Influence of storage and microwave heating on stability of soya spread lipids with linseed content

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Abstract: We have chosen to experiment with soya spreads containing linseeds, which enrich the spreads with essential fatty acids and valuable related substances. The soya spreads with linseeds represent an ideal combination for nutritionally valuable foods with health benefits. In this work we examined the influence of microwave heating and storage on stability of linseed oil and soya spread lipids. Industrially produced soya spreads (S1) with addition of 5, 7 and 10 % linseeds and linseed oil were stored without a protective atmosphere in refrigerator at 5 °C. The lipids of commercial soya spreads (S2) enriched with 2, 5 and 10 % ground linseeds and soya spread lipids (S2) without linseeds were microwave-heated. The data obtained from experiments have shown that the storing of linseed oil for 30 days in refrigerator has caused excess of the maximum acceptable peroxide value (Food Codex of SR). Microwave-heated lipids from commercial soya spreads (S2) enriched with 2 % ground linseeds have the best result of stability and protective factor, compared with lipids from 5 and 10 % linseeds enriching soya spreads (S2). After the 5, 10 and 20 min of microwave heating, the lipids of soya spread (S2) enriched with 2 % ground linseeds have the protective factor 1.02, 1.15 and 1.43 respectively, compared with lipids from soya spread (S2) without linseeds. The microwave heating for 5 min has been accompanied by a decreasing of hydroperoxides and by formation of secondary oxidation products.

Keywords: linseed, linseed oil, microwave heating, soya spreads, stability of lipids, storage experiment

Introduction

According to Riaz (2005), soya beans have beneficial effect in the prevention of various sorts of cancer formation, osteoporosis and heart diseases. Flax (*Linum usitatissimum*, L.) is a crop which is adaptable to climatic conditions in a wide range of temperature. It is grown for fibrous stalk which is used in the textile industry and for seeds with 45 % ratio of lipids, which are characterised by 58—60 % content of alpha-linolenic acid (Shahidi *et al.*, 2005). This essential fatty acid is in human organism built into the nerve cells and serves as a precursors of biosynthesis of valuable acids (Brenna *et al.*, 2009). Alpha-linolenic acid oxidizes 20—40 times faster than oleic acid and 2—4 times faster than linoleic acid (Frankel, 1993). Linseed also contains lignans, which act in mammalian body as hormones-phytoestrogens. They are able to suppress growth of cancer cells (Shahidi *et al.*, 2005). Diglucoside secoisolariciresinol (SDG) is the main lignan of linseed with good antioxidant potential (Schmidt, 2010). We have investigated how the addition of linseeds increases the stability of soya spreads by storing soya spreads enriched with linseed in a fridge and by heating their lipids in microwave. Microwaving and conventional heating change the chemical parameters, especially the content of free fatty acids, carbonyl compounds, polymers, unsaturated fatty acids, polar, volatile and non-volatile substances. According to Schmidt (2010), this chemical changes are more significant in microwave heating, compared with conventional heating. A serious problem is mainly the oxidative degradation of lipids that occurs during microwave heating, in which the hydroperoxides are formed 2—3 times faster than during conventional heating (Schmidt, 2010).

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Materials and Methods

The following materials were used: commercially produced linseed oil was cold-pressed (0.25 l packing, Slovakian producer: Marianna, Ivánka pri Dunaji), commercially packed linseeds (producer: Marianna, Ivánka pri Dunaji), industrially produced soya spreads (S1) with addition of 5, 7 and 10 % linseeds (Slovakian producer: Alfa bio, Banská Bystrica), commercially produced soya spread (S2) was enriched with 2, 5 and 10 % ground linseeds in laboratory (producer: Alfa bio, Banská Bystrica).
Chloroform p.a., diethyl ether p.a., glacial acetic acid p.a., methanol p.a. (Mikrochem, s. r. o., Slovakia), potassium hydroxide p.a., potassium chloride p.a., sodium sulphate p.a. (Lachema, Czech Repub-

lic), deionized water (prepared in the laboratory), nitrogen (Messer Tatragas, Slovakia).

