Effect of biologically active substances on oxidative stability of flaxseed oil

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Abstract The enrichment of flaxseed oil, which is a valuable plant source of PUFA omega-3, fat-soluble vitamins and other biologically active substances (BAS), makes it possible to strengthen the therapeutic and prophylactic effect of flaxseed oil. The study of the effect of BAS additives on the oxidative stability of flaxseed oil is an important step in the process of creating products based on enriched flaxseed oil. Experiments were conducted to investigate the influence of added BAS (coenzyme Q₁₀, β -carotene, lutein, zeaxanthin, α -tocopherol, α -tocopherol acetate, cholecalciferol, selenomethionine) on flaxseed oil oxidation stability. Kinetic data on accumulation of primary and secondary oxidation products, free fatty acids in flaxseed oil, as well as the consumption of BAS added to the oil during its storage, were obtained. Experimental results showed that the BAS could have both antioxidant and pro-oxidant properties depending on their chemical structure and concentration. Coenzyme Q₁₀, carotenoids and selenomethionine at concentrations higher than 100, 10 and 0.5 mg/100 g respectively, accelerate significantly ($p < 0.05$) the oxidation of flaxseed oil. An addition of 5 mg/100 g β -carotene inhibits formation of flaxseed oil oxidation products. The co-influence of synthetic and natural oxidation inhibitors with BAS on oxidative stability of flaxseed oil was studied. The fat-soluble esters of ascorbic acid and their compositions with natural antioxidants based on beans and soybeans appeared to be effective and safe stabilizers of flaxseed oil enriched with BAS. Resulting

from the studies, new oxidation-resistant functional food products based on flaxseed oil are launched into manufacturing.

Keywords Flaxseed oil · Oxidative stability · Biologically active substances · Antioxidants · Functional food

Introduction

At present, it is generally recognized that polyunsaturated fatty acids (PUFAs) omega-3 have the exceptional importance for maintaining physical and mental health, preventing a variety of diseases (Connor 2000). On the other hand, modern human diet is critically lacking omega-3 fatty acids (Simopoulos 2002). The consumption of flaxseed oil could significantly eliminate their deficiency and improve the diet since it is the richest plant source of alpha-linolenic acid (ALA) belonging to the family of the omega-3 PUFAs. Due to the high ALA content, flaxseed oil can affect eicosanoids production, procoagulant activity and other membrane-bound reactions and exhibit hypolipidemic, hypotensive, antiallergic, antiarrhythmic and thrombolytic properties. Numerous studies have shown that flaxseed oil contributes to the prevention and treatment of cardiovascular, oncological and some other diseases (Thompson and Cunnane 2003; Pan et al. 2012). Positive influence of flaxseed oil on human health could be strengthened by enriching the oil with fat-soluble vitamins and other biologically active substances (BAS) (tocopherols, carotenoids, coenzyme Q₁₀, selenium, vitamin D, etc.). Nowadays, human dietary patterns are insufficient in these BAS, which are known to be very important for regulating metabolic processes and proper functioning of some organs and systems (Cooke et al. 2002; Brenneisen

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et al. 2005; Ernster and Dallner 1995; Holick 2004). Therefore, the development and production of flaxseed oil-based functional foods is one of the possible ways to optimize human nutrition at the current stage. Due to the combination of high omega-3 PUFAs content with the presence of vitamins and vitamin-like substances in such products, they can quickly and effectively correct metabolic processes with deficiency of essential nutrients and be an effective factor in disease prevention.

At the same time, high levels of ALA cause high oxidation susceptibility of flaxseed oil, which results in the formation of toxic compounds, change in taste, odor and color of the oil, decrease in nutritional value in a short period of its storage. On the other hand, in conditions of antioxidant (AO) deficiency in a body, the intake of PUFAs can lead to the induction of lipid peroxidation (LPO), the formation of free radicals, which in turn leads to an increase in atherogenicity and carcinogenesis (Halliwell and Gutteridge 2007; Schultz and Yarbrough 2004). To ensure a positive effect on human health, long-term consumption of flaxseed oil and other polyunsaturated oils for therapeutic and prophylactic purposes should be accompanied by an additional intake of AOs that effectively inhibit the processes of LPO in the body.

Thus, the development and production of foods based on enriched flaxseed oil pursue the following main goals:

1. strengthening the therapeutic and prophylactic effects of flaxseed oil;
2. adequate provision of the body with antioxidants simultaneous with the PUFA intake;
3. increasing the stability of flaxseed oil to oxidative changes and thereby increasing the permissible shelf life.

