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IMPROVING THE STABILITY OF COLD-PRESSED OILS BY THEIR ENRICHMENT IN SEA-BUCKTHORN OIL*

Sylwester Czaplicki, Małgorzata Tańska, Dorota Ogrodowska

Chair of Food Plant Chemistry and Processing
University of Warmia and Mazury in Olsztyn

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A b s t r a c t

Cold pressed oils from pumpkin and amaranthus seed are valued because of their health--promoting effect. Of particular importance are the contents of sterols in pumpkin seed oil and squalene in amaranthus oil, among others. Because of their high susceptibility to oxidation, methods of prolonging their shelf life are needed. One of such methods is to enrich them in antioxidants naturally occurring in plant oils.

This study analysed the opportunities to use of rich in antioxidants sea-buckthorn oil, including terpenoids, to increase the oxidation stability of cold pressed amaranthus and pumpkin seed oils.

The experiment involved blends of amaranthus and pumpkin seed oils with 0.5–12.0% of sea-buckthorn oil. In the oils and in the obtained blends, the fatty acid composition, the contents of selected terpenoid derivatives (carotenoids, tocopherols, sterols, squalene) and the oxidation stability as the induction time using a Rancimat apparatus were determined.

Sea-buckthorn oil rich in selected terpenoid derivatives proved to be effective in prolonging the shelf life of pumpkin and amaranthus seed oils, wherein the strongest relationship of oil stability indicators were observed in connection with carotenoid contents.

POPRAWA STABILNOŚCI OLEJÓW TŁOCZONYCH NA ZIMNO POPRZEZ ICH WZBOGACANIE OLEJEM ROKITNIKOWYM

Sylwester Czaplicki, Małgorzata Tańska, Dorota Ogrodowska

Katedra Przetwórstwa i Chemii Surowców Roślinnych
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: oleje tłoczone na zimno, terpenoidy oleju rokitnikowego, stabilizacja oleju, olej amarantusowy, olej dyniowy.

Address: Sylwester Czaplicki, University of Warmia and Mazury in Olsztyn, pl. Cieszyński 1, 10-726 Olsztyn, Poland, e-mail: selek@go2.pl
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Abstrakt

Tłoczone na zimno oleje dyniowy i amarantusowy cenione są ze względu na ich oddziaływanie prozdrowotne. Szczególne znaczenie ma zawartość steroli w oleju dyniowym oraz skwalenu w oleju amarantusowym. Z uwagi na ich wysoką podatność na utlenianie poszukiwane są metody przedłużania ich trwałości. Jednym z takich sposobów jest wzbogacenie ich w przeciwutleniacze naturalnie występujące w olejach roślinnych.

Za cel pracy postawiono określenie możliwości wykorzystania oleju rokitnikowego bogatego w antyoksydanty, m.in. terpenoidowe, do zwiększenia stabilności oksydacyjnej tłoczonych na zimno olejów amarantusowego i dyniowego.

W doświadczeniu sporządzono blendy olejów amarantusowego i dyniowego z olejem rokitnikowym w ilości 0,5–12,0%. W olejach i otrzymanych blendach określono skład kwasów tłuszczowych, zawartość wybranych pochodnych terpenoidowych (karotenoidów, tokoferoli, steroli, skwalenu) oraz stabilność oksydacyjną wyznaczaną jako czas indukcji w aparacie Rancimat.

Olej rokitnikowy bogaty w wybrane pochodne terpenoidowe okazał się skuteczny w przedłużaniu trwałości olejów dyniowego i amarantusowego, przy czym najsilniejszą zależność wskaźników stabilności olejów obserwowano w powiązaniu z zawartością karotenoidów.

