PAPER

SEA-BUCKTHORN OIL IN VEGETABLE OILS STABILISATION

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ABSTRACT

The paper proposes the development of blends of vegetable oils with a high content of easily oxidizable unsaturated fatty acids with sea-buckthorn fruit oil as a natural method of their preservation. The predominant lipophilic compounds in sea-buckthorn oil included β-carotene, α-tocopherol, and β-sitosterol. Strong correlations were found between oxidative stability of the blends and α- and β-tocopherols, lutein and β-carotene concentration (r ranging from 0.96 to 0.99). The observed effect may result from both the particularly huge increase in the carotenoid content (from 64- to 171-fold) in the obtained blends, and the synergistic interaction between tocopherol mixtures and carotenoids.

Keywords: oils stabilisation, sea-buckthorn fruit oil, natural antioxidants

1. INTRODUCTION

Consumption of vegetable oils provides the body with energy and ingredients with structural, regulatory and protective functions (AYALA *et al.*, 2014). Lipid components form, *inter alia*, the structure of cell membranes; moreover, they are involved in the formation and functioning of nerve cells, regulation of intrasystemic metabolism, and maintaining the oxidation/reduction balance in the cells (MURRAY *et al.*, 1995). The composition of fatty acids and the profile of low-molecular lipophilic compounds depend on the botanical source of origin, and the method of oil production. Most oils contain predominantly unsaturated fatty acids which allow them to maintain the liquid state at room temperature. However, the presence of unsaturated fatty acids promotes the initiation of oxidation processes, which are particularly rapid for polyunsaturated acids. With an increase in the number of unsaturated bonds in the cell, the number of carbon atoms separating them also increases. The carbon-hydrogen bond near such a carbon atom is characterized by lower dissociation energy, which leads to easy formation of free radicals. According to COSGROVE *et al*. (1987) the oxidation of oleic acid is 50 times slower than that of linoleic acid, and 100 times slower than that of linolenic acid. The slightly lower rate of the oxidation of oleic acid as compared to linoleic acid (10-40 times lower rate) was specified in the study by MCCLEMENT and DECKER (2008).

Oxidation of an oil begins at the moment of the extraction thereof from a plant matrix. Due to the destruction of natural cell structures, they become susceptible to the action of enzymes, oxygen, light, and other free radical generators (SZUKALSKA, 2003). The basic and primary indicator of the oxidation of an oil is an increase in the value of peroxide value (PICURIC-JOVANOVIC *et al.*, 1999; BROADBENT and PIKE, 2003). For cold-pressed oils, the value of peroxide value as permitted by Codex Alimentarius (2005) is 15 $mEqO_2/kg$. The value of peroxide value, however, does not allow clear determination of freshness of an oil. Only the performance of additional analyzes on the secondary products of oil oxidation e.g. by the TBA test, determination of the anisidine value, or the measurement of absorbance of conjugated dienes and trienes allows a more precise determination of the degree of oxidation (JERZEWSKA, 1991).

Despite the presence of oxidizable unsaturated fatty acids, vegetable oils contain numerous stabilizing compounds exhibiting antioxidant action (CZAPLICKI *et al.*, 2011; OGRODOWSKA *et al.*, 2014; ROSZKOWSKA *et al.*, 2015). They get to the oil from the plant matrix, or are added at the packaging stage. The compounds of particular significance are terpenoid compounds such as tocols, sterols, carotenoids, and squalene. Most often, however, the content of natural antioxidants is not sufficiently high to fully protect the oil against oxidation. A study by CZAPLICKI *et al*. (2011) on nine popular bio-oils found that the content of unsaponifiable fraction ranged from only 0.48% (poppyseed oil) to 7.12% (amaranth oil). It therefore seems that vegetable oils valued for their unique compositions of fatty acids, such as linseed, borage, and evening primrose oils, should be enriched with either natural or synthetic antioxidants. Literature describes attempts to increase the oxidative stability of oils through the addition of compounds such as e.g.: tocopherols, tocotrienols, sesamol, butylated hydroxyl toluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), tertiarybutylhydroquinone (TBHQ) and ascorbyl palmitate (AP) (HAMDO *et al.*, 2014; HWANG and WINKLER-MOSER, 2013; ÖNAL-ULUSOY and ERGIN, 2002). Similarly, a positive effect on the oxidative stability of oils was observed in tests using grape seed extract, green tea extract, microalgae *Scenedesmus almeriensis* extracts, and extracts of herb such as rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), marcela (*Achyrocline satureioides*), and carqueja (*Baccharis trimera*) (POIANA, 2012; CHEN *et al.*, 2013; VIEITEZ *et al.*, 2013; LIMÓN *et al.*, 2015). However, the introduction of pure substances or lyophilized extracts into an oil results in the need for standardization

of their concentrations in the oil, which may be limited by the solubility of the compound or extract being added. It was demonstrated, *inter alia*, that in corn oil at a temperature of 25°C, only 3% sterols can be dissolved (VAIKOUSI *et al.*, 2007). The practice of introducing into oils substances which do not occur in them naturally gives rise to controversy associated with the loss of the "natural" characteristic of a product.
On the other hand, a natural manner of increasing the content of antioxidants in oils may be the development of their blends with oils being particularly rich in natural antioxidants. An oil which is characterized by an exceptionally high content of phytosterols, tocopherols, and carotenoids, is the oil extracted from sea-buckthorn fruits. This study attempted to determine the effects of the addition of sea-buckthorn oil as a stabilizer of linseed, borage, and evening primrose oils which are characterized by different compositions of fatty acids and contents of endogenous antioxidants. The following were assessed: the content of natural terpenoid antioxidants in the obtained blends, the composition of fatty acids, and their oxidative stability.