The present study was carried out to evaluate the effect of a number of BAS (vitamins E and D, coenzyme Q₁₀, carotenoids, selenomethionine) on the oxidative stability of flaxseed oil in order to develop new, oxidation-resistant functional food products based on flaxseed oil. Their use by humans could improve the diet and contribute to the prevention and treatment of a wide range of diseases.

Materials and methods

Materials

Flaxseed oil used in the study was supplied by Club Farm-Eco Ltd. Company (Belarus). The oil was produced by cold pressing with oil expeller from dry, purified flaxseeds (the oil temperature at the exit from the press did not exceed 38 °C), followed by settling for 24 h.

Haricot beans and soybeans were supplied by UE “Agromarket” company, and prior to their use they were dried in a drying cabinet at the temperature of (40 ± 1) °C to a humidity of no more than 8%, after which they were comminuted in a grain mill to the size of comminuted particles of 1–2 mm. Shredded beans and soybeans (1–2 mm) were put into glass bottles, in which flaxseed oil was then added, concentration of vegetable additive was equal to 0.8% (w/w).

The following reagents were used: DL- α -tocopherol ($\geq 96\%$, high performance liquid chromatography (HPLC) grade), coenzyme Q₁₀ ($\geq 98\%$, HPLC grade) from Sigma-Aldrich, DL- α -tocopherol acetate ($\geq 96\%$, HPLC grade), DL-selenomethionine ($> 98\%$, thin layer chromatography (TLC) grade), cholecalciferol (vitamin D₃) ($\geq 98\%$, HPLC grade), (6-O-palmitoyl-L-ascorbic acid (AP) ($\geq 99\%$) manufactured by Sigma; 6-O-stearoyl-L-ascorbic acid (AS) ($\geq 95\%$) produced by ChemExper. β -Carotene 30% FS, FloraGLO Lutein 20% SAF, OPTISHARP (Zeaxanthin) 20% FS were from DSM Nutritional Products Ltd.

The following analytical standards were used: a mixture of fatty acid methyl esters (FAMES) RM-2 (C16–C18) from Supelco, methyl heptadecanoate ($\geq 99\%$), 5- α -cholestane (97%, GC), coenzyme Q₉ ($\geq 96\%$, HPLC grade) from Sigma-Aldrich. Tocopherol Set (α -, β -, γ -, δ -tocopherols) was produced by Calbiochem (Merck), DL- α -tocopherol acetate (analytical standard from Supelco), lutein ($\geq 97\%$, HPLC grade), zeaxanthine ($\geq 95\%$, HPLC grade) from Sigma-Aldrich, β -carotene ($\geq 97\%$ (UV) from Sigma. Phytosterols: β -sitosterol ($\geq 95\%$), campesterol (70%), stigmasterol (95%), cycloartenol ($\geq 90\%$), was supplied from Sigma-Aldrich. The HPLC solvents were of chromatographic purity purchased from Sigma-Aldrich. All the reagents and standards used in this study were supplied by Belleshimkomplekt, Belarus.

Preparation of BAS solutions in flaxseed oil

Coenzyme Q₁₀, ascorbic acid esters were added to the flaxseed oil in the form of 1% (w/w) solutions in flaxseed oil prepared by dissolving an appropriate amount of a test compound with constant heating and stirring under N₂ atmosphere until a clear solution was obtained. β -Carotene, lutein and zeaxanthin were added to flaxseed oil in the form of 0.2% (w/w) flaxseed oil solutions obtained by dissolving oil suspensions of carotenoids in the flaxseed oil. The temperature required for preparing oil solution of Q₁₀ did not exceed 50 °C, of β -carotene – 65 °C, of lutein and zeaxanthin – 85 °C, of ascorbyl palmitate and ascorbyl stearate – 105 °C. Heating was carried out until additives were fully dissolved in flaxseed oil. 1%-solutions of α -tocopherol, α -tocopherol acetate and solutions of cholecalciferol (2 mg/100 g) in the oil were prepared without

heating. The solutions prepared in this way were added to the oil to obtain the required concentration. Selenomethionine oil solutions were prepared in the required concentration for research without heating.

