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ORIGINAL ARTICLE

Effect of lipophilic sea buckthorn extract on cream cheese properties

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Abstract The aim of this study was to evaluate the physico-chemical, microbiological, sensory properties and antioxidant activity of the functional cream cheese prepared with lipophilic extracts of sea buckthorn (*Hippophae rhamnoides* L.). The first step of the research consisted of an evaluation of the physico-chemical characteristics and the antioxidant capacity of the sea buckthorn lipophilic extracts. The sea buckthorn extracts had a significant antioxidant capacity (67.04 ± 2.67%), a content of total carotenoids of 8.27 ± 0.01 mg L^{-1} and a content of total polyphenols of 1842.86 ± 1.41 mg/100 g dry vegetal material. The addition of the sea buckthorn extracts did not negatively affect the fresh cream cheese's sensory characteristics. The addition of sea buckthorn extracts to the cream cheese resulted in an increase of antiradical activity and dry matter content, a decrease in acidity and higher growth inhibitition of germs.

Keywords Sea buckthorn · Lipophilic extract · Oxidative stability · Cream cheese

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Introduction

Nowadays, the local manufacturers of food products tend to replace synthetic additives with natural ones. A safe and effective possibility is to use biologically active compounds extracted from local natural resources. There is an increased interest in berries because they are characterized by a large area of cultivation and they are rich in nutritionally important antioxidants, vitamins and minerals (Roman et al. 2013).

The importance of the sea buckthorn as medicinal plant is determined by the content of biologically active compounds in fruits, leaves and even in the peel. The sea buckthorn is a natural concentrate of vitamins (C, P, B1, B2, E, K), carotenoids, folic acid, isoramnetol, unsaturated fatty acids and phytosterols, nicotinic acid, volatile oils, etc. (Wani et al. 2016; Cho et al. 2017; Kuhkheil et al. 2017). The sea buckthorn fruits have an orange color due to their content in carotenoids, which is much higher than in carrots or squashes (Creţu and Domaşenco 2005). The association of sea buckthorn fruits consumption and the prevention of cardiovascular diseases and cancer is justified by the rich content in antioxidants (carotenoids, vitamin C) and phytonutrients (Halliwell 1997; Lõugas 2006; Puupponen-Pimiä et al. 2002).

Biologically active compounds obtained from natural resources of plant origin that are stable and safe for consumption can be used as replacement for synthetic food additives. Thus, natural extracts of plant origin are good alternatives for synthetic food additives, with the added benefit of enriching the nutritional value of the food (Caleja et al. 2017; Pasqualone et al. 2015; Rasooli 2007). Some pastries, meat and dairy products with added natural plant extracts, spices or fruit powders were obtained and reported

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so far (Caleja et al. 2015a, b; Reddy et al. 2005; Shah et al. 2014).

Several techniques for the extraction of bioactive compounds from plants were reported, such as solvent accelerated extraction (He et al. 2018; Gomes et al. 2017), ultrasound-assisted extraction (Bimakr et al. 2017; Rivera-Mondragón et al. 2019), microwave-assisted extraction (Mahendran et al. 2018; Bonomini et al. 2018; Bakić et al. 2019) and supercritical fluid extraction (Escobedo-Flores et al. 2018; Ashfaq et al. 2019). For the quantitative analysis of bioactive compounds, UV–Vis molecular absorption spectrophotometry (Aryal et al. 2019; Grulichová et al. 2018; Amri and Hossain 2018; Voeora et al. 2019) is typically used to determine total content and chromatographic methods are used for determining the individual content (Rekik et al. 2018; Petry and Mercadante 2018; Nasrollahi et al. 2019).

Studies on the obtaining of lipophilic extracts from sea buckthorn fruits are increasing in number, because sea buckthorn represents a potential ingredient for the preparation of functional food products, rich in biologically active compounds. The sea buckthorn, shows an increased interest due to its organoleptic and physico-chemical characteristics.

Dairy products underlie a healthy diet for all age groups (Huth and Park 2012; Rozenberg et al. 2016). Cream cheese is a favorite dairy product of children, for whom the abuse of food additives of synthetic origin can lead to hyperactivity and nutritional allergies (Inetianbor et al. 2015; Almeida et al. 2014).

The aim of this study was to develop a functional fresh cream cheese by addition of lipophilic sea buckthorn extracts. The research was divided into two steps: firstly, optimization of the extraction process of the liposoluble compounds from sea buckthorn in order to obtain stable and high-quality lipophilic extracts and secondly, the preparation, characterization and determination of the stability of the obtained functional fresh cream cheese.