Introduction

Plant oils in the human diet constitute a source of essential unsaturated fatty acids. The property distinguishing them from other fats is their high content of polyunsaturated fatty acids. In the contemporary diet of highly developed countries, improper diet balancing is often observed in the proportion of omega-3 and omega-6 fatty acids, which is an identified risk factor for the occurrence of so-called "diseases of affluence" (SIMOPOULOS 2001, SIMOPOULOS 2002, MOZAFFARIAN et al. 2005). Supplementation of the diet using appropriate plant oils is recommended in the prevention of these diseases. In the oils, non-glycerol components are also present, including terpene derivatives characterised by biological activity in the body, as well as by antioxidant activity (KELLY 1999, QUILES et al. 1999, BERG et al. 2000, BERGER et al. 2004, TANG et al. 2005, GUPTA et al. 2011). Antioxidant properties of tocopherols have long been used for the preservation of fat products. Squalene and carotenoids, among others, are also known for their ability to inhibit the oxidation of lipids (MAŁECKA 1994, MUELLER and BOEHM 2011). The biological function of the natural components is also significant, and introducing plant terpenoids (carotenoids, phytosterols, squalene, tocopherols) into fat products could maintain the health safety of food. Cold pressed amaranthus seed oil is a rich source of tocopherols, squalene and phytosterols. However, despite the fact that some of them have potential antioxidant activity, it does not protect the oil from oxidation changes. Similarly, pumpkin seed oil, in spite of certain amounts of squalene, sterols and tocopherols, undergoes the action of antioxidant factors. Plant oils with a high percentage of polyunsaturated fatty acids are particularly susceptible to

oxidation changes. Using synthetically obtained antioxidants for preservation of the plant oils helps to prevent and inhibit on-going oxidation changes. However, there are doubts raised by contradictory studies indicating both the harmful effect of these compounds and a lack of such effect on human health. Both opinions seem to be correct, since the activity of these compounds depends on the ingested dose. This dose, in turn, may be too high considering the multitude of products preserved with synthetic antioxidants (WILLIAMS et al. 1999, SARAFIAN et al. 2002, SOUBRA et al. 2007, GULTEKIN and DOGUC 2013). Introducing natural antioxidants into the products would increase their shelf life while maintaining their natural character. Methods of preventing oxidation of lipids in exactly such a way have long been sought. In many studies, the source of antioxidants are often, for example, plant extracts rich in natural antioxidants (ECONOMOU et al. 1991). Sea-buckthorn oil used in the work is valued because of its broad spectrum of biological activity (SURYAKUMAR and GUPTA 2011). It is particularly rich source of α-tocopherol and β-carotene which have documented antioxidant properties (SIES and STAHL 1995, GOULSON and WARTHESEN 1999).

For this reason, this study sought to assess the opportunities to use rich in terpenoid derivatives sea-buckthorn oil as a factor increasing the oxidation stability of cold pressed amaranthus and pumpkin seed oils.

Material and Methods

The pumpkin seed oil, amaranthus seed oil and sea-buckthorn fruit oil were used in this study. The seeds (cleaned, without foreign odour, moisture content not more than 8%) and fruits (harvested at the stage of full ripeness, without foreign odour) were purchased from "Szarłat" company (Łomża, Poland). Oils from seeds were obtained by cold pressing (temperature < 45°C) the raw material on a IBG Monforts & Reiners, Komet CA59G (Germany) laboratory expeller equipped with a 4 mm diameter nozzle and purified by centrifugation at 8000 x g on a Eppendorf centrifuge (type 5810R, Eppendorf AG, Hamburg, Germany). Sea-buckthorn fruit oil were obtained from lyophilised oleosomes (isolated from fruit juice) by hexane extraction.

Blends used in experiment were prepared triplicate by 0.5, 1.0, 2.0, 4.0, 8.0, 12.0% of sea-buckthorn oil addition to analysed pumpkin and amaranthus seeds oils.

In stabilised oils initial state of their rancidity were analysed. The acid (AV), peroxide (PV), and *p*-anisidine (*p*-AV) values were determined in accordance with procedures of EN ISO 660:2009 (CEN 2009), EN ISO 3960:2012 (CEN 2012), and EN ISO 6885:2008 (CEN 2008), respectively.