2. MATERIALS AND METHODS

2.1. Chemicals

Chromatography-grade solvents: methanol, methyl tert-butyl ether (MTBE), iso-propanol, hexane, pyridine, N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were purchased from Sigma-Aldrich (St. Louis, MO, United States, supplier Poznań, Poland). Analytical-grade reagents: methanol, dichloromethane, chloroform, sulphuric acid, potassium hydroxide, sodium sulphate, powdered zinc (POCH, Gliwice, Poland) were used. Analytical standards: 5α-cholestane (97%), β-Apo-8'-carotenal (>96%) and fatty acids mixture were purchased from Sigma-Aldrich and tocopherols mixture (95%) from Merck (Darmstadt, Germany). Deionized water was obtained from HLP 5s deionizer (Hydrolab, Gdańsk, Poland).

2.2. Materials

The linseed oil, borage seed oil, evening primrose seed oil and sea-buckthorn fruits oil were used in this study.
Oils were obtained by pressing the raw material on a IBG Monforts & Reiners, Komet CA59G (Germany) laboratory expeller. The oils were purified by centrifugation at 8000 x g on a Eppendorf centrifuge (type 5810R). The oils blends were prepared by 15% of sea buckthorn oil addition to analysed linseed, borage seed and evening primrose seed oils.
In oils and prepared mixtures fatty acids compositions and bioactive compounds (carotenoids, sterols and tocopherols) concentrations were analysed as well as oxidative stability was also examined.

2.3. Determination of fatty acid composition

Ten micrograms of sample was dissolved in 1.5 mL of chloroform-methanol-sulphuric acid (100:100:1, v/v/v), transferred into 2 mL-pharmaceutical vials and sealed hermetically over a gas burner (ZADERNOWSKI and SOSULSKI, 1978). The fatty acids methylation was carried out by heating the vials at 70°C for 2 hours. After cooling, the vials were opened and the powdered zinc was added to decompose remained sulphuric acid. Obtained methyl esters were dried in a stream of nitrogen, purified in a hexane extraction and analysed by gas chromatography with a GC-MS QP2010 PLUS (Shimadzu,

Japan) system. Separation was performed on a BPX70 (25 m x 0.22 mm x 0.25 μm) capillary column (SGE Analytical Science, Victoria, Australia) with helium as the carrier gas at a flow rate of 0.9 mL/min. The column temperature was programmed as follows: a subsequent increase from 150°C to 180°C at the rate of 10°C/min, to 185°C at the rate of 1.5°C/min, to 250°C at the rate of 30°C/min, and then 10 min hold. The interface temperature of GC-MS was set at 240°C. The temperature of the ion source was 240°C and the electron energy 70 eV. The total ion current (TIC) mode was used in 50-500 m/z range. According to the shares of individual fatty acids oils oxidation index (U) was calculated using the formula given by COSGROVE *et al.* (1987):

$$U = (0.02 \cdot (C_{16:1}+C_{18:1}) + 1 \cdot C_{18:2} + 2 \cdot C_{18:3})/100.$$

2.4. Determination of carotenoids

Carotenoids in oils were analysed with a reversed phase high performance liquid chromatography (RP-HPLC) technique. The sample of oils was diluted in hexane contained β-Apo-8'-carotenal as an internal standard and saponified with 6 mL of 40% methanolic KOH solution in a shaker at room temperature in the dark for 16 h. Next, 30 mL of hexane to the sample was added and then the tube was filled up to 50 mL with 10% Na_2SO_4. The lower phase was separated, triple-rinsed with 10 mL of hexane and collected with the upper organic phase. The organic solvent was evaporated at 40°C under a nitrogen stream and dissolved in 2 mL of a methanol: dichloromethane (45:55 v/v) solution. The chromatographic analysis of carotenoids was conducted according to modified EMENHISER *et al.* (1995) method. Briefly, the analysis was carried out using a 1200 series liquid chromatograph manufactured by Agilent Technologies (Palo Alto, CA, USA), equipped with a diode array detector (DAD) from the same manufacturer. Separation was performed at 30°C on a YMC-C_{30} 250 x 4.6 mm, 5 μm column and YMC-C_{30} 10 x 4.6 mm, 3 μm precolumn (YMC-Europe GmbH, Germany). A methanol- methyl tert-butyl ether (MTBE) gradient was programmed as it is presented in Table 1.
The absorbance was measured at the wavelength of 450 nm. Carotenoids were identified, based on retention times of available standards (Sigma-Aldrich, USA), and by comparing the UV–Visible absorption spectra.

Table 1: HPLC gradient conditions established for analysis of carotenoids.

