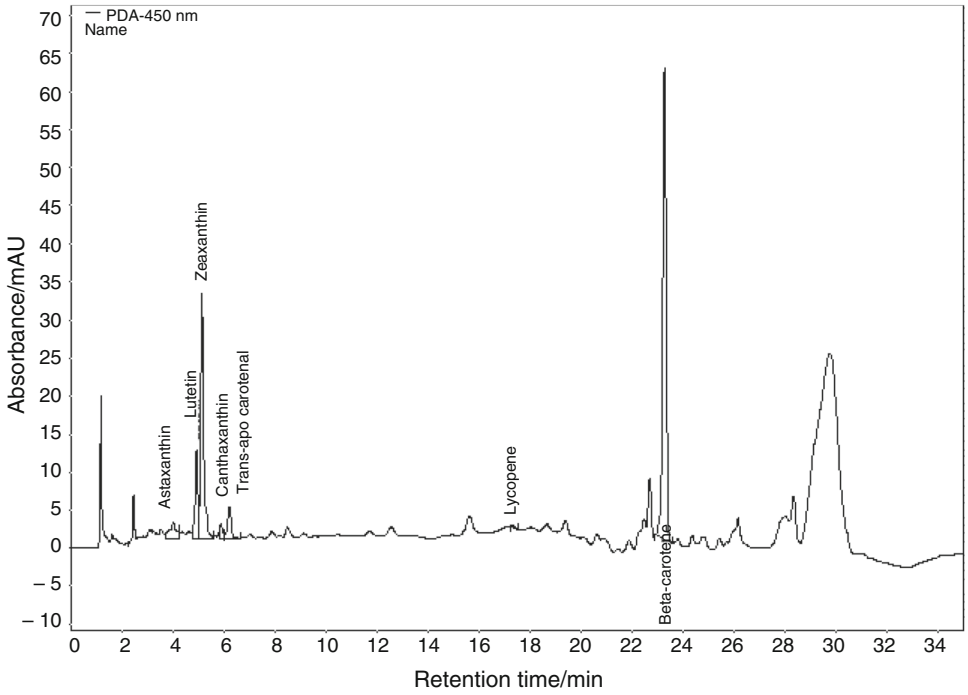

Fig. 1 HPLC chromatogram at λ = 450 nm of carotenoids in a sample of dried sea buckthorn by-products (* mAU = milli-absorbance units)

Table 1 Average carotenoid content of dried sea buckthorn by-products

Compound	Retention time/min	Content/mg 100 g^{-1}
Astaxanthin	4.017	0.478 ± 0.05
Lutein	4.912	0.696 ± 0.04
Zeaxanthin	5.135	6.960 ± 0.15
Canthaxanthin	5.883	0.136 ± 0.02
Trans-apo carotenal	6.212	0.236 ± 0.02
Lycopene	17.310	0.091 ± 0.01
β-Carotene	23.280	8.076 ± 0.35
Total carotenoids		16.673 ± 0.48

Beta-carotene and zeaxanthin were the main carotenoids found in dried sea buckthorn by-products (8.07 mg 100 g^{-1} and 6.96 mg 100 g^{-1}, respectively). These results are in good agreement with those obtained previously by Andersson et al. [35] who reported a total carotenoid content of 1.5–18.5 mg 100 g^{-1} fresh weight in four cultivars of sea buckthorn berries, while the main carotenoids were zeaxanthin, β-carotene, β-cryptoxanthin, lutein, lycopene and γ-carotene. Raffo et al. [36] found also that zeaxanthin (3–15 mg 100 g^{-1}), β-carotene (0.3–5 mg kg^{-1}) and β-cryptoxanthin (0.5–1.9 mg kg^{-1}) were the main carotenoids in various *H. rhamnoides* cultivars studied. Korekar et al. [37] reported a total carotenoid content between 0.1 and 14.4 mg 100 g^{-1} in seventeen natural population of sea buckthorn (*Hippophae rhamnoides* L.) from trans-Himalaya, while Teleszko et al. [38] found carotenoid concentrations between 6.19 and 23.91 mg 100 g^{-1} fresh weight in eight sea buckthorn (*Hippophaë rhamnoides* subsp. *mongolica*) cultivars.

Total phenolic content of dried sea buckthorn by-products was 279.18 ± 2.66 mg GAE 100 g^{-1}, while the radical scavenging activity of dried sea buckthorn by-products was 8.296 ± 0.36 mmol Trolox 100 g^{-1} as measured by the ABTS method.

Total carotenoid content in oils

Total carotenoid content of the oils is given in Table 2. Of the three oils, the extra virgin olive oil had the highest total carotenoid content (21.5 mg kg^{-1}), followed by extra virgin sunflower oil (6.3 mg kg^{-1}) and refined sunflower oil (4.8 mg kg^{-1}). After 50 min of ultrasonic-assisted extraction of 10% DSB, total carotenoid content in oils ranged between 137.83 mg 100 g^{-1} (RS) and 157.84 mg kg^{-1} (EVO). Chemat et al. [39] found a total carotenoid content of 63.84 mg L^{-1} of extract after 30 min UAE of 10% sea buckthorn by-product in 1 kg of sunflower oil.