Fatty acids derivatization was done according to method described by Zadernowski and Sosulski (1978). Methylated fatty acids were analysed by gas chromatography with a GC-MS QP2010 PLUS (Shimadzu, Japan) system. Separationı 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. Obtained results of fatty acids composition were used to oxidation index (U) calculation according to formula given by COSGROVE et al. (1987): $U = (0.02 \cdot (C_{16:1} + C_{18:1}) + 1 \cdot C_{18:2})/100$.

Carotenoids in oils were analysed with a reversed phase high performance liquid chromatography (RP-HPLC) technique according to method previously described by CZAPLICKI et al. (2016). Carotenoids separation was performed at 30°C on a YMC-C_{30} 150 x 4.6 mm, 5 μm column (YMC-Europe GmbH, Germany) with the use a 1200 series liquid chromatograph manufactured by Agilent Technologies (Palo Alto, CA, USA), equipped with a diode array detector (DAD). Gradient of methanol – methyl tert-butyl ether (MTBE) was used as a mobile phase. Carotenoids were identified based on retention times and by comparing the UV–Visible absorption spectra of available standards (Sigma-Aldrich, USA). For quantitative analysis of carotenoids to the oil samples internal standard of β-Apo-8'-carotenal was added.

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 previously described by CZAPLICKI et. al (2011). 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 5α-cholestane internal standard solution was added (0.4 mg/g). 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. After silylation sterols were separated on ZB-5MSi (Phenomenex Inc., Torrance, CA, USA) capillary column. The quantifications using the internal standard method was done with the use of total ion current (TIC) mode at 100–600 m/z range.

The tocopherols analysis was carried out by high performance liquid chromatography (HPLC), according to the method described by CZAPLICKI et

al. (2011). The analysis was performed using a 1200 series liquid chromatograph manufactured by Agilent Technologies (Palo Alto, CA, USA), equipped with a fluorescence detector. 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.

Induction time of oils was measured on a Rancimat apparatus 743 (Metrohm, Herisau, Switzerland). The analysis was performed according to method described by FARHOOSH (2007). 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.

Statistical analysis

The 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. In order to develop the prediction model for oxidative stability of oils the linear regression and coefficient of determination (R^2) were estimated for each bioactive compound.

Results and Discussion

This study assessed the opportunities to use of sea-buckthorn oil, which is rich in bioactive terpenoid derivatives (α-tocopherol, carotenoids), to preserve cold pressed oils. In the experiment, freshly-pressed oils from amaranthus and pumpkin seeds were used. The oils had low acid values (AV) defining the degree of hydrolysis of oils, which for amaranthus and pumpkin seed oils were 2.33 and 1.88 mg KOH $\cdot$ g^{-1} of oil, respectively. High acid value (6.06 mg KOH $\cdot$ g^{-1}) in case of sea-buckthorn fruit oil was connected with very high organic acids concentration in fruits used to oil production. All used oils were characterised by a low degree of oxidation, indicated by low peroxide (PV) and anisidine values (p-AV). In this case, lower values were observed for amaranthus seed oil (PV = 0.17 mEq $O_2 \cdot kg^{-1}$ of oil; p-AV = 0.25). Pumpkin seed oil, despite having

slightly higher oxidation values, met the requirements of the Codex Alimentarius Commission standard for cold-pressed and virgin oils, determined as 4 mg KOH g^{-1}, and 15 mEq O_2 kg^{-1} of oil, respectively (Codex Alimentarius Commission 2001).

Table 1
Rancidity indices of amaranthus and pumpkin seed oils before enrichment with sea-buckthorn oil

Specification	Acid value [mg KOH g^{-1}]		Peroxide value [mEq O_2 kg^{-1}]		Anisidine value [–]	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Amaranthus seed oil	2.33	0.04	0.20	0.06	0.86	0.08
Pumpkin seed oil	1.88	0.02	1.96	0.27	2.95	0.68
Sea-buckthorn fruit oil	6.06	0.01	0.17	0.01	0.25	0.02