Time [min]	Methanol [%]	MTBE [%]	Flow rate [mL/min]
0-5	95	5	1
25	72	28	1.25
33	5	95	1.25
40	95	5	1
60	95	5	1

2.5. Determination of phytosterols

The content of sterols in oils was determined by gas chromatography coupled with mass spectrometry (GC-MS QP2010 PLUS, Shimadzu, Japan) according to the method described

by VLAHAKIS and HAZEBROEK (2000). The sample was saponified by adding a 0.5 mL 2M NaOH methanolic solution at ambient temperature for 2 hours. Unsaponifiables were extracted with diethyl ether which was evaporated under nitrogen conditions. The dry residues were re-dissolved in 1.5 mL of n-hexane and a 0.2 mL of 5α-cholestane internal standard solution was added (0.4 mg/mL). After evaporation, the residues were re-dissolved in 100 μL of pyridine and 100 μL BSTFA (N,O-bis (trimethylsilyl) trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) and left in 60°C for 60 minutes to complete derivatization. One mL of hexane was then added to the sample and 1 μL of the obtained mixture was analysed. A ZB-5MSi capillary column was used for the separations of sterols with helium as a carrier gas at a flow rate of 0.9 mL/min. The injector temperature was set at 230ºC and the column temperature was programmed as follows: 70°C for 2 min, a subsequent increase to 230°C at the rate of 15°C/min, to 310°C at the rate of 3°C/min, and then 10 min hold. The interface temperature of GC-MS was set at 240°C. The temperature of the ion source was 220°C and the electron energy 70 eV. The total ion current (TIC) mode for quantification (100-600 m/z range) was used. The quantifications using the internal standard method were done.

2.6. Determination of tocopherols

The tocopherols analysis was carried out by high performance liquid chromatography (HPLC), according to the method described by CZAPLICKI *et al.* (2011). Briefly, 0.1 g of oil (± 0.001 g) was diluted in hexane in a 10 mL measuring flask. After subsequent centrifugation (10 min. at 25000 x g) in a 5417R-type Eppendorf centrifuge (Eppendorf AG, Hamburg, Germany), the sample was transferred to a chromatographic vial and 20 μL was injected into the chromatographic system. The analysis was performed using a 1200 series liquid chromatograph manufactured by Agilent Technologies (Palo Alto, CA, USA), equipped with a fluorescence detector from the same manufacturer. The separation was done on a Merck LiChrospher Si 60 column, 250 mm x 4 mm, 5 μm. A 0.7% isopropanol solution in hexane at a 1 mL/min flow rate was used as the mobile phase. The fluorescence detector was set at 296 nm for excitation and 330 nm for emission. Peaks were identified on the basis of retention times determined for α-, β-, γ- and δ-tocopherol standards (Merck, Darmstadt, Germany) separately, and their content was calculated using external calibration curves.

2.7. Determination of induction time

Induction time of oils was tested on a Rancimat apparatus 743 (Metrohm, Herisau, Switzerland). The analysis was performed according to method described by FARHOOSH (2007). Briefly, 2.5 g of oil in a reaction vessel was weighed and after capping the vessel was placed in a thermostated electric heating block at temperature 110°C. An air flow rate of 20 L/h was given. Determination of the induction time was based on the conductometric detection of volatile oxidation products. The time that elapsed until these oxidation products appeared was saved as the induction time.

2.8. Determination of initial state of oils rancidity

The acid (AV), peroxide (PV), and p-anisidine (p-AV) values were determined in accordance with procedures of CEN ISO 660:2009, CEN ISO 3960:2010, and CEN ISO 6885:2008, respectively.

2.9. Statistical analysis

The obtained results of all analysis (performed in triplicate) were statistically analysed using Statistica 12.0 PL software (StatSoft Inc., Kraków, Poland). In order to indicate the significance of differences between oil samples, unvaried analysis of variance (ANOVA) with a Duncan test at $p \leq 0.05$ significance level was used. The intra-sample quality variation of fresh oils and their blends with the sea buckthorn oil was assayed using principle component analysis (PCA) at $p \leq 0.05$ significance level.

3. RESULTS AND DISCUSSIONS

The initial rancidity of three used in this study highly unsaturated oils (linseed, borage seed, and evening primrose seed) was relatively low, with an AV value from 1.37 to 3.15 mg KOH/g, and a PV value from 0.93 to 3.76 mEq O_2/kg (Table 2). According to requirements of the Codex Alimentarius Commission these results met the standard for cold-pressed and virgin oils, determined as 4 mg KOH/g, and 15 mEq O_2/kg of oil, respectively. Values of a p-AV of these oils, which reflect the content of secondary products of lipid oxidation, varied from 2.00 to 7.85 (Table 2), and were similar for example to results for raspberry seed oil (OOMAH *et al*., 2000). Determined AV, PV, an p-AV values showed that used oils were of food-grade quality typical for other cold pressed and virgin oils.

Table 2: Rancidity indices of linseed, evening primrose, and borage oils before enrichment with sea-buckthorn oil.