Evaluation of oxidative stability

An accelerated kinetic method was used to evaluate the oxidation rate of flaxseed oil. Reference samples of the oil with a mass of (100 ± 0.1) g and test samples with the BAS additives and stabilizers were stored for 12 months and longer in the dark at room temperature (20 ± 5) °C and with free access of air oxygen. The ratio of the surface area that comes into contact with air to the oil volume was kept constant for the required time and was equal to 0.16 cm^{-1} . For each concentration of an additive, a set of oil samples was prepared. Periodically, at intervals of 1–2 months, three vials were withdrawn from each sample series to determine the amount of hydroperoxides and other indicators of the oil quality.

The flaxseed oil oxidative stability was also evaluated through standard accelerated oxidation procedure (ISO 6886 2016) using 892 Professional Rancimat apparatus. The oxidation procedure was performed at 100 °C, air flow rate of 20 l/h and oil sample weight of 3 g. The StabNet 1.0 software was used for automatic recording of oxidation induction period (IP). At least three IP measurements were performed for each sample, and the average value was calculated. The stabilization efficiency (stabilization factor) was evaluated by the ratio of oxidation induction periods of flaxseed oil with stabilizing additives and without them.

Acid, peroxide, p-anisidine and iodine values

The acid, peroxide, p-anisidine and iodine values (AV, PV, p-AnV, IV) of the oil samples were measured following ISO standard methods (ISO 660 2009; ISO 3960 2007; ISO 6885 2006; ISO 3961 2013).

Fatty acid composition

Fatty acid methyl esters were prepared in accordance with the standard method (ISO 12966-2 2011). To determine fatty acid (FA) composition of the oils, the respective methyl ester samples were analyzed by gas chromatography (GC) using a Shimadzu GC-2010 instrument fitted to flame-ionization detector (FID). Separation was made using a Stabilwax-DA (Restek) capillary column ($30 \text{ m} \times 0.53 \text{ mm ID}$, $1.0 \mu\text{m}$ film thickness). The flow rate of the carrier gas (nitrogen) was adjusted to 1.9 ml/min, the temperature of the detector was 240 °C, while the column temperature was programmed at a ramp of 5 °C/

min beginning from 120 to 240 °C, and then kept isothermally for 20 min at 240 °C. FA content was calculated using the internal standard method, methyl heptadecanoate was used as a standard.

Determination of minor components and BAS additives in flaxseed oil

Tocopherols content in flaxseed oil was determined according to the methods stipulated in ISO (ISO 9936 2008) using an HPLC instrument equipped with a fluorescence detector RF-10AXL (Shimadzu). A LiChrospher Si 60 column ($250 \text{ mm} \times 4 \text{ mm ID}$, particle size of $5 \mu\text{m}$) was utilized at 25 °C. The flow rate of the mobile phase, which was 5% *tert*-butyl ether solution in *n*-heptane, was adjusted to 1.0 ml/min.

Individual phytosterol content in the samples was determined using the GC method after samples saponification and isolation of the unsaponifiable fraction without derivatization of sterols as reported by Choong et al. (1999). Analysis was performed utilizing a Rtx-1 (Restek) capillary column ($30 \text{ m} \times 0.32 \text{ mm}$, $0.5 \mu\text{m}$ film thickness). The flow rate of nitrogen as a carrier gas was 1.1 ml/min, and FID temperature was maintained at 300 °C, while the column temperature was programmed at a ramp of 6 °C/min beginning from 150 to 300 °C, and then kept isothermally for 25 min at 300 °C. 5- α -cholestane was used as an internal standard.

Carotenoid content was determined using reversed-phase HPLC in gradient elution mode as reported by Aman et al. (2005). Chromatography was executed utilizing YMC C30 column ($150 \text{ mm} \times 4.6 \text{ mm}$, particle size of $5 \mu\text{m}$) and under gradient conditions with the following mobile phase composition: A—an acetone–water mixture (60:40, v/v), B—acetone; Linear gradient elution (1–10 min (B from 0 to 100%), 10–20 min (B 100%)) with a flow rate of 0.8 ml/min was used; The column oven was conditioned at 25 °C, UV–VIS detector was set to a wavelength of 446 nm. The method allows all carotenoids to be separated, including zeaxanthine and lutein.

The analysis of coenzymes Q content was performed by reversed-phase HPLC method in isocratic mode as reported by Qu et al. (2009). LiChrospher 100 RP-C18 column ($250 \text{ mm} \times 4 \text{ mm ID}$, particle size of $5 \mu\text{m}$) was used at 25 °C. The mobile phase was consisted of methanol, hexane, acetic acid, 2-propanol (345:80:8:8, v/v/v/v) and sodium acetate 3.4 g. The eluent flow rate was 1.0 ml/min and UV–VIS detector was set to a wavelength of 270 nm.