$\bar{x}$ – mean value, SD – standard deviation, $n = 3$

In oils, the most valuable are their unsaponifiable fraction components (carotenoids, squalene, sterols and tocopherols). There is many reports recommended their consumption in prevention of many diseases (TUCKER and TOWNSEND 2005, DEVARAJ and JIALAL 2006, FARVIN et al. 2006, GRATTAN 2013). Due to the oxidation of oils ingredients their value is lost during the storage. The fatty acid composition has a great effect on the susceptibility of oils to oxidation. In the discussed oils, a large proportion of fatty acids were unsaturated acids (about 70%). The acid composition of pumpkin seed oil suggests its greater susceptibility to oxidation. Its fatty acids are about 55% linoleic acid, which is about twice as susceptible to oxidation as monounsaturated oleic acid (COSGROVE et al. 1987), whose proportion in this oil constituted nearly 18%. Amaranthus seed oil had a slightly lower proportion of unsaturated acids, but the proportions of oleic acid (27%) and of linoleic acid (40%) were observed to be more favourable in these terms. The proportion of unsaturated acids in oil from sea-buckthorn fruit reached over 60%, but linoleic acid constituted only 12%. Such a proportion of fatty acids has an effect on the susceptibility of oils to oxidation changes. The oxidation index computed based on the relation formulated by COSGROVE et al. (1987) for amaranthus seed oil and pumpkin seed oil reached the values of 0.41 and 0.56, while the value computed for sea-buckthorn oil was only 0.16 (Table 2). This indicates that using sea-buckthorn oil as a source of natural antioxidants will also have a preserving effect on oils, by decreasing the value of the oxidation index.

Table 2

Fatty acids composition, oxidation indices and main bioactive compounds content in oils

Compound/discriminant	Amaranthus seed oil		Pumpkin seed oil		* Sea buckthorn fruit oil	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Fatty acids [%]						
palmitic	27.08^{a}	0.57	19.65^{b}	3.13	36.31^{c}	0.01
palmitoleic	nd^{a}		nd^{a}		40.97^{b}	0.04
stearic	5.01^{a}	0.07	7.43^{b}	1.01	0.55^{c}	0.01
oleic	27.27^{a}	0.21	17.73^{b}	0.15	8.77^{c}	0.11
linoleic	40.65^{a}	0.71	55.20^{b}	1.41	12.12^{c}	0.13
Oxidation index [–]	0.41^{a}	0.01	0.56^{b}	0.04	0.16^{c}	0.00
Carotenoids [mg · 100 g^{-1}]						
lutein	0.13^{a}	0.04	0.50^{b}	0.09	3.24^{c}	0.49
all-trans β-carotene	0.07^{a}	0.09	0.40^{b}	0.03	118.36^{c}	9.58
other carotenoids	0.04^{a}	0.01	0.26^{b}	0.02	74.82^{c}	6.25
total carotenoids	0.24^{a}	0.07	1.16^{a}	0.11	206.04^{b}	15.63
Tocopherols [mg · 100 g^{-1}]						
α-tocopherol	26.80^{a}	2.04	11.40^{b}	0.38	144.14^{c}	4.10
β-tocopherol	25.18^{a}	1.90	4.00^{b}	0.00	3.98^{c}	0.23
γ-tocopherol	9.20^{a}	0.83	49.69^{b}	0.21	4.63^{c}	0.32
δ-tocopherol	9.67^{a}	0.82	nd^{b}		0.75^{c}	0.00
total tocopherols	70.86^{a}	2.50	65.09^{b}	1.87	153.50^{c}	4.10
Sterols [mg · 100 g^{-1}]						
campesterol	18.41^{a}	0.52	nd^{b}		7.87^{c}	0.45
Δ5-avenasterol	251.14^{a}	1.68	nd^{b}		20.46^{c}	1.54
β-sitosterol	377.17^{a}	9.46	71.22^{b}	3.47	536.30^{c}	1.25
Δ7-stigmastenol	319.93^{a}	3.84	9.40^{b}	0.56	nd^{c}	
Δ7-stigmasterol	210.62^{a}	13.29	nd^{b}		nd^{b}	
Δ7-avenasterol	49.76^{a}	3.57	3.39^{b}	0.16	nd^{c}	
other sterols	71.93^{a}	2.02	53.42^{b}	8.26	262.81^{c}	16.61
total sterols	1299.0^{a}	0.12	137.4^{b}	0.68	855.9^{d}	8.95
Squalene [mg · 100 g^{-1}]	2560.8^{a}	358.65	310.6^{b}	12.5	nd^{c}	
Induction time [h]	4.46^{a}	0.06	7.50^{b}	0.32	>48^{c}	