	Acid value [mg KOH/g oil]		Peroxide value [mEq O_2/kg oil]		Anisidine value [-]	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Linseed	1.37	0.02	0.93	0.05	2.00	0.26
Evening primrose	1.57	0.01	3.76	0.11	7.85	0.61
Borage	3.15	0.22	1.45	0.10	4.61	0.52

The fatty acid compositions of the studied linseed, borage seed, and evening primrose seed oils, and of their blends with sea-buckthorn oil, are presented in Table 3. It was found that linseed, borage seed, and evening primrose seed oils were characterized by a high percentage of polyunsaturated fatty acids (PUFA). The oils being richest in these acids included linseed oil (73%) and evening primrose oil (82%), and the total share of acids containing at least one unsaturated bond in these oils amounted to nearly 90%. In turn, borage oil was characterized by the highest share of γ-linolenic acid (7%) of all the studied oils, and unsaturated fatty acids were also represented by oleic (26%) and linoleic acid (33%). The oils used in the experiment are valued for their composition of fatty acids; however, both the great number of unsaturated bonds and the degree of their unsaturation have an adverse effect on the oxidative stability of an oil. The susceptibility of the oils under study to oxidation is expressed as an oxidizability index calculated according to the formula proposed by COSGROVE *et al*. (1987). Borage oil turned out to be the oil being least susceptible to oxidative changes; the oxidizability index for this oil amounted to 0.61. In turn, linseed oil, which contained almost 60% of α-linolenic acid, exhibited the highest value of the oxidizability index (1.33). As compared with e.g.

rapeseed oil (ROSZKOWSKA *et al.*, 2015), the oxidizability indices of the oils under study had values higher by 65, 143, and 259% for, respectively, borage oil, evening primrose oil, and linseed oil.

Table 3: Fatty acids composition (%), the main bioactive compounds content in oils [mg/100 g of oil], and their oxidation index [-] and induction time [h].

	Linseed oil		Borage seed oil		Evening primrose seed oil		Sea buckthorn fruit oil	
Compound	$\bar{x}$	**SD**	$\bar{x}$	**SD**	$\bar{x}$	**SD**	$\bar{x}$	**SD**
palmitic ($C_{16:0}$)	7.33^{a}	1.13	19.60^{b}	1.25	7.98^{a}	0.11	36.31^{c}	0.01
palmitoleic ($C_{16:1}$)	nd^{a}		nd^{a}		nd^{a}		40.97^{b}	0.04
stearic ($C_{18:0}$)	3.28^{a}	0.75	7.46^{b}	0.44	2.28^{c}	0.05	0.55^{d}	0.01
oleic ($C_{18:1}$)	15.88^{a}	1.8	26.40^{b}	1.44	7.12^{c}	1.34	8.77^{c}	0.11
linoleic ($C_{18:2}$)	14.49^{a}	0.32	32.64^{b}	1.41	75.47^{c}	1.88	12.12^{d}	0.13
α-linolenic ($C_{18:3}$)	59.02^{a}	3.36	nd^{b}		nd^{b}		1.28^{c}	0.04
γ-linolenic ($C_{18:3}$)	nd^{a}		13.91^{b}	1.73	7.17^{c}	0.39	nd^{a}	
Σ PUFA	73.51^{a}	3.68	46.55^{b}	3.14	82.64^{c}	2.27	13.40^{d}	0.17
Oxidizability index	1.33^{a}	0.07	0.61^{b}	0.05	0.90^{c}	0.03	0.16^{d}	0.00
lutein	0.22^{a}	0.01	0.09^{a}	0.01	0.07^{a}	0.01	3.24^{b}	0.49
all-trans β-carotene	0.22^{a}	0.05	0.07^{a}	0.05	0.14^{a}	0.04	118.36^{b}	9.58
other carotenoids	0.04^{a}	0.01	0.01^{a}	0.01	0.09^{a}	0.08	74.82^{b}	6.25
total carotenoids	**0.48**^{a}	**0.06**	**0.18**^{a}	**0.07**	**0.30**^{a}	**0.12**	**206.04**^{b}	**15.63**
α-tocopherol	6.21^{a}	0.30	nd^{b}		26.42^{c}	0.17	144.14^{d}	4.10
β-tocopherol	nd^{a}		nd^{a}		nd^{a}		3.98^{b}	0.23
γ-tocopherol	37.02^{a}	1.01	11.95^{b}	0.21	40.41^{c}	0.86	4.63^{d}	0.32
δ-tocopherol	nd^{a}		100.25^{b}	0.50	nd^{a}		0.75^{a}	0.00
total tocopherols	**43.23**^{a}	**1.32**	**112.20**^{b}	**0.71**	**66.83**^{c}	**0.69**	**153.50**^{d}	**4.10**
campesterol	40.44^{a}	0.63	38.30^{a}	3.04	57.35^{b}	3.08	7.87^{c}	0.45
Δ5-avenasterol	13.37^{a}	0.53	43.42^{b}	0.77	62.92^{c}	5.95	20.46^{d}	1.54
β-sitosterol	110.21^{a}	0.29	37.96^{b}	2.05	595.94^{c}	6.46	536.30^{d}	1.25
Δ7-stigmastenol	nd^{a}		nd^{a}		5.95^{b}	0.28	nd^{a}	
Δ7-stigmasterol	14.75^{a}	0.35	nd^{b}		nd^{b}		nd^{b}	
cycloartenol	162.38^{a}	0.53	51.02^{b}	4.74	nd^{c}		28.50^{a}	0.63
other sterols	45.44^{a}	1.64	37.13^{a}	1.22	8.58^{b}	0.19	262.81^{c}	16.61
total sterols	**386.58**^{a}	**0.83**	**207.81**^{b}	**7.49**	**730.74**^{c}	**4.06**	**855.94**^{d}	**8.95**
Induction time	2.44^{a}	0.23	3.91^{b}	0.25	3.97^{b}	0.20	>48^{c}	

nd – not detected

Values within a row with different letters are significantly different ($p \leq 0.05$).