Instruments and equipment

Analytical balance Mettler AJ 150 (Mettler — Toledo, Switzerland), automatic titrator Basic Titrino 794 with combined Pt-ring electrode (Metrohm Ltd., Switzerland), centrifuge MPW 2 with rotating radius 14.5 cm (Poland), filter paper No. 390 (Filtrak, Germany), chromatographic station CSW 32 (DataApex, s. r. o., Czech Republic), magnetic stirrer MST basic (IKA Werke GmbH, Nemecko), microwave oven EME 1960, 750W (Elektrolux, Great Britain), grinding machine Labor Müszeripari Müvek, type QC 108 (thickness of grinding 250 μm), Rancimat 743 (Metrohm Ltd., Switzerland), rotary vacuum evaporator (Model Unipan 350, Poland), spectrophotometer Shimadzu 1601 (Shimadzu Corporation, Japan), drying-oven KWC 100 (Poland) and Simax laboratory glasses.

Analytical methods

The peroxide value was determined iodometrically and expressed in mmol 0.5 O_2 kg^{-1} of fat (AOCS Cd 8-53). The equivalence point of titration was determined by potentiometric titration (Basic Titrino 794). The acid value was determined by alkalimetric titration and expressed in mg KOH g^{-1} of fat (AOCS Cd 3d-63). The iodine value was determined iodometrically according to Hanuš and expressed in g I_2 100 g^{-1} of fat (ČSN 58 01 01). *p*-Anisidine value express the content of aldehydes in fats, especially 2-alkenals, by measuring the absorbance of the reaction product (IUPAC II.D.26.).

The stability of fats was determined by the method of accelerated oxidation with the apparatus Rancimat 743 at a constant temperature of fat (110 °C) and air flow (20 dm^3 h^{-1}), which bubbled through the sample. The stability of fat is expressed by induction period in hours (AOCS Cd 12b-92). The induction period is expression of the resistance of lipids to oxidation. It is exactly the time during which oxidation takes place at a constant and very slow speed (Velasco *et al.*, 2004). The antioxidant activity of the spread lipids enriched with linseeds is expressed as a protection factor (PF) calculated as a ratio of the induction period of fat with the addition of linseeds (IP_A) and without the addition of linseeds (IP_0):

$$P_F = IP_A / IP_0 \quad (1)$$

Analysis of fatty acids content in lipids

Fatty acids were derived to methyl esters according the method of Christopherson and Glass (1969). To separate methyl esters the gas chromatograph Hewlett Packard 5890 series II (Palo Alto, USA) with a capillary column Supelcowax 10 (30 m × × 0.53 mm; 1.0 μm film) was used. The sample was analyzed under the conditions: carrier gas — helium (7 cm^3. min^{-1}), the injection temperature was 250 °C, FID detector 260 °C.

Procedures

The linseed oil and commercial soya spreads (S1) were stored in a clear glass bottle (oil) and in a flask (spreads), without a protective atmosphere, in a refrigerator at 5 °C (average value). The commercial soya spreads (S2) were enriched by adding 2, 5 and 10 % ground linseeds with a thickness of grinding 250 μm. The lipids from the spreads were extracted by the method of Folch *et al.* (1957). Then, the lipids were put into a beaker, that was placed onto a rotating plate of a microwave oven and they were heated in the oven for 2, 5, 10 and 20 minutes.

The extraction procedure: The sample weighing 25 g was mixed with 50 cm^3 of methanol on the magnetic stirrer for 3 min at 300 r min^{-1} (RPM). After that time chloroform in the amount of 100 cm^3 was added into the beaker and the beaker with its content was stirred for another 20 min. The sample was uniformly spilled into the centrifuge tubes and centrifuged for 10 min at 3500 r min^{-1} (relative centrifugal force 1990 x g). After the centrifugation, the upper (polar) layer was removed by pipette and the lower lipid phase (dissolved in chloroform) was poured over the cake. Then the cake was submitted to the methanol:chloroform (volume ratio 1:2) extraction. The aqueous solution of KCl (0.88 %) was added into the extract and the lower chloroform phase was separated in a separatory funnel. The chloroform phases from the first and the second extractions were combined together. For a better separation it is necessary to use KCl (aqueous solution, 0.88 %) in the amount of one quarter of the obtained volume of the extract. Then the sample was shaken and centrifuged for 10 min at 3500 r min^{-1} (1990 x g). After this time, the upper layer (without cake) was removed with pipette and micropipette. It was necessary to add the solution containing methanol and water in the volume ratio 1:1 in the amount of one quarter of the obtained volume of the extract. The sample was centrifuged under the above-mentioned conditions for 10 min. After the centrifugation, the upper layer was removed with a pipette and micropipette. Anhydrous sodium sulphate was added to the chloroform extract and then the product was filtered. The chloroform was evaporated under reduced pressure in a rotary vacuum evaporator.