Materials and methods

Extraction of the assimilating pigments and polyphenols

The "Elizaveta" variety fruits were harvested in the AGROSARGAL agricultural production cooperative, Sărata-Galbenă village from the Hânceşti district situated in Center area of the Republic of Moldova. The Sărata-Galbenă village is located at 46°44′02″ latitude, 28°31′15″ longitude and 86 meters altitude above sea level. The climate was temperate-continental, differing in its unstable character. The average annual air temperature was 9 °C. The absolute minimum temperature was recorded in January (− 29 ± 1 °C), and the maximum temperature in July (38 ± 1 °C). The average annual amount of precipitation was 525 ± 25 mm/year. The field of cultivation of sea buckthorn berries was located in the steppe area of southern Moldova, with the predominance of typical low humiferous and carbonate cernozomes.

For this research, cylindrical fruits, light-orange colored, with a sweet sour taste, harvested in the second decade of October 2016 were used. The mass of 100 fresh fruits was 64 ± 15 g, having 17.2 ± 0.14% dry weight, 9.32 ± 0.20°Brix soluble solids in fruit pulps and total titratable acidity of fruits 1.9 ± 0.1 g citric acid/100 g fruit. The fruits were air-dried until their humidity reached 7.8 ± 0.2%, then grounded together with pips and sieved. The powder thus obtained had a granularity of 85 ± 15 µm.

Reagents Folin-Ciocâlteu, 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) and *p*-anisidine were purchased from Merck (Germany). Gallic acid (GA) and hexane were bought from Sigma-Aldrich (Germany).

The extractions were carried out in deodorized refined sunflower oil (1 g of dry vegetal material extracted with 12 mL of commercial sunflower oil). The extractions were performed using stirring and sonication extraction techniques. Two different temperature (20 °C and 45 °C) and three different extraction times (0.5 h, 1.0 h, and 1.5 h) were tested. The obtained extracts were centrifuged at 7000 rpm for 10 min and after that were decanted. The resulting oily extracts were kept in dark glass bottles at 4 °C prior to use and analysis.

Characterization of the obtained extracts

Determination of the assimilating pigments

For the determination of the assimilating pigments (chlorophyll *a*, *b* and total carotenoids), the absorbance of the obtained extracts were measured using a T80 + Spectrometer (PG Instruments, U.K.) at 663 nm (for chlorophyll *a*), 647 nm (for chlorophyll *b*) and 470 nm (for total carotenoids) wavelengths versus the deodorized refined sunflower oil used as blank. The content of carotenoids was determined by the following equations (Sarolic et al. 2014; Tesfaye et al. 2017):

$$C_a\left(\text{mg L}^{-1}\right) = (12,25 \times A_{663.2}) - (2,79 \times A_{646.8})$$

$$C_b\left(\text{mg L}^{-1}\right) = (21,5 \times A_{646.8}) - (5,1 \times A_{663.2})$$

$$C_{a+b}\left(\text{mg L}^{-1}\right) = \frac{(1000 \times A_{470} - 1.82 \times C_a - 85.02 \times C_b)}{198}$$

where $A_{663.2}$, the absorbance of the extract at 663.2 nm; $A_{646.8}$, the absorbance of the extract at 646.8 nm; A_{470}, the absorbance of the extract at 470 nm.

Determination of the total content of polyphenols

Determination of the total content of polyphenols was performed according to Sturza (2006) on the same spectrophotometer, at 765 nm wavelength, using a 10 mm quartz cuvette. The results of the total content of polyphenols, expressed in mg GA/100 g of dry weight (DW) vegetal material, were obtained using the GA calibration curve ($y = 1.4x + 0.0037$, $R^2 = 0.999$).

Determination of the antioxidant activity using free radical DPPH

Determination of the antioxidant activity of the lipophilic extracts was performed using a DR5000 UV–Vis spectrophotometer (Hach Lange, Germany) and expressed as % inhibition of DPPH using the following equation (Sarolic et al. 2014):

$$AA\% = \frac{A_0 - A_t}{A_0} \times 100\%$$

where A_0, the absorbance of the DPPH solution at the initial time of 0 s; A_t, the absorbance of the DPPH solution after 30 min.

Determination of the peroxide value (PV)

The determination of the peroxide value was performed by the volumetric method and the results obtained were calculated according to the following equation (Method Cd 8-53 2003):

$$PV = \frac{(S - B) \times N \times 1000}{\text{mass of sample, g}},\ \text{mEq } O_2/\text{kg}$$

where S, the volume of titrant, mL of sample; B, the volume of titrant, mL of blank; N, the normality of sodium thiosulfate solution.