The total carotenoid content of EVO oils was higher with more than 29% after 50 min UAE than after 10 days of maceration, while in the sunflower oils, this increase ranged between 9.4 and 33.3%. In a study on enrichment of edible oils with sea buckthorn by-products, Chemat et al. [39] found also that sonication extracted more material than maceration. This was attributed to the acceleration of the mass transfer of sea buckthorn by-products constituents in the oil as a consequence of ultrasonic cavitation [16].

Table 2 Total carotenoid content, peroxide value and ABTS antioxidant activity of the control oils and of the oils enriched in carotenoids after extraction of dried sea buckthorn (*Hippophaë rhamnoides*) by-products

	Total carotenoids/mg kg^{-1}		Peroxide value/meq kg^{-1}		ABTS antioxidant activity/mmol Trolox kg^{-1}	
	UAE	Maceration	UAE	Maceration	UAE	Maceration
EVO	21.50 ± 0.89^{A}	21.50 ± 0.89^{A}	45.50 ± 1.66^{BC}	45.50 ± 1.66^{B}	5.55 ± 0.21^{A}	5.55 ± 0.21^{A}
EVO + 2.5%DSB	$76.91 \pm 1.82^{B}_{b}$	$56.57 \pm 2.77^{B}_{a}$	$46.44 \pm 1.78^{C}_{a}$	$51.43 \pm 2.31^{C}_{b}$	$5.37 \pm 0.30^{A}_{a}$	$5.73 \pm 0.19^{A}_{a}$
EVO + 5.0%DSB	$98.84 \pm 3.09^{C}_{b}$	$73.69 \pm 2.78^{C}_{a}$	$43.65 \pm 0.98^{B}_{a}$	$42.14 \pm 1.88^{B}_{a}$	$5.27 \pm 0.15^{A}_{a}$	$6.75 \pm 0.26^{B}_{b}$
EVO + 10.0%DSB	$157.84 \pm 6.67^{D}_{b}$	$122.28 \pm 4.33^{D}_{a}$	$35.18 \pm 1.21^{A}_{a}$	$37.52 \pm 1.26^{A}_{a}$	$5.35 \pm 0.34^{A}_{a}$	$6.90 \pm 0.22^{B}_{b}$
EVS	6.30 ± 0.28^{A}	6.30 ± 0.28^{A}	153.53 ± 6.44^{A}	153.47 ± 6.44^{A}	4.47 ± 0.20^{A}	4.47 ± 0.20^{A}
EVS + 2.5%DSB	$40.77 \pm 1.94^{B}_{a}$	$45.49 \pm 1.42^{B}_{b}$	$159.11 \pm 5.81^{AB}_{a}$	$162.56 \pm 6.66^{A}_{a}$	$4.55 \pm 0.17^{A}_{a}$	$4.72 \pm 0.14^{A}_{a}$
EVS + 5.0%DSB	$74.79 \pm 3.38^{C}_{b}$	$56.11 \pm 2.06^{C}_{a}$	$166.21 \pm 6.77^{B}_{a}$	$161.62 \pm 5.09^{A}_{a}$	$4.83 \pm 0.22^{AB}_{a}$	$4.74 \pm 0.18^{A}_{a}$
EVS + 10.0%DSB	$143.93 \pm 5.56^{D}_{b}$	$121.28 \pm 5.51^{D}_{a}$	$161.58 \pm 4.89^{AB}_{a}$	$159.39 \pm 5.79^{A}_{a}$	$5.12 \pm 0.24^{B}_{a}$	$4.77 \pm 0.21^{A}_{a}$
RS	4.80 ± 0.21^{A}	4.80 ± 0.21^{A}	116.82 ± 3.66^{A}	116.82 ± 3.66^{A}	$4.16 \pm 0.09^{A}_{a}$	$4.16 \pm 0.09^{A}_{a}$
RS + 2.5%DSB	$43.41 \pm 1.65^{B}_{b}$	$38.17 \pm 1.56^{B}_{a}$	$112.86 \pm 4.08^{A}_{a}$	$141.54 \pm 5.56^{C}_{b}$	$4.21 \pm 0.16^{A}_{a}$	$4.33 \pm 0.19^{AB}_{a}$
RS + 5.0%DSB	$82.65 \pm 3.35^{C}_{b}$	$64.75 \pm 2.93^{C}_{a}$	$121.65 \pm 5.77^{A}_{a}$	$128.52 \pm 3.68^{B}_{a}$	$4.28 \pm 0.19^{A}_{a}$	$4.56 \pm 0.15^{BC}_{a}$
RS + 10.0%DSB	$137.83 \pm 6.65^{D}_{b}$	$120.34 \pm 5.13^{D}_{a}$	$141.08 \pm 6.14^{B}_{a}$	$143.45 \pm 4.87^{C}_{a}$	$4.59 \pm 0.17^{B}_{a}$	$4.63 \pm 0.14^{C}_{a}$

*Values in the same column for the same type of oil followed by different superscript upper-case letters are significantly different at $P < 0.05$

**Values in the same row for the same property followed by different subscript lower-case letters are significantly different at $P < 0.05$

Carotenoid content of the oils increased significantly by increasing incorporation of dried sea buckthorn by-products. The results revealed a linear dependence between the carotenoid content of the oils after extraction and the per cent incorporation of dried sea buckthorn by-products. Its correlation coefficient ranges from 0.966 to 0.997.