Statistical analysis

The data obtained were statistically processed using Statistica v. 10 software (StatSoft, Poland). The results were

expressed as mean $\pm$ standard deviation (SD) from 3 independent parallel experiments. The data were analyzed using the *t* test for independent samples. The differences were considered to be significant at $p < 0.05$.

Results and discussion

Compounds capable to enhance the therapeutic and prophylactic effects of flaxseed oil were selected as the additives for the oil enrichment. Some of the substances could show synergy with natural and synthetic antioxidants in inhibition of oxidative changes in flaxseed oil, and increase its shelf life. Such BAS as α -tocopherol, α -tocopherol acetate, β -carotene, lutein, zeaxanthin, coenzyme Q₁₀, selenomethionine, cholecalciferol (vitamin D₃) were used.

Characteristics of the investigated flaxseed oil

General specifications of the tested flaxseed oils are presented in Table 1. Low peroxide, acid and anisidine values characterizing the degree of oxidative deterioration of vegetable oils (content of hydroperoxides, free fatty acids and total content of secondary carbonyl oxidation products, respectively) indicate high quality of flaxseed oil samples taken for testing. According to the experimental data, the flaxseed oil contains a large amount of PUFAs; the share of ALA (omega-3) is 76.0–80.7% of the total amount of FA. The oil also contains the complex of minor components—tocopherols, carotenoids, coenzymes Q, phytosterols, phospholipids and a number of other compounds, largely ensuring the oxidative stability of the oil. The main components of the endogenous antioxidant system are tocopherols. Tocopherols are the most common AO in vegetable oils; they compete with unsaturated oils for peroxide radicals. Lipid peroxy radicals react with tocopherols much faster than with lipids. One tocopherol molecule can protect about 10^3 – 10^8 polyunsaturated fatty acid molecules at low peroxide value (Kamal-Eldin and Appelqvist 1996). In the studied flaxseed oil, the fraction of γ -tocopherol, which is the main form of vitamin E in food products, accounts for 91.9–94.1% of the total amount of tocopherols. In most cases γ -tocopherol behaves as an antioxidant in vegetable oils that stronger than α -tocopherol (Kamal-Eldin and Appelqvist 1996; Elisia et al. 2013). In the studied oil samples the concentration of native γ -tocopherol is in the range of 1.19×10^{-3} – 1.40×10^{-3} mol/l. The main carotenoid of the flaxseed oil is lutein, the content of which is 71.7–73.3% of the total amount of carotenoids, the share of β -carotene accounts for 8.2–11.9%.

When enriching flaxseed oil with BAS, it is necessary to investigate their effect on the oxidative stability of the oil.

Table 1 Characteristics of the studied flaxseed oil

Characteristics	Values
Fatty acids (g/100 g)	
C 16:0	4.5–5.9
C 18:0	3.1–4.4
C 18:1	13.1–20.1
C 18:2	15.3–16.6
C 18:3	52.6–63.8
Other	0.2–0.4
Total PUFA	69.2–79.0
Tocopherols (mg/100 g)	
γ	49.4–58.6
α	1.4–1.7
δ	2.3–2.6
Total	53.7–62.3
Carotenoids (mg/100 g)	
β -carotene	0.2–0.4
Lutein	1.9–2.6
Other	0.5–0.6
Total	2.6–3.6
Coenzymes (mg/100 g)	
Q ₁₀	2.9–4.2
Q ₉	1.1–2.1
Phytosterols (mg/100 g)	
β -sitosterol	159.5–208.3
Campesterol	103.4–131.8
Cycloartenol	196.4–217.4
Other	68.0–86.2
Total	527.4–643.7
PV (meq O ₂ /kg)	0.8–1.2
AV (mg KOH/g)	0.6–1.0
p-AnV	0.5–1.1
IV (g I ₂ /100 g)	177.4–192.5
IP at 100° (h)	3.8–5.6

Such a study is an important step in the process of creating products based on enriched flaxseed oil.

Effect of BAS additives on oxidation stability of flaxseed oil

The effect of BAS on the oxidative stability of flaxseed oil depending on the concentration and composition of the additives has been studied. For this purpose, kinetic curves of the accumulation of free fatty acids, primary and secondary oxidation products in flaxseed oil were obtained. Likewise, kinetic curves of the consumption of BAS during the storage of the oil with BAS additives under conditions of accelerated oxidation with free access of air at room

temperature were plotted. The data characterizing the process of oxidation under these conditions make it possible to simulate the process of oxidative “aging” of flaxseed oil, which takes place after opening a consumer pack and being exposed to air oxygen. Concentrations of BAS, used to enrich flaxseed oil, were selected with consideration of the recommended daily intake values of BAS (EFSA 2017; Sarmiento et al. 2016).