Sea-buckthorn oil, used as a stabilizer, owed its resistance to oxidation to, *inter alia*, the relatively low share of polyunsaturated fatty acids. The oxidizability index calculated for this oil only amounted to 0.16, and the induction time in the Rancimat test was longer than 48 h (in a temperature of 110°C), which demonstrates its extraordinary resistance to oxidative changes. However, this resistance is owed not only to the characteristics of fatty acids but also to the abundance of natural antioxidants. The average content of carotenoids in sea-buckthorn oil amounted to 206 mg/100 g, and the predominant one was β-carotene (65%). In addition to β-carotene, sea-buckthorn oil also contained, *inter alia*, α-carotene, lutein, zeaxanthin, and β-cryptoxanthin. Sea-buckthorn oil also contained tocopherols at an amount of 144 mg/100 g, with the predominant α homologue (94%), and phytosterols at an amount of 856 mg/100 g, with the predominant β-sitosterol (63%).

Linseed, evening primrose, and borage oils were characterized by significantly lower contents of these antioxidants. The carotenoid content did not exceed the value of 0.48 mg/100 g and, in the extreme case, was ca. 1100 times lower than that in sea-buckthorn oil. Sea-buckthorn oil was also significantly richer in tocopherols, as it contained, respectively, 3.6-, 1.4-, and 2.3-times more of these compounds than, in turn, linseed, borage seed, and evening primrose seed oils. In the group of these compounds, γ-tocopherol (linseed and evening primrose oils), and δ-tocopherol (borage seed oil) were predominant. As regards phytosterols, the total content thereof being similar to that of sea-buckthorn oil was found in evening primrose seed oil, while linseed oil and borage seed oil contained 2.21- and 4.12-times less of those compounds, respectively. The predominant phytosterols in the enriched oils included cycloartenol (linseed oil and borage seed oil) and β-sitosterol (evening primrose seed oil).

The addition of sea-buckthorn oil resulted in an over 64-, 171-, and 103-fold increase in the carotenoid content of linseed oil, borage seed oil, and evening primrose seed oil, respectively, with a particularly apparent increase in the share of all-trans β-carotene (Table 4).

The enrichment with sea-buckthorn oil resulted in an increase in the content of this compound to a level ranging from approx. 3 mg/100 g in an evening primrose seed oil blend to almost 5 mg/100 g in a linseed oil blend. The enrichment with sea-buckthorn oil also contributed to an increase in tocopherol content. This change was biggest for linseed oil (45%), and the content of α-tocopherol in this oil increased over three-fold. Borage seed oil, which was the only one containing no α-tocopherol, was enriched with this component to an amount of almost 29 mg/100 g. At the same time, the addition of sea-buckthorn oil caused a significant increase in the content of β-sitosterol in linseed oil (58%) and borage seed oil (197%). Evening primrose seed oil was the only oil in which no significant change to the concentration of this sterol was noted, with the total increase in the share of sterols by 2.57%.

At the same time, the enriched oils were characterized by significantly lower oxidizability index (a decrease by 12-30%), and the induction time being increased by approx. 21–32% (Table 4). The noted relative increase in the stability of oil was statistically significant, and reached the highest value for linseed oil. However, the actual induction time of this oil only increased to a value of 3.21 h (from the initial value of 2.44 h), which may be explained by the particularly high content of PUFA. This phenomenon may be explained not only by the change to fatty acid concentration but also by the more than 3-fold increase in the concentration of α-tocopherol, and over 60-fold increase in the concentration of carotenoids.

Table 4: Fatty acids composition (%), the main bioactive compounds content [mg/100 g of oil] in oils blends with sea buckthorn oil and their oxidation index [-] and induction time [h].