Determination of the acid value (AV)

Determination of AV was performed by the volumetric method and the results obtained were calculated according to the following equation (Method Cd 3d-63 1999):

$$AV = \frac{V_{KOH} \cdot N_{KOH} \cdot 5.611}{m},\ \text{mg KOH/g}$$

where V_{KOH}, the volume of the potassium hydroxide solution, mL; N_{KOH}, the concentration of the potassium hydroxide solution, mol/dm^3; m, the mass of the sample, g.

Determination of the p-anisidine value (AnV)

The AnV is an indicator of the degree of lipid oxidation, especially of the stable secondary products formed by lipid oxidation in foods. The measurements were performed using the DR5000 UV–Vis spectrophotometer (Hach Lange, Germany) and isooctane as blank. The AnV was determined according to the equation (Method Cd 18-90 1997):

$$p - A.V. = \frac{25 \times (1,2As - Ab)}{m},$$

where p – A.V., represents the AnV; A_s, the absorbance of the sample; A_b, the absorbance of the blank; m, the mass of the sample, g.

Determination of conjugated dienes and trienes

In order to determine the amount of conjugated dienes (CD) and trienes (CT) a mass of 0.01 g lipophilic extract was weighed into a 25 mL volumetric flask and the was dissolved in hexane, mixed thoroughly, and brought to volume. The absorbance of the samples were measured using a 10 × 10 mm quartz cuvette at 236 nm and 273 nm wavelengths. CD and CT values were calculated using the following equations (Method Ti 1a-64 1993):

$$C_{CD/CT} = A_{236/273}/(\varepsilon \times 1)$$
$$CD/CT_{val} = \left[C_{CD/CT} \times \left(2.5 \times 10^4\right)\right]/W$$

where $C_{CD/CT}$, CD concentration in mmol/mL; $A_{236/273}$, the absorbance of the lipid solution at 236 and 273 nm; ε, molar absorptivity ($2.525 \times 104\ M^{-1}\ cm^{-1}$); 1, path length of the cuvette in cm (1 cm); 2.5×10^4, conversion factor, μmol; W, weight of the sample, g.

The results were expressed in μmol of CD and CT per gram of sample.

Preparation of cream cheese

Cream cheese samples were prepared under laboratory conditions following the classic recipe: low-fat farmer's cheese, 35% fat cream, sugar, and GRINSTED EggXit 502 stabilizer. Also, tartrazine (E102) (0.01% of the cream cheese's mass) or a lipophilic extract of sea buckthorn (2.2% of the cream cheese's mass) was added as colorants into the cream cheese. The sea buckthorn extracts were added to the cooled to 20 ± 2 °C cream cheese in order to prevent oxidation of the bioactive compounds within the extracts. The uniform incorporation of the extracts into the cream cheese was performed by homogenizing the samples for 5–10 min. The cream cheese samples were packaged in 200 g glass cups and sealed with aluminium foils. The resulting cream cheese samples were stored at 4 ± 2 °C.

The characterization of cream cheese

Physico-chemical characterization

The physico-chemical characteristics (the mass fraction of the dry matter content, fat content, pH, titratable acidity, and dynamic viscosity) of the cream cheese samples were determined according to the Official Methods of Analysis of AOAC International (2016).

Sensory analysis

The sensory analyses of the obtained cream cheese samples were performed according to SR ISO 6658:2017.

Determination of the antioxidant activity of the cream cheese

The antioxidant activity of the cream cheese was measured according to Brand-Williams et al. (1995). The antiradical activity of the products was determined in vitro in order to simulate gastric digestion in the presence of pepsin (150 mg/100 g of product), at pH = 2.0 ± 0.1 (1.5 M HCl), a temperature of 37.0 ± 0.1 °C, under stirring of 60 min^{-1} for 2 h (Miller et al. 1981). The samples were centrifuged 10 min at 6000 rpm, after which were filtered and tested as well (Brand-Williams et al. 1995). All assays were performed in triplicate at room temperature of 20 ± 1 °C. Based on the Monsen's model which showed that in vitro scientific results can be correlated with in vivo results to a degree which varies between 60 and 70% (Monsen 1988), we assume a similar correlation degree to be valid also for our in vitro results.

Microbiological analysis of the cream cheese

The microbiological analyses were performed in accordance with the Microbiological Criteria Rules for Foodstuff (G. D. of R. M. No. 221 2009). The total number of germs microorganisms was estimated.

Statistical analysis

For both the lipophilic sea buckthorn extracts and for the cream cheese obtained, the mean values and the standard deviations were calculated from 3 parallel experiments. One-way ANOVA and post hoc Tukey test were used for statistical comparison. The considered significance level was $p \leq 0.05$.