Taking into consideration the presence of endogenous minor components in flaxseed oil, it is necessary to account for the possibility of intermolecular interactions of AO in oxidation processes with vitamins, other BAS and selecting antioxidants ensuring effective stabilization of the oil in case of its enrichment. Interactions among antioxidants can be synergistic, antagonistic, or merely additive. Thus, coenzyme Q₁₀ in its reduced form (ubiquinol) participates in the regeneration of tocopherol and these two AOs were found to manifest synergism (Quinn et al. 1999). In the case of flaxseed oil enrichment with coenzyme Q₁₀, one should not ignore the possibility of antagonism between γ -tocopherol (phenolic AO) and coenzyme (quinone AO) (Storozhok et al. 1995). Tocopherol showed synergistic effects with β -carotene to decrease the autoxidation (Palozza and Krinsky 1992) and photosensitized oxidation of soybean oil (Choe and Min 2006). However, Henry et al. (1998) showed that there was no cooperative interaction between α -tocopherol and β -carotene in delaying the onset of safflower seed oil oxidation at 75 °C. It was also stated that antagonism between α -tocopherol and β -carotene was exhibited in the oxidation of esters of polyunsaturated fatty acids (Storozhok et al. 1995). α -Tocopherol showed synergism with ascorbic acid, ascorbyl palmitate (Kamal-Eldin and Appelqvist 1996; Niki 1996) as well as with phospholipids (Storozhok et al. 1995; Choe and Min 2006).

Coenzyme Q₁₀

Data on the changes in the content of hydroperoxides and secondary oxidation products for one of the samples of flaxseed oil with additives of coenzyme Q₁₀ at different concentrations during the storage period are shown in Fig. 1. According to Fig. 1, the oxidative stability of flaxseed oil added with coenzyme Q₁₀ depends on the concentration of the additive. There were no significant differences ($p > 0.05$) in concentration of hydroperoxides and secondary oxidation products between additives free oil and oil enriched with 100 mg/100 g coenzyme Q₁₀ (graphs 3 and 4, Fig. 1). As for the additive concentrations higher than 100 mg/100 g, the accumulation of hydroperoxides and secondary oxidation products, as well as free fatty acids (FFA), is intensified in these samples if compared to the oil without additives. The content of oxidation products in the oil with 150 mg and 200 mg/100 g

additives is significantly ($p < 0.05$) higher than in the oil without additives. Thus, after storage for 12 months the concentration of hydroperoxides increases by 35% in the oil containing 150 mg/100 g coenzyme, 200 mg/100 g—by 85% if compared with the additives free oil. Based on the data on the change in PV during storage the oxidation induction period values of flaxseed oils without additives and with BAS additives were found. Addition of 200 mg/100 g of coenzyme to the studied oil samples decreases induction period by 1.5–1.7 times in comparing to the oil without additives.

The effect of coenzyme Q₁₀ additives on the accumulation of FFA in flaxseed oil is less obvious than the influence of oxidation products. Thus, after storage for 12 months the acid value increases for the oil sample without additives from 0.60 ± 0.05 (for the initial oil) to 0.82 ± 0.09 mg KOH/g; for the oil containing 100 and 150 mg/100 g coenzyme Q₁₀ there were no significant differences ($p > 0.05$) in AV in comparison with the additives free oil sample. Significant differences ($p < 0.05$) in FFA content were observed between the oil containing 200 mg/100 g coenzyme and additives free oil. AV of the oil enriched with 200 mg/100 g coenzyme increased up to 1.18 ± 0.11 mg KOH/g during storage for 12 months, which was 44% higher than the same of additives free oil.