	Linseed oil		Borage seed oil		Evening primrose seed oil	
Compound	$\bar{x}$	**SD**	$\bar{x}$	**SD**	$\bar{x}$	**SD**
palmitic ($C_{16:0}$)	10.87[a]	0.83	27.15[b]	1.97	13.69[c]	1.99
palmitoleic ($C_{16:1}$)	5.68[a]	0.01	7.10[b]	0.53	7.17[b]	0.69
stearic ($C_{18:0}$)	2.96[a]	0.37	6.74[b]	0.47	1.87[c]	0.19
oleic ($C_{18:1}$)	15.19[a]	0.17	25.22[b]	0.75	8.19[c]	0.29
linoleic ($C_{18:2}$)	14.39[a]	0.21	24.94[b]	1.11	63.32[c]	2.09
α-linolenic ($C_{18:3}$)	50.91[a]	1.18	nd[b]		nd[b]	
γ-linolenic ($C_{18:3}$)	nd[a]		8.87[b]	0.05	5.77[c]	5.77[c]
Σ PUFA	65. 30[a]	1.39	33.81[b]	1.16	69.09[a]	3.15
Oxidizability index	1.17[a]	0.03	0.43[b]	0.01	0.75[c]	0.04
lutein	0.67[a]	0.04	0.56[a]	0.10	0.55[a]	0.13
all-trans β-carotene	17.94[a]	0.20	17.81[a]	0.77	17.87[a]	0.47
other carotenoids	11.26[a]	0.16	11.23[a]	0.80	11.30[a]	0.46
total carotenoids	31.31[a]	0.29	31.06[a]	1.9	31.16[a]	0.46
α-tocopherol	26.90[a]	2.18	21.62[b]	1.26	44.08[c]	0.60
β-tocopherol	0.60[a]	0.43	0.60[a]	0.44	0.60[a]	0.06
γ-tocopherol	32.16[a]	1.05	10.85[b]	0.54	35.04[c]	0.19
δ-tocopherol	0.11[a]	0.00	85.33[b]	1.64	0.11[a]	0.00
total tocopherols	59.77[a]	1.65	118.40[b]	2.87	79.83[c]	0.85
campesterol	35.55[a]	1.81	33.74[a]	2.12	49.93[b]	3.94
Δ5-avenasterol	14.43[a]	3.25	39.98[b]	2.15	56.55[c]	3.52
β-sitosterol	174.12[a]	6.16	112.71[b]	8.99	586.99[c]	29.61
Δ7-stigmastenol	nd[a]		nd[a]		5.06[b]	0.44
Δ7-stigmasterol	12.54[a]	0.66	nd[b]		nd[b]	
cycloartenol	142.30[a]	6.05	47.64[b]	3.51	4.28[c]	0.39
other sterols	78.05[a]	7.55	70.98[a]	5.30	46.71[b]	2.45
total sterols	456.98[a]	11.25	305.03[b]	10.15	749.52[c]	14.28
Induction time	3.21[a]	0.23	4.80[b]	0.25	4.81[b]	0.18

nd – not detected

Values within a row with different letters are significantly different ($p \leq 0.05$).

A PCA analysis confirmed strong correlation between the induction time for the oil and the shares of palmitoleic acid (0.98) and palmitic acid (0.77) being typical of sea-buckthorn oil (SHAFI *et al.,* 2008) (Fig. 1a), and between the induction time and the content of α- and β-tocopherol as well as lutein and β-carotene – correlations within a range of 0.96–0.99 (Fig. 2a). Stability of the oils was affected, to a much smaller extent, by the content of β-sitosterol, with the correlation coefficient being only equal to 0.41.