Results and discussions

Sea buckthorn extracts

Assimilating pigments content

The content of the assimilating pigments (chlorophylls and total carotenoids) extracted from sea buckthorn in sunflower oil is shown in Fig. 1. The quantities of chlorophyll *a* and *b* extracted from the sea buckthorn by stirring are statistically smaller than those obtained by sonication. Also, the highest amount of total carotenoids extracted from sea buckthorn was obtained by sonication. Using sonication at 20 °C temperature, extraction of buckthorn with sunflower oil yielded 2.79 ± 0.04 mg/l chlorophyll *a*, 4.73 mg/l chlorophyll *b*, at 1.5 h extraction time (Fig. 1c), and 8.27 ± 0.07 mg/l total carotenoids, at 1 h extraction time (Fig. 1b).

Data with the highest content of chlorophyll *a*, *b*, and total carotenoids obtained by stirring and sonication are shown in Fig. 2.

The carotenoids are compounds which have a special role in slowing the photo-oxidation process and can provide oxidative stability to food products. The variation of the content of carotenoids extracted in sunflower oil is largely influenced by the methods used and the extraction conditions. The content of carotenoids may vary between 1 and 20 mg/l but usually does not exceed 10 mg/l (Tesfaye et al. 2017). The reported content of carotenoids in sea buckthorn varied between 10.0 and 15.6 mg/100 g of sea buckthorn in Lithuania (Viskelis et al. 2012), between 5 and 8 mg/l in the Dagestan Republic (Teleszko and Wojdylo 2015), and between 0.62 mg/l and 2.39 mg/l in Poland (Kamalovna 2013). The results obtained in the present study prove that the extraction method used provides a high extraction degree in the case of lipophilic compounds. It was established that the highest carotenoids content (8.27 ± 0.01 mg/l) was extracted by sonication technique, at the temperature of 20 °C and 1.0 h extraction time.

The total content of polyphenols

The analysis of the total content of polyphenols from sea buckthorn extracts showed different values for stirring and sonication techniques at temperatures of 20 °C and 45 °C. The results also varied depending on the extraction times (0.5 h, 1.0 h, and 1.5 h), which is explained by the impact of the extraction technique on the content of bioactive organic compounds extracted, (Fig. 3). It was established that the highest content of total polyphenols (1842.86 ± 0.01 mg/100 g DW plant) was extracted by