The prooxidant effect of coenzyme Q₁₀ in flaxseed oil can be related to the possibility of coenzyme involvement in LPO processes in flaxseed oil. In particular, coenzyme can participate in electron transfer reactions with the formation of semiquinone anion-radicals, which in turn promote the development of chain processes of lipids oxidation, entering into redox reactions with the hydroperoxide substrates:

Carotenoids

Figure 2 presents kinetic curves of hydroperoxides and secondary oxidation products accumulation in flaxseed oil with β -carotene additives in various concentrations during the storage period. The addition of β -carotene to the flaxseed oil in concentration of 5 mg/100 g (9.3×10^{-5} mol/l) gives the stabilizing effect: accumulation of oxidation products slows down, after 12 months their concentration decreases twice if compared to the oil without additives. When the concentration of the additive is increased up to 10 mg/100 g, no significant differences ($p > 0.05$) between the oxidative stability of flaxseed oil with the

Fig. 1 Accumulation of hydroperoxides (a) and secondary oxidation products (b) during storage of flaxseed oil with coenzyme Q₁₀ additives at room temperature and with free air access: 1—CoQ₁₀, 200 mg/100 g; 2—CoQ₁₀, 150 mg/100 g; 3—no additives; 4—CoQ₁₀, 100 mg/100 g

Fig. 2 Changes in peroxide value (a) and *p*-anisidine value (b) of flaxseed oil with additives of β-carotene stored at room temperature and with free air access: 1—Car, 25.0 mg/100 g; 2—Car, 20.0 mg/

100 g; 3—Car, 15.0 mg/100 g; 4—no additives; 5—Car, 10.0 mg/100 g; 6—Car, 5.0 mg/100 g

additive and the control sample (oil without the additive) appear. A further increase in the content of β-carotene leads to a significant ($p < 0.05$) intensification of oxidation and oxidative destruction processes in flaxseed oil: after 12 months storage the concentration of hydroperoxides in the oil containing 25 mg/100 g β-carotene increases by 2.6 times if compared with the additive free oil. The induction period values for the studied samples of flaxseed oil with 25 mg/100 g of β-carotene were 1.3–1.5 times lower as compared with the oil without additives.

Similar dependences were obtained for the flaxseed oil enriched with lutein in the concentration range of 5–25 mg/100 g and zeaxanthin in the concentration range of 1.5–15.5 mg/100 g: at concentrations above 5 mg/100 g these carotenoids have a pro-oxidant effect, i.e. the accumulation of oxidation products in their presence is significantly ($p < 0.05$) intensified, that indicates a decrease in the oxidative stability of flaxseed oil. Figure 3 shows the dependence of PVs on the concentration of the lutein

Fig. 3 Dependence of content of hydroperoxides in flaxseed oil from concentration of additives of a lutein after 6 (2) and 10 (1) months of storage of oil at the room temperature and with free air access

additive in flaxseed oil stored for 6 and 10 months. The concentration of hydroperoxides in oil enriched with lutein over 10 mg/100 g significantly ($p < 0.05$) increases after 6 months storage if compared with the oil without additives: 1.8, 2.4 and 3.4 times more for 15, 20 and 25 mg/100 g lutein correspondingly. After 10 months storage the concentration of hydroperoxides in oil with lutein additive over 5 mg/100 g increases if compared with the control sample: its values are 2.2, 4.2, 7.1 and 11.1 times higher with lutein 10, 15, 20 and 25 mg/100 g correspondingly.

It was previously shown that β -carotene was a pro-oxidant in soybean oil oxidation process in the dark (Lee et al. 2003). The prooxidant effect of carotenoids in flaxseed and other vegetable oils is caused by the possibility of their participation in LPO processes. Carotenoids have a conjugated polyene chain with alternating double bonds in their structure. Due to the conjugation of π -bond electrons, these compounds can be easily oxidized and reduced to form stable ion radicals. The β -carotene radical cation, which has a higher one-electron reduction potential (1060 mV) than the PUFA (600 mV), can act as a pro-oxidant due to the detachment of the hydrogen atom from PUFA and generation of new fatty acid radicals (Lee et al. 2003). In addition, peroxide lipid radicals may be added to β -carotene molecules and produce carotene peroxy radical ($\text{ROO}-\text{Car}\cdot$), especially at higher than 150 mm Hg of oxygen (Burton and Ingold 1984). The carotene peroxide radical reacts with oxygen and then with lipid molecules, and produces lipid alkyl radicals, which propagate the chain reaction of lipid oxidation (Iannone et al. 1998):

During the storage of flaxseed oil, the content of carotenoids is reduced due to the ongoing oxidation processes. The loss of carotenoids increases with an increase in the initial concentration of the additive, as shown in Fig. 4 by the example of β -carotene. After 12 months of storage of the flaxseed oil containing β -carotene at the concentration of 5, 10, 15 and 25 mg/100 g (initial concentration of the additive), the loss of the additive was equal to 39.1, 43.4, 55.7 and 66.8% respectively.