* Sea buckthorn fruit oil characteristic data was published in work of CZAPLICKI et al. (2016)

$\bar{x}$ – mean value, SD – standard deviation, $n = 9$

Means in the same line with difierent letters are significantly difierent (P=0.05).

nd – not detected

The chemical characteristics of the oils proved that the preserved oils were poor in carotenoids (Table 2), with the dominant carotenoid being lutein – whose content was 0.13 and 0.50 mg · 100 g^{-1} in amaranthus and in pumpkin seed oils, respectively. Sea-buckthorn oil is an unusually valuable source of these components. The total carotenoid content of this oil was 206 mg · 100 g^{-1}, of which over 50% was β-carotene.

For tocopherols, the observed differences were not significant (Table 2). The content of these components in amaranthus and pumpkin seed oils was about 71 and 65 mg · 100 g^{-1}, respectively, and in amaranthus seed oil α- and

β-tocopherol dominated and their contents were comparable (about 25–27 mg · 100 g^{-1}). Tocopherols of pumpkin seed oil are 76% γ homologue, and the remaining 17.5% and about 6% are α- and β-tocopherol, respectively. Taking into account the antioxidant activity of tocopherols (MADHAVI et al. 1995), their favourable proportions can be observed in sea-buckthorn oil, in which nearly 94% constitutes α-tocopherol. The content of this homologue reached 144 mg · 100 g^{-1} in sea-buckthorn oil, which is nearly 13-fold higher than in pumpkin seed oil.

In terms of phytosterol contents, sea-buckthorn oil also exceeded pumpkin seed oil (Table 2). In both oils, the dominant compound was β-sitosterol, but in pumpkin seed oil its content was about 7.5-fold lower (71.22 mg · 100 g^{-1}). It was different for amaranthus seed oil, whose dominant sterol was β-sitosterol (about 320 mg · 100 g^{-1}), but its content was close to the contents of Δ7-stigmastenol and Δ5-avenasterol, and somewhat higher than of Δ7-stigmasterol. Although the content of the dominant β-sitosterol in sea-buckthorn oil was about 1.4-fold higher, in terms of the total sterol content, amaranthus seed oil was 1.5 times richer. Analysing the effect of an addition of sea-buckthorn oil on the phytosterols content in the obtained blend, it was found that it resulted in an increase in the total sterol content and in the β-sitosterol content in the composition with pumpkin seed oil, but it had a negative effect on the sterol content in the enriched amaranthus seed oil.

The situation was similar for squalene, which was not found in sea-buckthorn fruit oil (Table 2). Pumpkin seed oil was characterised by a squalene content in the amount of 310.6 mg · 100 g^{-1}, which is a high content among plant oils. Amaranthus seed oil, in turn, regardless of the method it is obtained with, is the richest plant source of squalene (CZAPLICKI et al. 2012). The squalene content in amaranthus seed oil is about 2,560 mg · 100 g^{-1} and, as in the case of pumpkin seed oil, an addition of sea-buckthorn oil lowered the squalene content in the product.

Taking into account the contents of the analysed bioactive substances and fatty acids composition, it is not surprising that the oxidation stability of sea-buckthorn oil was the highest (Table 2). The induction time for this oil exceeded 48 hours. The measurement results of the induction time of the other oils indicated amaranthus seed oil to be more susceptible to oxidation (4.46 h). The induction time determined for pumpkin seed oil was close to the times observed for cold pressed rapeseed oil (ROSZKOWSKA et al. 2015). Both of the studied oils proved to be far less stable than sea-buckthorn oil. BHATNAGAR et al. (2009) and HAMED and ABO-ELWAFA (2012) described the preservation of plant oils by mixing them with other, oxidatively-stable. In these studies, the authors preserved oils by introducing, e.g. the natural antioxidant of sesame oil (sesamin). However, they also considered the importance of the oxidative

stabilisation of oil by changing its fatty acid composition. Increasing the proportion of saturated fatty acids also resulted in an increase in the oxidation stability of the blend.