Antioxidant activity

The peroxide value and ABTS antioxidant activity of the oils are given in Table 2. The extra virgin olive oil showed the highest antiradical activity, of 5.55 mmol Trolox kg^{-1} against 4.47 mmol Trolox kg^{-1} and 4.16 mmol Trolox kg^{-1} for extra virgin sunflower oil and refined sunflower oil, respectively. The enrichment determined the increase of the ABTS antioxidant activity, but the increases were found significant mostly at 10% DSB incorporation.

Peroxide value

Oxidative stability of oils and fats is one of the most important parameters for their quality assessment. The peroxide value is commonly used to assess the stability or rancidity of fats by measuring the amount of peroxides and hydroperoxides formed in the initial stages of lipid oxidation [40].

After UV irradiation, the highest peroxide value was found in extra virgin sunflower oil, while the lowest peroxide value was found in extra virgin olive oil. Although sunflower oil is rich in tocopherols, it is almost free of phenolic compounds, while olive oil contains both tocopherols and phenolic compounds as antioxidants [2]. The high phenolic content of the olive oil may be responsible for its higher antioxidant activity and oxidative stability. The extraction of DSB in extra virgin olive oil determined a slight decrease in the peroxide value, but differences were found significant only at 10% DSB incorporation. This result is probably related to the fact that at lower addition levels, the increase of the carotenoid content was not sufficient for the detection of an important increase in oil stability as a result of the antioxidant effect of these pigments. Similar findings were reported by Gouveia et al. [41] in oils coloured by pigments extracted from microalgae.

Refined sunflower oil was more stable at UV accelerated oxidation than extra virgin sunflower oil. This could be due to the enrichment of the commercial refined sunflower oil with tocopherol. In extra virgin sunflower oil, the extraction of DSB generally determined the increase of the peroxide value, but differences were found not to be significant. However, in refined oil, enrichment of oil in carotenoids through extraction from DSB determined a significant increase of the peroxide value. These contradictory changes in the peroxide value could be assigned to the antioxidant or pro-oxidant behaviour of carotenoids that occurs under certain conditions. Carotenoids could increase the lipid peroxidation, this pro-oxidant activity of carotenoids being related to the presence of other antioxidants such as polyphenols and tocopherols [42]. As stated in previous studies, manifestation of antioxidant or pro-

oxidant behaviour is the result of various ratios among the carotenoids and the other compounds involved [4].

Thermal analysis and calorimetry

The thermal stability and degradation behaviour of the 5% sea buckthorn carotenoid-enriched edible oils (refined sunflower oil, cold-pressed sunflower oil and extra virgin olive oil) were investigated using a simultaneous nonlinear heating programme under dynamic air atmosphere. For comparative purposes, the initial edible oils and the sea buckthorn by-products powder were also thermally investigated in the same experimental conditions.

In Fig. 2, the TG, DTG and DSC curves for the thermal analysis of the pure refined sunflower oil (named "control")—obtained in the conditions described above—are presented; on the other hand, those for the sunflower oil enriched in sea buckthorn carotenoids are presented in Fig. 3. In order to understand better the influence of the carotenoids on the stability of the edible oils, the thermal analysis of the sea buckthorn powder was performed also in dynamic air atmosphere, and it is presented in Fig. 4.

Besides the thermal analysis of the refined sunflower oil, the thermoanalytical curves corresponding to the other two oils (extra virgin olive oil and cold-pressed sunflower oil) were also recorded; they undergo comparable thermal decomposition pathways; therefore, they are presented separately in the "Supplementary Materials" part—SM1a,b, while their sea buckthorn carotenoid-enriched versions are presented in SM2a,b. Also in the "Supplementary Materials" part, the DSC curves containing the characteristic thermal parameters for the refined sunflower oil, cold-pressed sunflower oil, extra virgin olive oil (SM3a–c), and for the sea buckthorn carotenoid-enriched edible oils (SM4a–c) are presented.

All these thermoanalytical curves indicate the total absence of metals (no residue when the recording is finished at 600 °C), the lack of water and of other liquid compounds in the samples and fairly good thermal stability—Fig. 2, SM1a,b, Fig. 3, SM2a,b.