Among the products of carotenoids transformations in lipid model systems and vegetable oils hydroxy- and epoxycarotenes were found (Anguelova and Warthesen 2000). In lipid model systems, high-molecular-weight compounds containing β -carotene were also discovered; these compounds may be formed in reactions involving β -carotene radical cations, free radicals of fatty acids and/or triplet oxygen (Tsuchihashi et al. 1995).

Fig. 4 Changing of β -carotene concentrations in flaxseed oil during storage at room temperature and with free air access depending on initial concentration of additive: 1—25 mg/100 g, 2—15 mg/100 g, 3—10 mg/100 g, 4—5 mg/100 g

Selenomethionine, α -tocopherol and cholecalciferol

The dependence of peroxide and anisidine values of flaxseed oil without additives and with selenomethionine additives in the concentration range of 0.22–1.10 mg/100 g on oil storage time at room temperature and with free access of air oxygen has been studied. The data obtained have shown that there were no significant differences ($p > 0.05$) in accumulation rate of primary and secondary oxidation products between the oil without the additives and oil with the additives of 0.22–0.5 mg/100 g selenomethionine. At the additive concentration of 1.1 mg/100 g the values of PV and AnV of the oil increased after 12 months storage by 21.5% and 49.6% correspondingly, if compared with the additive free oil. An addition of 1.10 mg/100 g selenomethionine to the studied samples of oil decreased the induction period of oxidation by 1.20–1.3 times in comparison to the oil without additives.

It was stated that the addition of α -tocopherol and α -tocopherol acetate at concentrations of 30–150 mg/100 g as well as vitamin D₃ at concentrations of 50–150 μ g/100 g do not significantly ($p > 0.05$) alter the oxidative stability of flaxseed oil.

Thus, the studies have shown that vitamins and other BAS used for enrichment of flaxseed oil can both inhibit the processes of oxidation and oxidative destruction of flaxseed oil lipids and accelerate them depending on the nature of the additive and its concentration range.

The combined effect of additive BAS and stabilizers on the oxidative stability of flaxseed oil

With the aim of looking for an effective way to ensure antioxidant protection of BAS-enriched flaxseed oil, at the next stage we studied combined effect of BAS additives, synthetic and natural oxidation inhibitors on the oxidative stability of flaxseed oil. It was shown earlier that fat-soluble esters of ascorbic acid—ascorbyl palmitate (AP) and ascorbyl stearate (AS), as well as natural stabilizing compositions based on bean and soybean seeds, effectively inhibit the oxidation and oxidative destruction of flaxseed oil lipids (Shadyro et al. 2017). These AOs also displayed high efficiency in inhibiting oxidative changes in flaxseed oil containing the studied BAS. The values of the stabilization factor for flaxseed oil supplemented with coenzyme Q₁₀ (100 mg/100 g) at concentrations of AP and AS of 0.02 and 0.04% (w/w) were equal to 1.9–2.6 and 2.3–3.7 respectively. These values were calculated from the values of the induction period under conditions of accelerated oxidation at the temperature of 100 °C. Figure 5 shows the changes in the concentration of hydroperoxides and secondary oxidation products during the storage period of one of the flaxseed oil samples without additives, and the samples containing coenzyme Q₁₀ and AP, as well as stabilizers based on bean seeds (STH), soybean (STS), AP and STH composition.

It is clearly seen in Fig. 5 that even under conditions of accelerated oxidation (free access of air oxygen) at room temperature AP and its compositions with plant stabilizers effectively slow down oxidative processes in flaxseed oil enriched with coenzyme Q₁₀: after 12 months storage the concentration of hydroperoxides in oil containing coenzyme Q₁₀ decreases by 5.7 times in presence of 0.02% AP, and by 8 times in presence of 0.02% AP and 1.2% STF; at the same time concentration of secondary oxidation