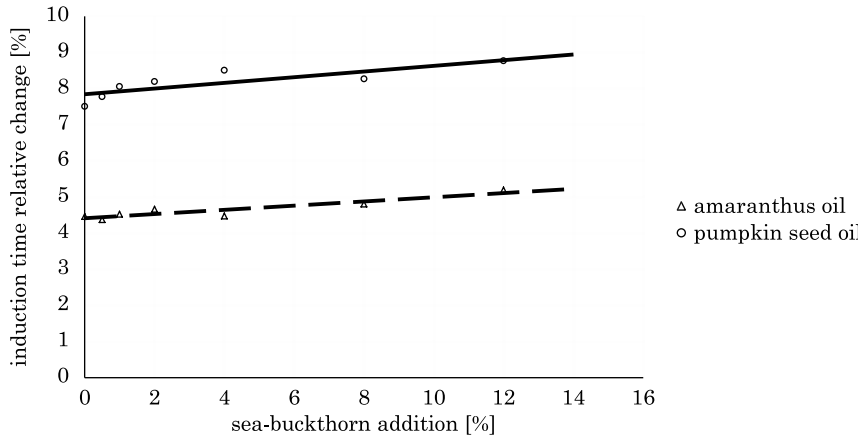

Fig. 1. Relation observed for induction time relative changes and percentage of sea-buckthorn oil addition in enriched oils

Table 3
The relation between the relative change in the induction time and the amount of the sea-buckthorn oil addition

	Linear regression equation	R^2	Induction time increase [min · 1%$^{-1}$ of sea-buckthorn fruit oil]
Amaranthus seed oil	$y = 1.2171x + 0.4102$	0.94	3.65
Pumpkin seed oil	$y = 1.0448x + 4.2568$	0.74	6.30

R^2 – regression coefficient, $n = 9$

Figure 1. presents the relation which was observed following analysis of enriched amaranthus and pumpkin seed oils. An addition of sea-buckthorn oil resulted in positive changes in the induction time of the studied oils. In the case of amaranthus seed oil, a slightly lower slope of the curve was observed compared to that obtained for pumpkin seed oil. In order to objectively compare the preservation efficiency of both oils, regression equations were determined for the relation between the relative change in the induction time and the percentage of the addition of sea-buckthorn oil. For pumpkin seed oil, the equation was characterised by a 10-fold higher value of the shift coefficient (Table 3). This suggests that the efficiency of the use of an addition of sea-buckthorn oil as a source of antioxidants is much higher for pumpkin seed

oil than for amaranthus seed oil, even for the lowest of the applied concentrations of sea-buckthorn oil. The induction times for the oils with enrichment levels from 0.5 to 12% increase gradually, and at the maximal addition they already assume similar values of about 16.5%. This represents a 50% increase in the stability obtained by GÁMEZ-MEZA et al. (1999) who, in order to preserve soy oil, used a 0.02% addition of butylated hydroxyanisole (BHA). The same concentration of tertiary butyl hydroquinone (TBHQ) used in their experiment resulted in a nearly three-and-a-half-fold increase in the induction time value. Numerous reports emphasise the efficiency of synthetic antioxidants in the preservation of plant oils (KHAN and SHAHIDI 2001, AZEEZ et al. 2013).

An increase in the induction time for the enriched oils, which was determined as an increase in the number of minutes as a result of the addition of each percent of the added sea-buckthorn oil is presented in Table 3. For amaranthus seed oil, this value reached 3.65 min · $1\%^{-1}$ and it was almost twice lower than for pumpkin seed oil (6.3 min · $1\%^{-1}$). These observations indicated that the addition of sea-buckthorn oil results in a change in the fatty acids composition and the contents of substances dissolved therein. In order to determine the effect of the amount of an addition of particular bioactive components of the studied oils on the stability of the obtained blends, the relations were determined between the contents of the components and the induction times determined for the mixtures.