Overall, the edible oils have three decomposition steps, with characteristic number of reactions, and each of them with their characteristic decomposition temperature ranges and mass loss percentages (Fig. 2 and SM1a,b). The olive oil has a higher thermo-oxidative stability of the aliphatic contained volatile groups (onset temperature 189 °C—SM3c) if compared with the other two sunflower oils (onset temperature 153 °C for the refined sunflower oil—SM3a, and onset temperature 160 °C for the cold-pressed sunflower oil—SM3b), but its overall thermal stability in air atmosphere is not as good (first step major peak temperature 314 °C—SM3c) as for the two sunflower oils (first step major peak temperature 325 °C for the refined sunflower oil—SM3a and first step major peak temperature 344 °C for the cold-pressed sunflower oil—SM3b). Also, the olive oil (SM1b) has a very small amount of lost mass in the second decomposition step, if compared to the refined and cold-pressed sunflower oils (Fig. 2 and SM1a).

In general, the oils enriched in sea buckthorn carotenoids have a similar thermal behaviour to the initial oils, with the same three decomposition steps and following comparable transformations (Fig. 3 and SM2a,b); this is

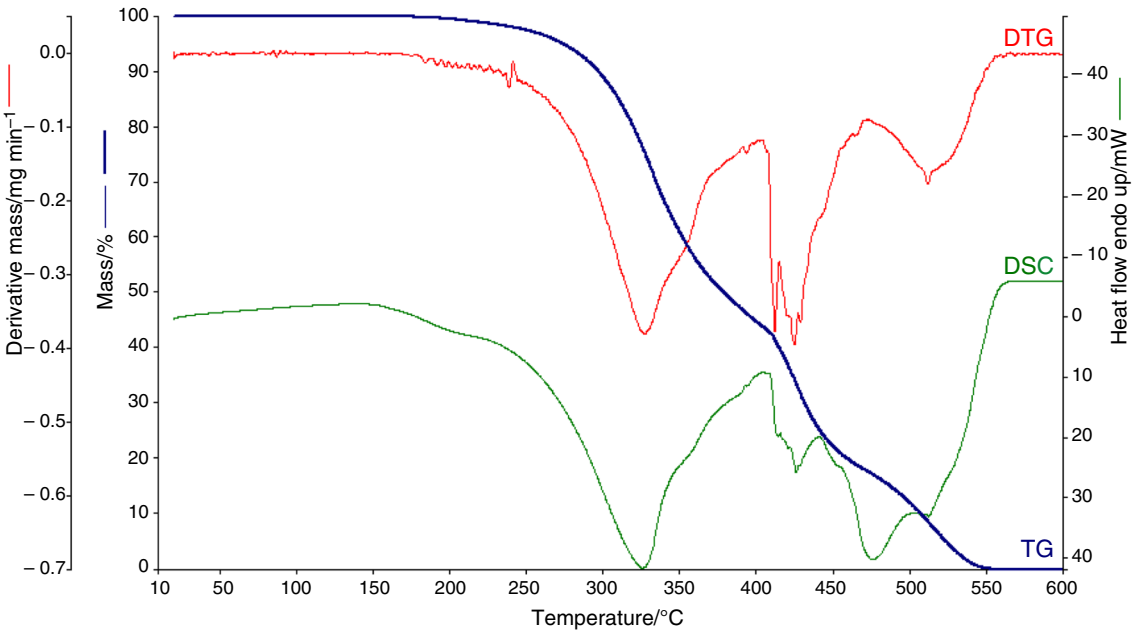

Fig. 2 The thermoanalytical curves of pure refined sunflower oil (control) in dynamic air atmosphere at 10 K min^{-1}