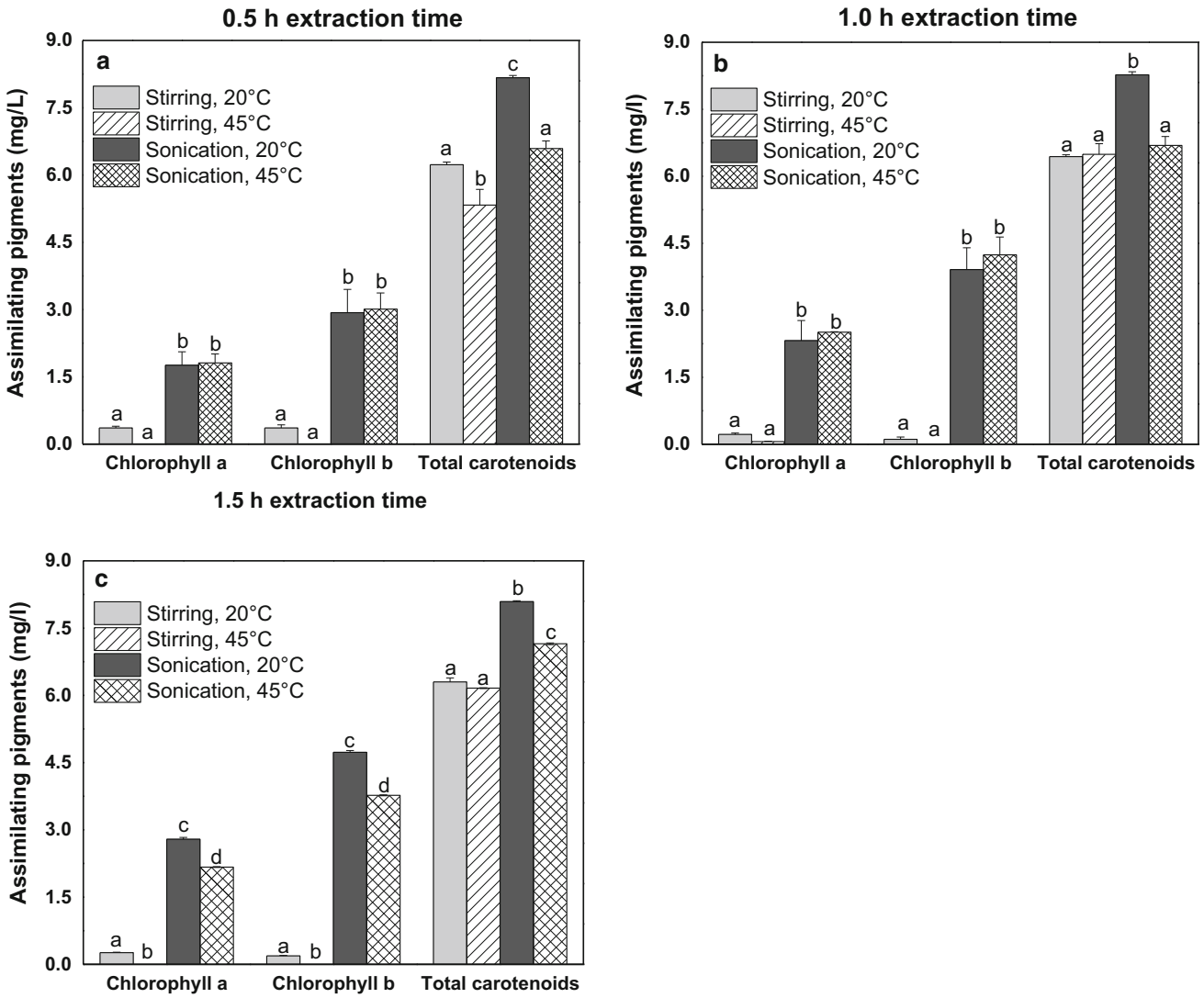

Fig. 1 Assimilating pigments content (chlorophylls and total carotenoids, mg/l) obtained from sea buckthorn (*Hippophae rhamnoides* L.) in sunflower oil, by stirring and sonication, at 0.5 h (**a**) 1.0 h (**b**) and 1.5 h (**c**) extraction times. The letters above the columns "a, b, c, d" are for statistical differences ($p \leq 0.05$) between sonication and reflux techniques tested for different temperatures

sonication technique with an extraction time of 1.5 h and a temperature of 20 °C. According to Vasilyeva, Guseva, & Batueva (2016), the total content of polyphenols varies between 250 and 330 mg/100 g DW of sea buckthorn.

The antioxidant capacity

The analysis of sea buckthorn extracts with DPPH free radicals allows the evaluation of the antioxidant capacity of the biologically active compounds. The sea buckthorn extracts have a distinct antioxidant capacity compared to sunflower oil samples, the antioxidant capacity of the sea buckthorn extracts was about 67.04 ± 2.67% (Fig. 4). The evaluation of the antioxidant activity after 3 months showed an essential reduction in antioxidant capacity for both sea buckthorn extracts and sunflower oil samples. However, even in this case the sea buckthorn extracts were characterized by a considerably higher antioxidant capacity than that of the sunflower oil samples (32.13 ± 4.02% for the sea buckthorn extracts compared to 20.26 ± 0.31% for the sunflower oil). Compared to the sunflower oil samples (Fig. 4), the sea buckthorn extract has superior antioxidant qualities and thus has an increased potential for the food industry and for consumption.

The increased antioxidant activity of the sea buckthorn extracts is due to its chemical composition, rich in carotenoids, vitamin C and phenolic compounds that have the ability to capture the radicals.

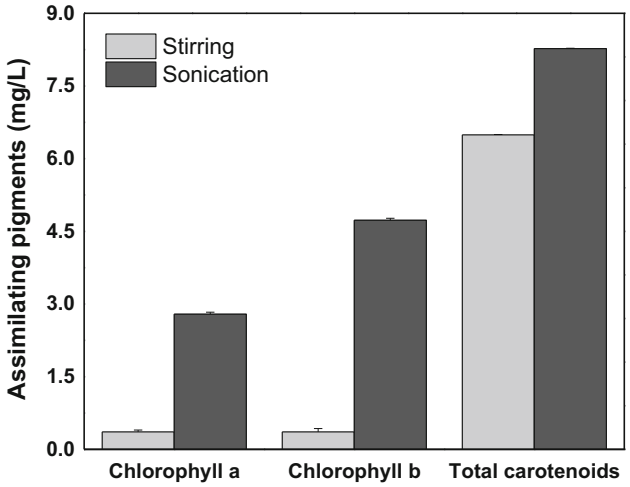

Fig. 2 The highest content of the assimilating pigments (chlorophylls and carotenoids, mg/l) obtained from sea buckthorn (*Hippophae rhamnoides* L.) in sunflower oil, by stirring and sonication