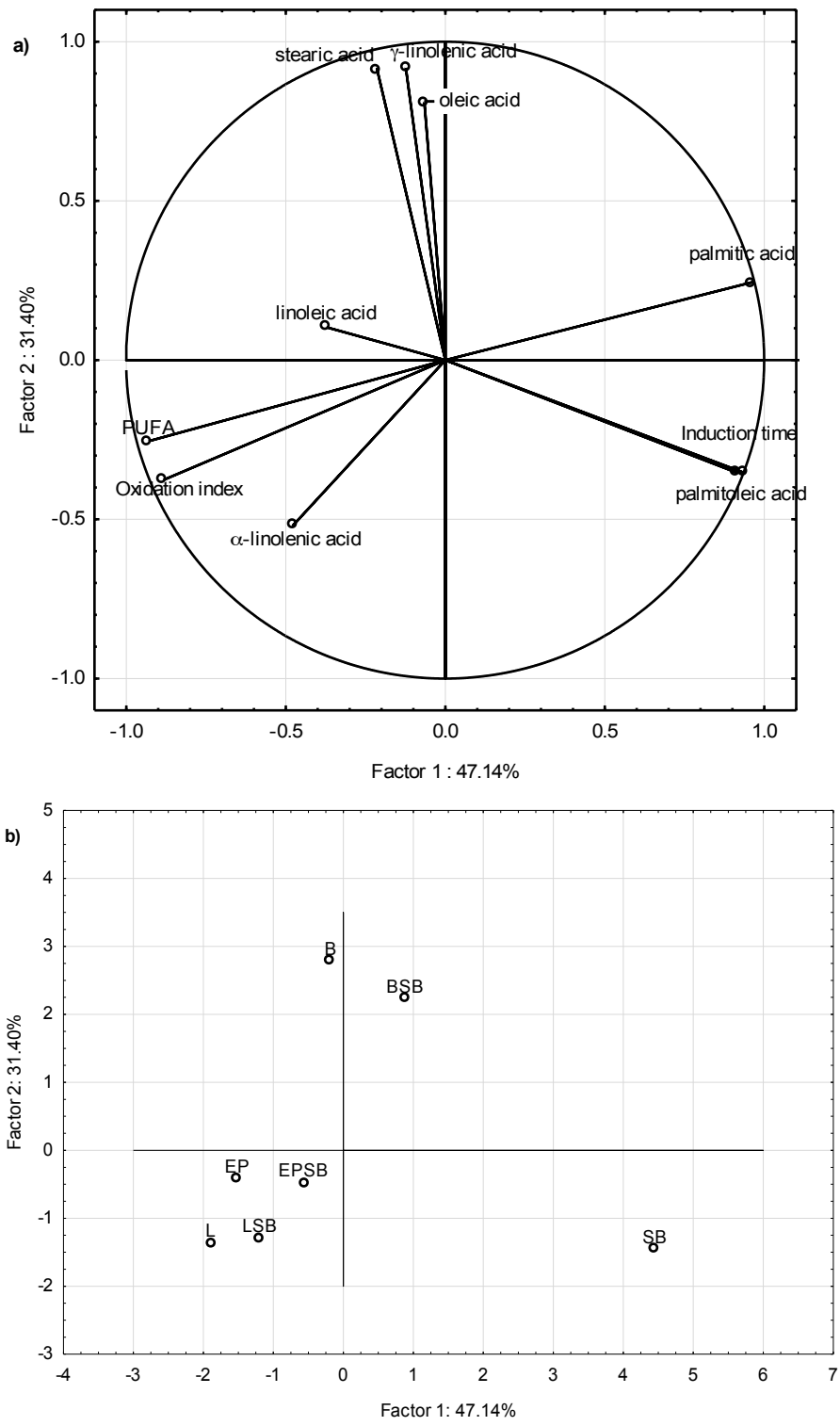

Figure 1: a) PCA loading plot of tested variables; b) Score plot of the two first principal components after PCA analysis of fatty acid composition, oxidation index and induction time of fresh oils and their blends with sea buckthorn fruit oil.
B – borage seed oil, BSB – borage seed oil enriched with sea buckthorn fruit oil, EP – evening primrose seed oil, EPSB - evening primrose seed oil enriched with sea buckthorn fruit oil, L – linseed oil, LSB - linseed oil enriched with sea buckthorn fruit oil, SB - sea buckthorn fruit oil.

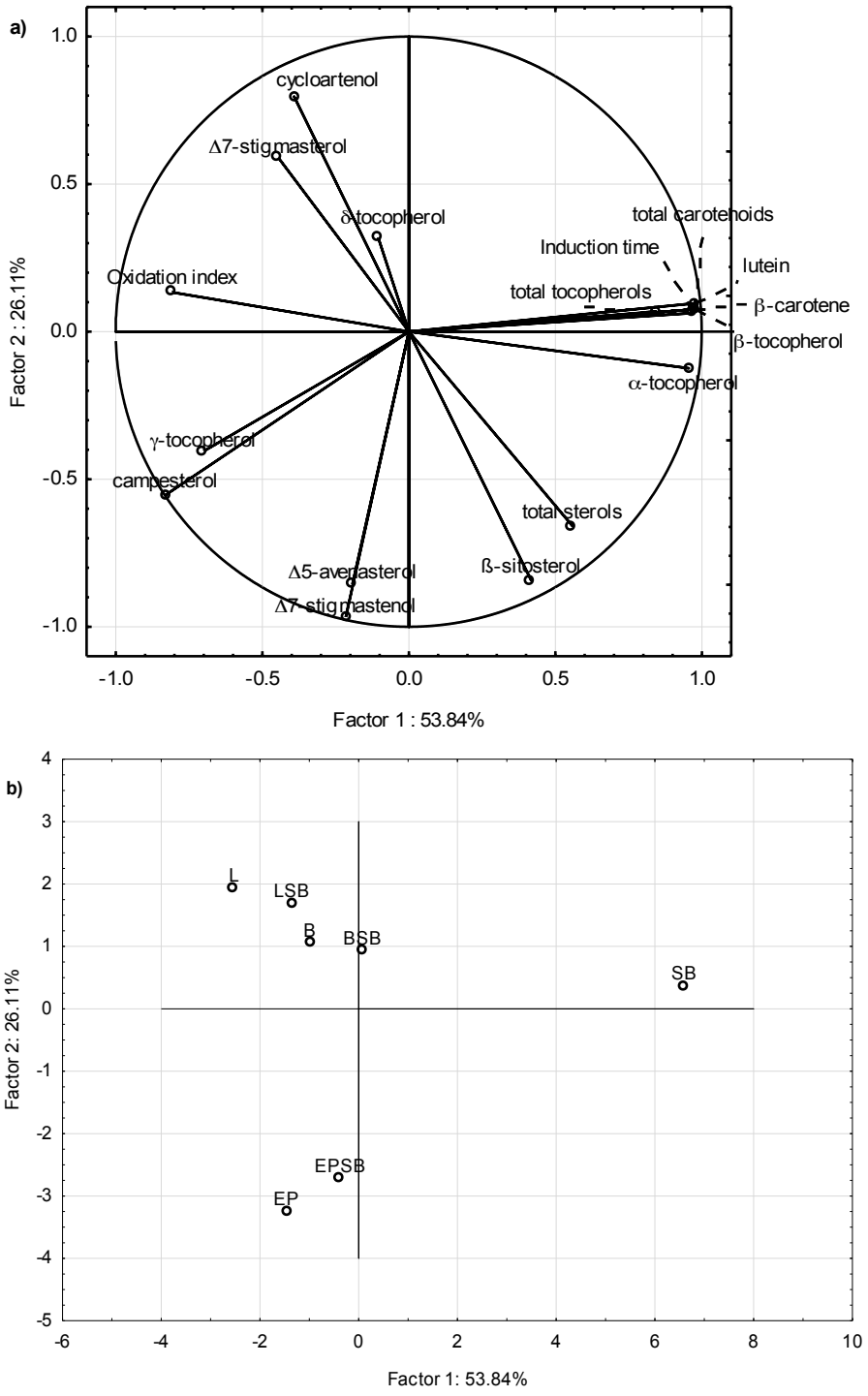

Figure 2: a) PCA loading plot of tested variables; b) Score plot of the two first principal components after PCA analysis of bioactive compounds content, oxidation index and induction time of fresh oils and their blends with sea buckthorn fruit oil.
B – borage seed oil, BSB – borage seed oil enriched with sea buckthorn fruit oil, EP – evening primrose seed oil, EPSB - evening primrose seed oil enriched with sea buckthorn fruit oil, L – linseed oil, LSB - linseed oil enriched with sea buckthorn fruit oil, SB - sea buckthorn fruit oil.