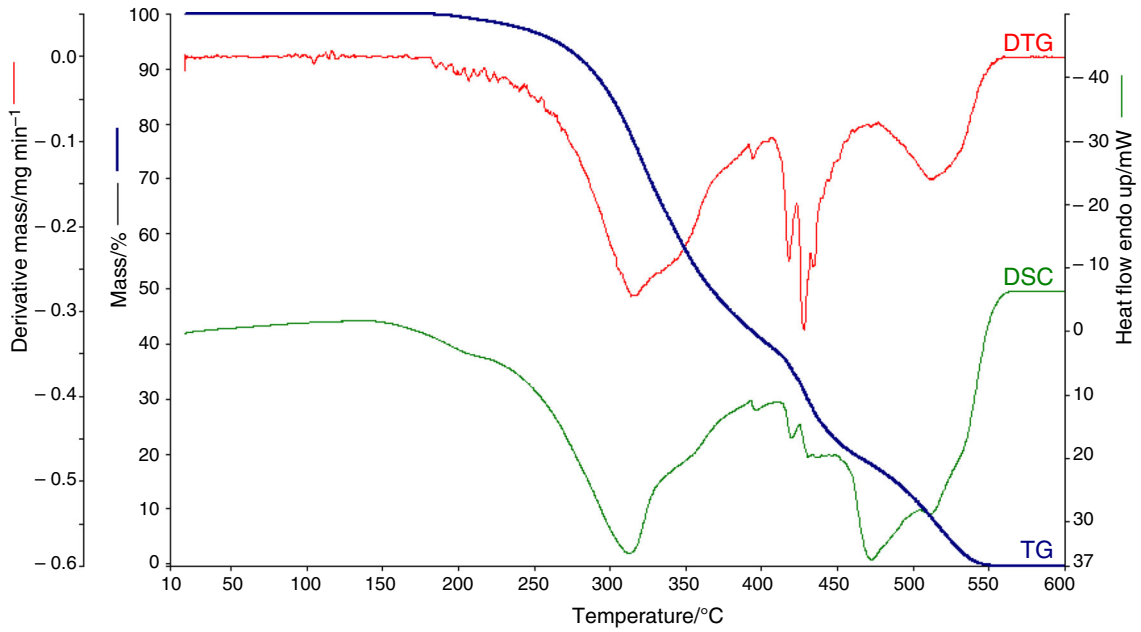

Fig. 3 The thermoanalytical curves of sea buckthorn carotenoid-enriched refined sunflower oil in dynamic air atmosphere at 10 K min^{-1}

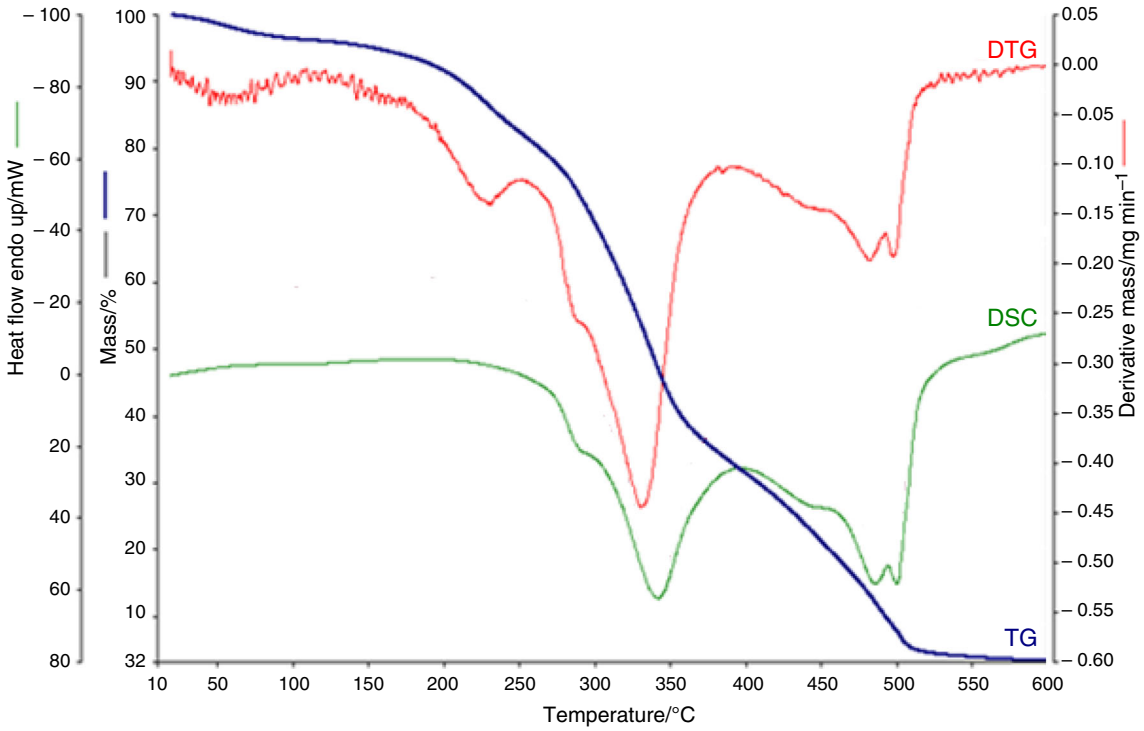

Fig. 4 The thermoanalytical curves of sea buckthorn powder in dynamic air atmosphere at 10 K min^{-1}

obvious especially for the thermal decomposition of the mass monitored by the TG curves, where for the first step the mass loss pathway is very close and only with a few degrees displaced at lower temperatures (Fig. 5a–c). After losing 5% water, the thermal decomposition of the sea buckthorn powder (Fig. 4) resembles by chance to a high extent to the decomposition of the raw oils (Fig. 2) and takes place in two major steps (Fig. 4) that are very close to their first and third steps (Fig. 2); thus, the thermal behaviour of the carotenoid-enriched oils (Fig. 3) remains similar to the behaviour of the initial oils, encompassing the percentages of carotenoids from the sea buckthorn that