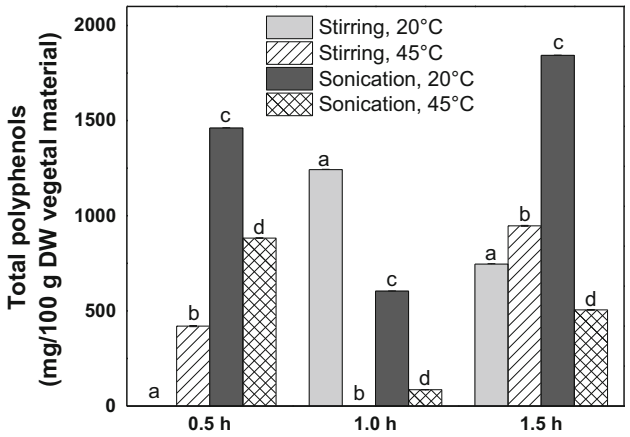

Fig. 3 The total content of polyphenols (mg/100 g DW vegetal material) obtained from sea buckthorn (*Hippophae rhamnoides* L.) in sunflower oil, by stirring and sonication, at 0.5 h, 1.0 h, and 1.5 h extraction times. The letters above the columns stand for statistical differences as in Fig. 1

Quality indices and stability of the obtained extracts

The basic quality indices (AV, PV, *p*-anisidine, K_{236}, K_{273}, $K_{236/273}$) were determined for the sea buckthorn extracts obtained by sonication technique, at the temperature of 20 °C and 1.0 h extraction time. The quality parameters were determined according to standardized analysis methods (Method Ti 1a-64 1993) and are presented in Table 1.

According to normative documents, the AV value should not exceed 0.6 mg KOH/g (Viskelis et al. 2012). The AV in the investigated samples ranged from 0.080 ± 0.018 mL KOH/g to 0.19 ± 0.02 mL KOH/g. The sea buckthorn extracts showed the highest value of AV, which is explained by the increased free fatty acid content. The PV index expresses the degree of lipid

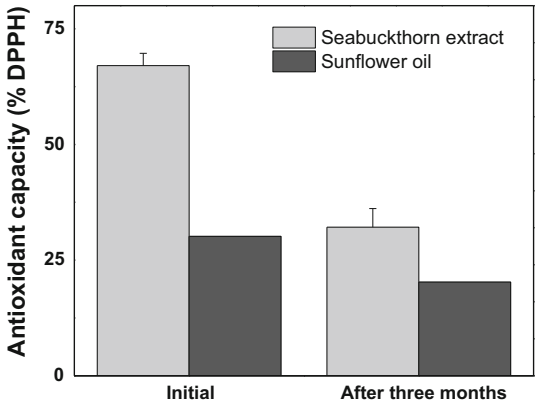

Fig. 4 The variation in time of the antioxidant capacity (% of inhibited DPPH) of the sea buckthorn (*Hippophae rhamnoides* L.) lipophilic extract in comparison with the sunflower oil

oxidation of the extract and should not exceed 10 mEq O_2/kg (G. D. of R. M. no. 434 2010). In or study, PV values of the sea buckthorn extracts ranged from 1.31 ± 0.04 to 2.23 ± 0.07 mEq O_2/kg. In order to determine the content of secondary products of lipid oxidation (aldehydes and ketones), the *p*-anisidine value was determined. The results obtained ranged from 8.06 ± 0.32 to 9.44 ± 0.27. The small value confirms the antioxidant effect of the biologically active compounds contained in the sea buckthorn.

The K_{236} index indicates the content of CD and secondary products of the oxidation that absorb at the wavelength of 236 nm. For the investigated sample the recorded values varied from 0.16 ± 0.05 to 0.27 ± 0.05. The K_{273} index indicates the content of CT and products of the oxidation that absorb at the wavelength of 273 nm. In this case, values ranging from 0.08 ± 0.01 to 0.12 ± 0.07 were obtained.

The K_{236} and K_{273} indices for foods with high lipid content should not exceed 2.50 and 0.25 respectively (Method Ti 1a-64 1993). The data presented above are within the permissible limits, and even more considerably lower, which justifies the biological value of the lipophilic sea buckthorn extracts.

In order to evaluate the changes of the physico-chemical characteristics and the quality indices with time, the obtained extracts were analyzed after a period of 3 months. The results obtained showed that the presence of biologically active compounds in the lipophilic sea buckthorn extracts play a positive role in slowing down the oxidative process and thus in increasing the shelf life.