Figure 2 presents the relations observed between the determined induction times of the studied oils enriched to a different degree with α-tocopherol, β-carotene and β-sitosterol, with a decrease in the squalene content. The β-carotene content in amaranthus seed oil with a 12% addition of sea-buckthorn oil increased to almost 13 mg · 100 g^{-1}, which is a value nearly 66-fold higher than the initial value. At the same time, a 66% increase was observed in the α-tocopherol content and a 12% increase in the β-sitosterol content. The content of squalene of which this oil is a rich source decreased by about 103 mg · 100 g^{-1} of oil. It is only a four percent decrease in the squalene content.

The obtained data were analysed by determination of the relation between the contents of particular antioxidants and the induction times of the mixtures (Table 4). It was found in amaranthus seed oil that lengthening the induction time by 0.73 hours had the greatest effect on the β-carotene content. This can be explained by the fact that the percent change in the β-carotene content was greatest. The literature emphasises the antioxidant properties of both β-carotene and of its metabolites (MUELLER and BOEHM 2011).

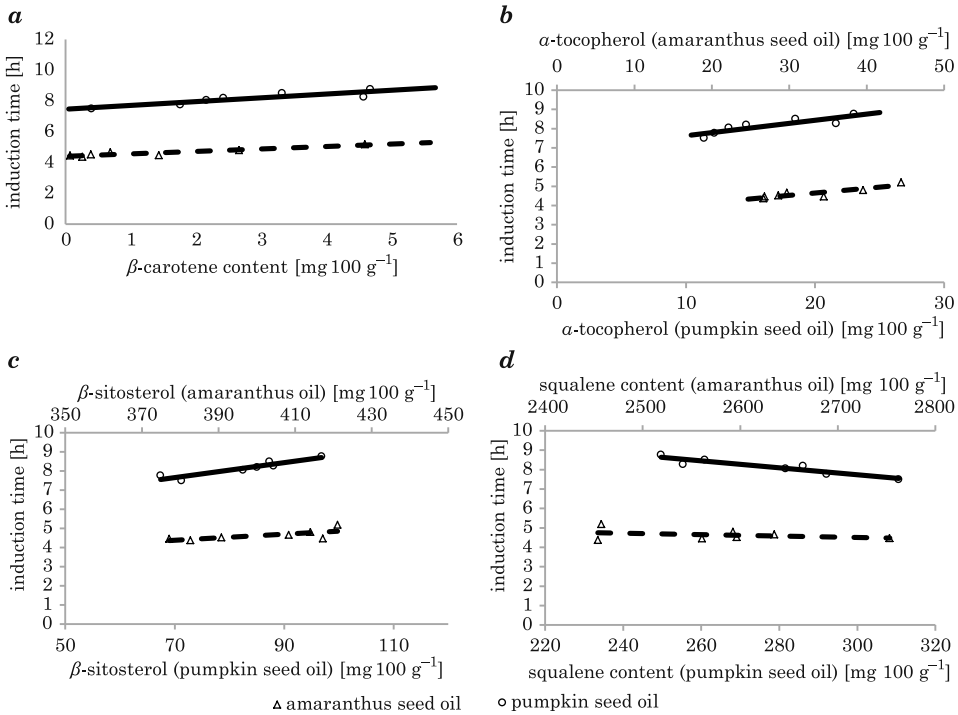

Fig. 2. Relations observed for induction time and bioactive compounds content in enriched oils

Table 4

Linear regression equations and determination coefficients (R^2) for the relation between the bioactive compound concentration and enriched oils induction time