products decreases by 3.9 and 4.7 times correspondingly. Esters of ascorbic acid and their compositions with stabilizers based on legume seeds also effectively inhibit the oxidation and oxidative destruction processes in flaxseed oil enriched with carotenoids, selenomethionine and other studied BAS additives: concentration of primary and secondary oxidation products in BAS-enriched oil with stabilizing additives decreases by 4–8 times after 12 months storage if compared to non-stabilized oil. With the use of the above-mentioned stabilizers, the losses of both endogenous BAS of flaxseed oil, such as tocopherols, carotenoids, coenzymes Q, and the BAS additives used to enrich the oil, are substantially ($p < 0.05$) reduced. Thus, when the flaxseed oil containing coenzyme Q₁₀ (total concentration of 105.6 ± 5.2 mg/100 g) is kept for 12 months under conditions of free access of ambient air, the content of coenzyme Q₁₀ is reduced by 40.5%, while in the presence of AP (0.04%) the loss of coenzyme Q₁₀ decreases to 6.5% during the same time. During the storage of the flaxseed oil without the additives under the same conditions, the total content of native tocopherols (58.4 ± 2.1 mg/100 g) decreases by 18.5% in 6 months, and by 52.7% in 12 months. The AP addition in the amount of 0.04% decreases the loss of tocopherols to 8.9% in 12 months of storage. When α -tocopherol (0.05%) was used for enriching the flaxseed oils the total concentration of tocopherols was equal to 108.4 ± 4.8 mg/100 g; the loss of tocopherols during 12 months of storage in the enriched flaxseed oil was equal to 54.5%. In the presence of 0.04% AP additive tocopherol losses got decreased to 10.31%, and α -tocopherol loss was a bit higher than the loss of γ -tocopherol. After 12 months of storage the loss of β -carotene in the enriched flaxseed oil (initial concentration of β -carotene was 15.8 ± 0.6 mg/100 g) decreased from 55.8% for non-stabilized oil to 7.5% for the oil stabilized with the AP (0.04%), and to 5.1% for the oil

Fig. 5 Changes in peroxide value (a) and *p*-anisidine values (b) of flaxseed oil with the additive of coenzyme Q₁₀ (100 mg/100 g) and stabilizers at the room temperature and with free air access: 1—no

additives; 2—CoQ₁₀; 3—CoQ₁₀ + STF, 1.2%; 4—CoQ₁₀ + STS, 1.2%; 5—CoQ₁₀ + AP, 0.02%; 6—CoQ₁₀ + STF, 1.2% + AP, 0.02%

stabilized with the composition of AP and vegetable stabilizer STH. The loss of lutein and zeaxanthin in the oil enriched with them was equal to 5–8% in presence of stabilizers. Concentration of selenomethionine, cholecalciferol and α -tocopherol acetate did not change ($p > 0.05$) during 12 months storage of the oil with these additives.

Hence, fat-soluble derivatives of ascorbic acid and their compositions with vegetable stabilizers based on beans and soybeans effectively inhibit the oxidation and oxidative destruction of PUFAs, reducing the concentration of oxidation products by 4–8 times and the loss of vitamins, coenzymes and other BAS by 5–11 times during 12 months storage, thus increasing shelf life, improving the effectiveness of products based on enriched flaxseed oil.

Practical application of research results

Results of the study were used for development of formulations and technologies of production of oxidation resistant functional products based on flaxseed oil enriched with coenzyme Q₁₀, carotenoids, organic selenium, vitamins E and D₃. The shelf life of the new products based on flaxseed oil is 12 months. The production of these foods was launched in 2014–2015 at one of the enterprises in Belarus. New products based on flaxseed oil are recommended for enriching the body with omega-3 PUFAs, vitamins and other BAS.

Conclusion

The additives of biologically active substances can significantly ($p < 0.05$) change the rate of accumulation of free fatty acids, primary and secondary oxidation products in flaxseed oil, thus exhibiting both antioxidant and pro-oxidant properties. The actual result depends on the nature of an additive and its concentration in the oil. β -Carotene at concentration of 5 mg/100 g inhibits formation of oxidation products of flaxseed oil lipids. Coenzyme Q₁₀, carotenoids (β -carotene, lutein, zeaxanthin) and selenomethionine at concentrations higher than 100, 10 and 0.5 mg/100 g respectively, accelerate the oxidation of flaxseed oil. The additives of α -tocopherol and α -tocopherol acetate at concentrations of 30–150 mg/100 g, as well as cholecalciferol (vitamin D₃) at concentrations of 50–150 μ g/100 g do not significantly ($p > 0.05$) alter the oxidative stability of flaxseed oil. Ascorbic acid esters of ascorbyl palmitate and ascorbyl stearate, as well as their compositions with vegetable stabilizers based on beans and soybeans, effectively inhibit the processes of lipid oxidation, reducing the concentration of oxidation products by 4–8 times and the loss of vitamins, coenzymes and other BAS by 5–11 times during 12 months storage, thus

increasing shelf life, improving the effectiveness of products based on enriched flaxseed oil.

Relying on the research results, formulations and methods have been developed to produce functional foods resistant to oxidation and based on flaxseed oil.

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