The relationship between the induction time for an oil and its potential oxidizability is rarely noted, since an oil is a complex mixture of compounds. Strong correlations could

possibly be noted for a pure phase of proper lipids. However, BHATNAGAR *et al*., (2009) demonstrated that the addition of coconut oil to refined sunflower oil and rice bran oil had a positive effect on the stability of blends due to a change to the proportions of fatty acids. Correlations between the induction time (stability of an oil) and the antioxidants content were found significantly more often (MATEOS *et al*., 2005). Hovewer, the in the case of mixture of antioxidants the resultant activity depends not only on their content and composition, but also on their synergistic or antagonistic activity, as well as lipophilic or hydrophilic properties (KMIECIK *et al*., 2011). A relationship between the stability of an oil and the content of β-carotene, being similar to that noted in this study, was found earlier by GOULSON and WARTHESEN (1999) in relation to high oleic rapeseed oil. They demonstrated that β-carotene significantly inhibited the oxidation of this oil in the dark at a concentration of approx. 5 mg/100 g, while with the simultaneous exposure, the antioxidant effect was already observed at a concentration of approx. 2.75 mg/100 g. However, the impact of particular antioxidant components is not only dependent on the concentrations at which they occur. Synergistic interactions which were demonstrated, *inter alia*, between α-tocopherol and β-carotene, are also important (SCHROEDER *et al*., 2006). Tocopherols are considered to be the main lipid antioxidants. CHOE and MIN (2006), referring to numerous studies, report that the tocopherols' capacity to quench free radicals depends on their structure and the concentration in the oil. According to the cited authors, the highest activity as regards quenching free radicals is exhibited by δ-tocopherol, and the value for this activity decreases for γ-, β- and α-tocopherol, respectively. The addition of α-tocopherol at an amount of 10 mg/100 g of oil may, in certain cases, even accelerate the oxidation (CHOE and MIN, 2006). In turn, with concentrations of tocopherols exceeding $4 \cdot 10^{-3}$ M, the activity of particular homologues does not differ significantly (JUNG *et al*., 1991).

Sterols were the predominant group of compounds in the unsaponifiable fraction of all oils under study. The noted low coefficient of correlation between their concentrations and the induction time indicates a weaker resultant effect of the action of these compounds on the oxidation mechanism. Earlier studies indicate that these compounds may exhibit both pro- and antioxidative action. A weak pro-oxidative action was demonstrated for, *inter alia*, β-sitosterol (LAMPI *et al*., 1999), while ergosterol, lanosterol, stigmasterol and cholesterol exhibit no antioxidative properties in relation to thermally oxidized safflower oil (SIMS *et al*., 1972). On the other hand, antioxidative activity is exhibited by sterols containing an ethylidene group within their structure (LAMPI *et al*., 1999). This group is found in, *inter alia*, Δ5-avenasterol which, in the oils tested as part of this study, occurred at an amount ranging from approx. 13 mg/100 g (linseed oil) to 63 mg/100 g (evening primrose oil). The noted increase in the content of Δ5-avenasterol by approx. 8% in enriched linseed oil could have affected the increase in the stability of this oil in relation to a non-enriched oil. The mechanism of antioxidative action of sterols having an ethylidene group involves their capacity to easily split off a proton, and form stable tertiary radicals. The resulting compounds are so durable that they do not initiate autooxidation (MAŁECKA, 1995). Our recent study showed that the thermal degradation of rapeseed oil sterols was faster than tocopherols and carotenoids (ROSZKOWSKA *et al*., 2015).

Analysis of plot score (Fig. 1b) confirmed a close similarity of linseed oil and evening primrose seed oil, and blends of these oils with sea-buckthorn oil. Then, a separate group comprised both borage seed oils (enriched and natural). Both groups of oils were clearly distinguished from sea-buckthorn oil in terms of the composition of fatty acids. On the other hand, other chemical components analysis (Fig. 2b) showed a close similarity of linseed oil and borage seed oil, and their blends with sea-buckthorn oil. In this case, a separate group comprised natural and enriched evening primrose seed oils.

4. CONCLUSIONS

Results of the study suggest that the development of blends of oils containing valuable and unique polyunsaturated fatty acids with sea-buckthorn oil being rich in antioxidant compounds increases the oxidative stability of these oils. In addition, the enrichment with sea-buckthorn oil contributes to both significant increase in the content of carotenoids (mainly β-carotene), α-tocopherol and β-sitosterol, and relative decrease in the share of polyunsaturated acids. Enriched oils gain new sensory qualities (the color derived from carotenoids) and health-promoting properties (the inhibition of radical formation, and the presence of numerous antioxidants); moreover, they have an extended shelf life.

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