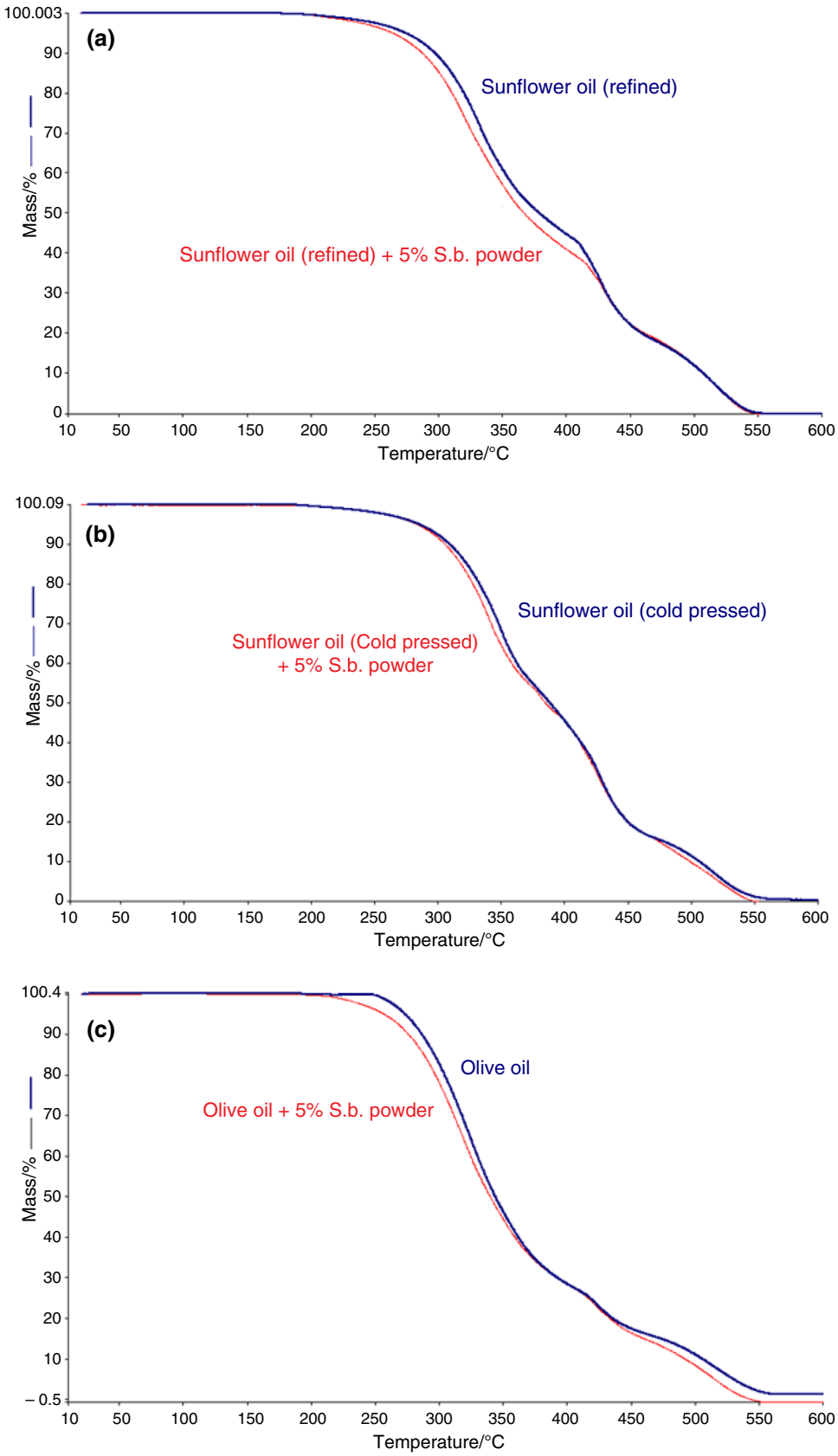

Fig. 5 The comparative thermogravimetric (TG) curves of the pure edible oils versus the sea buckthorn carotenoid-enriched edible oils in dynamic air atmosphere at 10 K min^{-1}; (**a** refined sunflower oil, **b** cold-pressed sunflower oil, **c** extra virgin olive oil)

do not affect too much the pathway, but only the lost quantities and the thermal parameters (Fig. 3).

The first thermal decomposition step of all oils starts with an exothermic loss of the volatile compounds with low molecular weights, for the refined sunflower oil this volatilisation process beginning at 182 °C—the TG and DTG curves in Fig. 2, while for the oil enriched in sea buckthorn carotenoids at 175 °C—the TG and DTG curves in Fig. 3. The first thermal decomposition step continues with a second exothermic reaction, which is the main part of this step in both mass loss and thermal effect (Fig. 2 vs. Fig. 3). While the lost mass is 57% in the case of the first thermal decomposition step of the refined sunflower oil (Figs. 2, 5a), by enriching with carotenoids results in a mass loss of 62% for the first step (Figs. 3, 5b), this difference of 5% is actually the exact amount of sea buckthorn that was added to the initial oil. The second and third steps of thermal decomposition of the refined sunflower oil + carotenoids are not at all affected by the extraction of carotenoids from the sea buckthorn, mainly because most of the carotenoids were already thermally decomposed and lost during the first step, while the quantity remained after the first step is indeed very low. An overall similar thermal behaviour is observed for the other two systems (oil + carotenoids vs. oil)—Fig. 5b, c, the thermogravimetric curves being only slightly disturbed by the presence of the carotenoids and only due to their just-earlier thermal decomposition. Thus, it is very likely that the carotenoids do not interact greatly with the oils, therefore not affecting too much the thermodynamic stability of the systems with their presence.