According to the results obtained (Table 1), PV and AV showed only an insignificant increase to 1.45 ± 0.28 mEq O_2/kg and 0.21 ± 0.01 mg KOH/g respectively, which is explained by the inhibition of the oxidative process, by the biologically active compounds contained in the lipophilic sea buckthorn extracts.

Table 1 The quality indices (AV and PV, *p*-anisidine, K_{236}, K_{273}, and K_{236}/K_{273} ratio) of the lipophilic sea buckthorn (*Hippophae rhamnoides* L.) extracts and sunflower oil

Quality indices	Lipophilic sea buckthorn extract		Sunflower oil
	Initial	After 3 months	
AV, mg KOH/1 g fat material	0.19 ± 0.02	0.21 ± 0.01	0.080 ± 0.018
PV, mEq O_2/kg	1.31 ± 0.04	1.45 ± 0.28	2.23 ± 0.07
p-anisidine	8.06 ± 0.32	8.23 ± 0.20	9.44 ± 0.27
K_{236}	0.16 ± 0.05	0.168 ± 0.014	0.27 ± 0.05
K_{273}	0.12 ± 0.07	0.057 ± 0.006	0.08 ± 0.01
K_{236}/K_{273}	1.46 ± 0.49	2.97 ± 0.51	3.38 ± 0.01

The oxidation degree of the samples expressed by the amount of primary and secondary oxidation products formed after 3 months of storage time was evaluated by determining the *p*-anisidine value, K_{236} and K_{273} indices. The obtained values (8.23 ± 0.32, 0.168 ± 0.014, and 0.057 ± 0.006, respectively) indicate the stagnation of the lipid oxidation process due to the enrichment of the oil with biologically active compounds with antioxidant capacity.

Cream cheese with lipophilic sea buckthorn extracts addition

The results obtained from the sensory analysis showed that all cream cheese samples obtained were rated "very good" and fulfill the quality conditions (SR ISO 6658:2017). The best sample was the cream cheese with the content of lipophilic sea buckthorn extract, which showed no perceptible defects (Table 2).

Although the sensory analysis of food gives us an overview of the quality of analysed products such as appearance, consistency, taste, smell, etc. these data are not enough to appreciate a food product as being qualitative and safe for consumption. Of primary importance are the physico-chemical analyses and the microbiological stability of the food products. The dry matter content and the fat content were determined once because their values did not change during storage. The physico-chemical characteristics such as the dry substance content and the fat content had values that fall within the limits stipulated in the normative documents for fresh cow cheese (G. D. of R. M., No. 611 2010).

It was found that the extract used had a direct influence on the dry matter content of the cream cheese. The lowest content of dry substance was obtained in the case of the cream cheese sample with synthetic dye (27.15%) followed by the cream cheese with sea buckthorn lipophilic extract (28.85%). The fat content of the cream cheese samples increased to 11.95% when lipophilic extracts were added, compared to the control sample (9.95%). Thus, the fat content of the extracts directly influenced the fat content of the cream cheese.

Table 2 shows the increase of the acidity due to lactic fermentation in cream cheese, but also due to the hydrolysis of triglycerides in the cream with free fatty acids formation. Data showed that titratable acidity linearly increased during storage. The extracts used directly influenced the acidity of the cream cheese. The lipophilic sea buckthorn extracts decreased the acidity of the cream cheese, the initial acidity being 111°T compared to 115°T of the control sample (after the first day of production). This decrease was maintained even after 10 days of storage (121°T for the cream cheese with lipophilic sea buckthorn extract and 127°T for the control cream cheese extract). After 10 days of storage, the titratable acidity of the cream cheese containing sea buckthorn lipophilic extract increased by 1.09 times while that for the control sample increased by 1.10 times. Even in the 10th day of storage, the titratable acidity had values that fall within the limits of the normative documents for fresh farmer cheese, max. 220°T (G. D. of R. M. No. 611 2010). The active acidity decreased linearly during 10 days of storage. The active acidity decreased from 5.15 to 4.95 for the samples with the addition of synthetic dye and from 5.20 to 5.02 for the samples with added sea buckthorn lipophilic extracts.

The rheological properties of the cream cheese are influenced by temperature, presence of additives, fermentation time, quality of raw material, and presence of stabilizers (Costin et al. 2005). The cream cheese is a fluid, elastic-viscous mass. The viscosity of the cream cheese samples obtained was determined at the fortification speed of 75 min^{-1}. After 10 days of storage, the viscosity of the obtained samples has changed insignificantly. This was due to the presence of the stabilizer which has the property of stabilizing of the emulsions but also works as a thickening agent.