Bioactive compound	Amaranthus seed oil		Pumpkin seed oil	
	linear regression equation	R^2	linear regression equation	R^2
β-carotene	$y = 0.2478x + 7.4655$	0.81	$y = 0.1594x + 4.4088$	0.87
α-tocopherol	$y = 0.0364x + 3.4403$	0.79	$y = 0.0802x + 6.8343$	0.77
β-sitosterol	$y = 0.0111x + 0.1944$	0.49	$y = 0.0391x + 4.9181$	0.87
Squalene	$y = -0.0179x + 13.105$	0.87	$y = -0.0009x + 6.9575$	0.11

R^2 – regression coefficient, $n = 9$

In pumpkin seed oil, together with an over 11-fold increase in the β-carotene content, an increase in the induction time by 1.26 hours was observed. This change was also connected with relatively smaller changes in the contents of other antioxidants. The β-sitosterol content increased by about 36% and the α-tocopherol content increased as much as 2-fold. At the same time, the content of squalene, as a result of its lack in sea-buckthorn oil,

decreased by 20%. Analysing the information presented in Table 4, it is possible to find that the change in the squalene content had the least effect on the oxidation stability of pumpkin seed oil.

As presented in Figure 3, preservation of amaranthus seed oil with sea-buckthorn oil results in a change in its natural colour. The high carotenoid content in sea-buckthorn oil is the reason that its 4% addition is noticeable and its 8–12% proportion may be a reason for its lack of acceptance among consumers. However, this colouration does not have to be perceived as a flaw. Cold pressed pumpkin seed oil also has intense colouration, which is not an obstacle in its wide use in gastronomy and as a health-promoting dietary supplement. What is important is the benefit resulting from increasing the shelf life of oils thanks to the use of natural antioxidants of sea-buckthorn oil. In both preserved oils, supplementation with sea-buckthorn oil at the level of 12% resulted in a 16% increase in the stability of oils measured by the induction time. The literature also describes the use of natural antioxidants of oils for stabilisation during frying. Lavender and thyme herbs also have a positive effect on the stabilisation of sunflower oil. In sunflower oil, by reacting with free radicals created under the effect of heating, antioxidants of the herbs may prevent degradation of tocopherols (BENSMIRA et al. 2007). A similar antioxidant effect was found by the use of cassia essential oil used as cooking oil and its optimum content was 0.012% (DU and LI 2008).

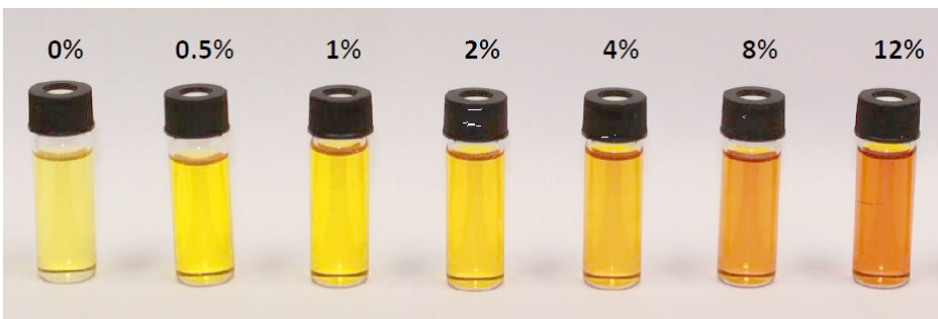

Fig. 3. The amaranthus oil with varying degree of sea-buckthorn oil enrichment

Conclusions

The results of the presented studies confirm the effectiveness of an addition of sea-buckthorn oil as a source of natural antioxidants, especially α-tocopherol and carotenoids, to improve the oxidation stability of cold pressed amaranthus and pumpkin seed oils. Supplementation with sea-buckthorn oil

has the greatest effect on changes in the contents of carotenoids. The data indicate greater dependence between these changes and the oxidation stability of the preserved oils than for tocopherols.

The results indicate that on the obtained blended stability has a greater influence natural antioxidants concentration than fatty acid composition. This thesis is confirmed by the enriched amaranthus and pumpkin seed oil "oxidation index" and "induction time" values relation. Pumpkin seed oil significantly exceeded amaranthus seed oil in its carotenoid content, and was richer in polyunsaturated fatty acids.

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