However, the calorimetric study may provide more insights regarding the interaction between the carotenoids and the oils; thus, in the DSC curves (e.g. Fig. 6), the first step major peak temperature is at 325 °C for the refined sunflower oil—SM3a, while for the refined sunflower oil enriched in sea buckthorn carotenoids, it is at 312 °C—SM4a. It results that by adding carotenoids from sea buckthorn to the refined sunflower oil, the thermokinetic instability of the system increases (Fig. 6). A similar behaviour is encountered for the other two edible oils (cold-pressed sunflower oil and olive oil) versus their carotenoid sea buckthorn-enriched versions—SM3b,c versus SM4b,c.

The extraction of carotenoid compounds in the oil from sea buckthorn by-products results in a very small decrease of about 4–5 °C in the oxidation onset temperature and of about 11–12 °C in the temperature of the DSC peak (Table 3), if compared with the higher effect that the carotenoids from dry tomato by-products have [14]. These results are well correlated with those obtained previously [14] for the extraction of carotenoids from dry tomato by-products, exerting a much lower pro-oxidant effect and thus not causing a significant decrease in the thermal oxidative stability of the sunflower oils and olive oil.

Colour

Colour is an important factor for appeal and a determinant of consumer acceptability, image, market size and value [41]. The sea buckthorn pulp and peel are very rich in carotenoids, which give the berry its orange colour. Extraction of carotenoids from DSB significantly ($P < 0.05$) modified the colour of the oils. Colour parameters L^* (lightness), a^* (redness) and b^* (yellowness) of oil samples are presented in Table 4.

In extra virgin olive oil, the extraction of DSB determined the increase of lightness (L^*), while in sunflower oils, the L values decreased; therefore, the extraction resulted in the darkening of these oils. The a^* and b^*

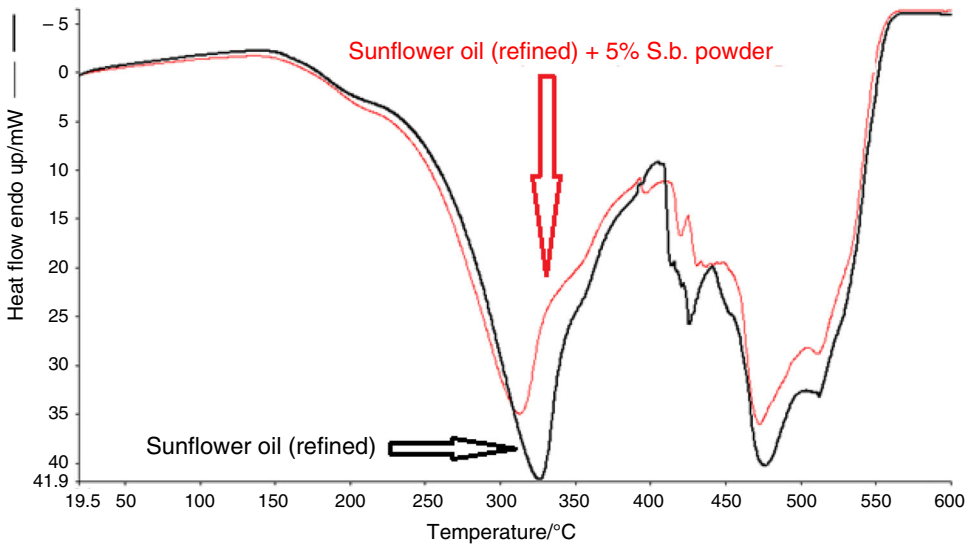

Fig. 6 The differential scanning calorimetry (DSC) curves of the refined sunflower oil versus the sea buckthorn carotenoid-enriched refined sunflower oil in dynamic air atmosphere at 10 K min^{-1}

Table 3 Thermal parameters of the oxidation DSC onset and DSC peak

Oil type	Initial oils		5% Carotenoid-enriched (DSB) oils	
	DSC oxidation onset temperature/°C	DSC oxidation peak temperature/°C	DSC oxidation onset temperature/°C	DSC oxidation peak temperature/°C
RS	153	325	148	312
EVS	176	344	172	334
EVO	189	314	177	303

RS refined sunflower oil, *EVS* extra virgin (cold-pressed) sunflower oil, *EVO* extra virgin olive oil

Table 4 CIELab parameters of the control oils and of the oils enriched in carotenoids after extraction of dried sea buckthorn (*Hippophaë rhamnoides*) by-products