The microbiological analysis was also performed to assert with certainty that the obtained samples are qualitative and safe for consumption (G. D. of R. M. No. 221 2009). The results showed that on the 10th day of storage, the sample with the addition of synthetic dye had a rapid increase in the total number of germs. However, in all samples examined, the total number of germs does not exceed the regulated allowance of 100,000 CFU/g. The

Table 2 Changes of the physico-chemical quality indicators, microbiological stability and in vitro antiradical activity of the cream cheese during storage

Cream cheese	Dry matter content, %	Fat content, %	Average score on organoleptic analysis		pH		Titratable acidity, °T		Dynamic viscosity, mPa × s		Total viable count*, (CFU/g) × 10^2		Antiradical activity, %
	After 1 day	After 1 day	After 1 day	After 10 days	After 1 day	After 10 days	After 1 day	After 10 days	After 1 day	After 10 days	After 1 day	After 10 days	After 10 days
With 0.01% colorant tartrazine (control sample)	27.15 ± 0.07^{a}	9.95 ± 0.05^{a}	19.6	14.6	5.15 ± 0.01^{d}	4.95 ± 0.01^{d}	115.0 ± 1.4^{b}	127.0 ± 1.4^{d}	13,399	13,760	4	54	8.68 ± 0.55^{a}
With 2.5% sea buckthorn lipophilic extract	28.85 ± 0.07^{c}	11.95 ± 0.05^{b}	20.0	16.7	5.20 ± 0.01^{e}	5.02 ± 0.01^{d}	111.0 ± 1.4^{a,b}	121.0 ± 1.4^{c}	11,258	11,520	4	20	28.89 ± 0.37^{b}

The letters "a, b, c, d" are for statistical differences ($p \leq 0.05$), between the control sample and the cream cheese with addition of lipophilic sea buckthorn extract

*Nutrient agar

slower rate of growth of germs in the cream cheese samples with added extract is due to phenolic compounds and carotenoids which are known to have bacteriostatic and bactericidal effects (Kaushal and Sharma 2011; Zheng et al. 2009).

The biological value of the cream cheese samples was determined by simulating digestion under gastric conditions and determining antiradical activity using free radical DPPH after 10 days of storage (Zheng et al. 2009). In the case of the cream cheese samples with added sea buckthorn lipophilic extract, the antiradical activity increased by 3.3 times compared with the control samples, increase which was attributed to the carotenoids.

For the determination of the shelf life of the cream cheese, we took into account also the evolution of physico-chemical, microbiological and sensory parameters over time. The results obtained showed that on the 10th day of storage the physico-chemical parameters and the microbiological stability fell within the limits stipulated by the current standards (G. D. of R. M. No. 611 2010). Also, the sensory analysis during the preservation showed that the cream cheese with the addition of synthetic dye kept its the sensory characteristics like "good" until the 8th day while those with the extracts of sea buckthorn until the 10th day of storage.

Conclusion

The objective of the present study was to investigate the effect of the sea buckthorn lipophilic extracts on cream cheese properties. In order to obtain an extract from sea buckthorn with a high content of liposoluble compounds stirring and sonication extraction techniques and different temperature regimes (20 °C and 45 °C) and extraction times (0.5 h, 1.0 h and 1.5 h) were tested. After optimization of the extraction method, it was established that the highest content of biologically active lipophilic compounds was obtained by sonication for 1 h at 20 °C. The content of the total carotenoids extracted was 8.27 ± 0.01 mg/l, the polyphenols content was 1842.86 ± 1.41 mg/100 g DW and the antioxidant capacity of the sea buckthorn extract was about 67.04%. Also, the stability of the obtained extract in time was evaluated. For this purpose, the antioxidant capacity and the quality indices (IP, IA, K236, K273, p-anisidine) were evaluated after a period of 3 months. The results obtained showed that the presence of biologically active compounds in the lipophilic sea buckthorn extract slowed the oxidative process and increased the shelf life.

The optimized lipophilic extract of sea buckthorn was added as colorant into the cream cheese. Following the addition of the extract, it was found that the cream cheese

was positively influenced by sea buckthorn lipophilic extracts increasing the antiradical activity and inhibiting the growth of the germ in the cream cheese. Moreover, the addition of sea buckthorn extracts did not adversely affect the sensory characteristics of the product.

This research demonstrates the possibility of using sea buckthorn lipophilic extract in the production of functional foods. A particular interest is the possibility of replacing synthetic antioxidants with natural ones obtained from local horticultural sources in order to provide consumers with safe and stable food products.

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