	L^*		A^*		B^*	
	UAE	Maceration	UAE	Maceration	UAE	Maceration
EVO	78.76 ± 0.62^{A}	78.76 ± 0.62^{A}	-2.10 ± 0.12^{A}	-2.10 ± 0.12^{A}	38.29 ± 5.26^{A}	38.29 ± 5.26^{A}
EVO + 2.5%DSB	$83.19 \pm 1.48^{B}_{a}$	$83.13 \pm 1.29^{B}_{a}$	$-0.76 \pm 0.39^{B}_{a}$	$-0.58 \pm 0.41^{A}_{a}$	$47.42 \pm 3.68^{B}_{a}$	$49.95 \pm 1.79^{B}_{a}$
EVO + 5.0%DSB	$84.02 \pm 1.04^{B}_{b}$	$80.36 \pm 1.17^{AB}_{a}$	$0.10 \pm 0.47^{B}_{a}$	$1.67 \pm 0.46^{B}_{b}$	$58.14 \pm 3.03^{C}_{a}$	$61.39 \pm 3.93^{C}_{a}$
EVO + 10.0%DSB	$80.92 \pm 1.34^{A}_{a}$	$79.61 \pm 2.43^{A}_{a}$	$4.10 \pm 1.27^{C}_{a}$	$4.52 \pm 1.78^{C}_{a}$	$70.08 \pm 2.23^{D}_{a}$	$66.87 \pm 5.03^{C}_{a}$
EVS	83.21 ± 0.61^{C}	83.21 ± 0.61^{B}	-0.77 ± 0.20^{A}	-0.77 ± 0.20^{A}	18.55 ± 2.18^{A}	18.55 ± 2.18^{A}
EVS + 2.5%DSB	$78.48 \pm 0.80^{AB}_{a}$	$79.75 \pm 1.05^{A}_{a}$	$1.02 \pm 0.23^{AB}_{a}$	$0.43 \pm 0.59^{A}_{a}$	$49.38 \pm 4.20^{B}_{a}$	$48.85 \pm 1.86^{B}_{a}$
EVS + 5.0%DSB	$80.08 \pm 1.57^{B}_{a}$	$78.90 \pm 0.83^{A}_{a}$	$2.64 \pm 1.16^{B}_{a}$	$2.78 \pm 0.58^{B}_{a}$	$58.38 \pm 5.91^{BC}_{a}$	$61.29 \pm 0.67^{C}_{a}$
EVS + 10.0%DSB	$76.42 \pm 1.25^{A}_{a}$	$77.99 \pm 1.64^{A}_{a}$	$7.13 \pm 2.33^{C}_{a}$	$6.51 \pm 2.23^{C}_{a}$	$65.25 \pm 9.85^{C}_{a}$	$71.07 \pm 6.42^{D}_{a}$
RS	83.52 ± 1.52^{B}	83.52 ± 1.52^{AB}	-0.12 ± 0.15^{A}	-0.12 ± 0.15^{A}	9.21 ± 0.57^{A}	9.21 ± 0.57^{A}
RS + 2.5%DSB	$82.67 \pm 0.51^{B}_{a}$	$83.82 \pm 1.35^{B}_{a}$	$-0.29 \pm 0.17^{A}_{a}$	$-0.36 \pm 0.28^{A}_{a}$	$46.90 \pm 1.05^{B}_{a}$	$42.75 \pm 4.61^{B}_{a}$
RS + 5.0%DSB	$80.40 \pm 0.80^{A}_{a}$	$81.71 \pm 2.12^{AB}_{a}$	$2.63 \pm 0.67^{B}_{a}$	$1.81 \pm 1.38^{B}_{a}$	$61.94 \pm 4.83^{C}_{a}$	$55.39 \pm 8.68^{C}_{a}$
RS + 10.0%DSB	$79.73 \pm 0.70^{A}_{a}$	$80.75 \pm 0.79^{A}_{a}$	$5.62 \pm 0.59^{C}_{a}$	$5.25 \pm 0.64^{C}_{a}$	$69.51 \pm 0.87^{D}_{a}$	$69.11 \pm 3.66^{D}_{a}$

*Values in the same column for the same type of oil followed by different superscript upper-case letters are significantly different at $P < 0.05$

**Values in the same row for the same parameter followed by different subscript lower-case letters are significantly different at $P < 0.05$

values increased significantly with increase in levels of added DSB, indicating increase in redness and yellowness appearance, respectively. These changes could be attributed to the extraction of carotenoids, predominantly β-carotene and zeaxanthin, in the oils. Besides the nutritional aspects related to the increase of the oil functionality, the enrichment with carotenoids of the oils leads to the improvement of the chromatic characteristics of the oils which may increase consumer attractiveness and confidence.

Conclusions

Based on the results of this study, sea buckthorn by-products are a good source of carotenoids, mainly zeaxanthin and β-carotene. Extraction of these by-products in edible vegetable oils enhanced significantly the carotenoid content and contributed to the increase of radical scavenging activity of the oils. Ultrasonic-assisted extraction led to a significantly higher recovery of carotenoids from dried sea buckthorn by-products than maceration. The thermodynamic stability of the sunflower oils and olive oil is not greatly affected by the enrichment with carotenoids from sea buckthorn by-products, while only the thermokinetic stability may diminish; therefore, the thermal oxidative stability of the carotenoids-edible oils systems is fairly good, presumably the shelf life being the same as in the case of the initial edible oils, with only a high increase in temperature that may eventually lower it. One may conclude that the carotenoids from sea buckthorn by-products may be safely used for their colouring effect and for the appeal to enhance the acceptability and valorisation of the edible oils in order to be further sold.

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