Statistical analysis

The statistical analysis was carried out using the program Statgraphics Plus, version 3.0 for Windows (Manugistic Inc., USA). In this work were determined the chemical parameters of every sample three times. The mean value of measured data was determined with confidence interval (95.0 %), which was calculated using a one-variable analysis of data. The figures were created using the program Origin, version 6.1 (Origin Lab Corporation, USA).

Results and discussion

Content of fatty acids in linseed oil and soya spread lipids

The lipids of commercial soya spreads (without the addition of linseeds) contained 29 % linoleic acid, 7 % alpha-linolenic acid and 43 % oleic acid. It is caused by the presence of rapeseed oil, according producer formulation.

The lipids of commercial soya spreads (S2) enriched with 2, 5 and 10 % laboratory ground linseeds contained 10, 11, and 17 % alpha-linolenic acid respectively and on average 34 % linoleic acid (see Table 1.). The lipids of industrially produced soya spreads (S1) with addition of 5, 7 and 10 % linseeds contained 10, 12 and 15 % alpha-linolenic acid respectively and on average 27 % linoleic acid (see Table 2.). We have obtained lipids with higher levels of alpha-linolenic acid compared to the lipids from industrially produced soya spreads S1 by using a laboratory ground linseeds (fineness of grinding 250 μm) added to the soya spreads S2. The linseed oil contained 31 % linoleic acid and 36 % alpha-linolenic acid (see Table 2.). The total unsaturation

Tab. 1. Chemical parameters and presence of fatty acids in lipids of commercial soya spreads (S2) enriched with ground linseeds.

Lipids of soya spreads (S2) enriched with ground linseeds:		0 %	2 %	5 %	10 %
Chemical parameters					
Iodine value	[g I_2 100 g^{-1} of fat]	115.83 ± 0.51	124.94 ± 0.57	128.24 ± 0.66	134.43 ± 0.69
Acid value	[mg KOH g^{-1} of fat]	1.75 ± 0.04	1.32 ± 0.07	1.72 ± 0.05	1.54 ± 0.06
Peroxide value	[mmol 0.5 O_2 kg^{-1} of fat]	0.31 ± 0.08	4.06 ± 0.06	3.94 ± 0.03	4.09 ± 0.02
Fatty acids	[area %]				
palmitic acid	(16:0)	8.5	7.0	7.7	6.4
stearic acid	(18:0)	3.3	3.3	3.1	3.0
arachidic acid	(20:0)	0.7	0.5	0.4	0.4
behenic acid	(22:0)	0.6	0.4	0.3	0.3
palmitoleic acid	(16:1-9c)	1.4	0.8	1.6	0.4
oleic acid	(18:1-9c)	42.6	38.8	36.8	34.4
gondoic acid	(20:1-11c)	1.0	0.8	0.7	0.7
linoleic acid	(18:2-9c, 12c)	29.0	33.8	33.8	33.9
α-linolenic acid	(18:3-9, 12, 15c)	7.0	9.7	11.1	16.9
others		5.9	4.9	4.5	3.6

Tab. 2. Chemical parameters and presence of fatty acids in linseed oil and the lipids of industrially produced soya spreads (S1) with added linseeds.

Linseed oil and lipids of soya spreads (S1) with added linseeds		Linseed Oil	Lipids of soya spreads (S1) with added linseeds:		
			5 %	7 %	10 %
Chemical parameters					
Iodine value	[g I_2 100 g^{-1} of fat]	156.40 ± 0.89	126.90 ± 0.40	129.40 ± 0.68	133.15 ± 0.66
Acid value	[mg KOH g^{-1} of fat]	0.16 ± 0.07	0.76 ± 0.04	0.79 ± 0.06	0.84 ± 0.08
Peroxide value	[mmol 0.5 O_2 kg^{-1} of fat]	4.01 ± 0.08	1.78 ± 0.04	1.91 ± 0.02	2.00 ± 0.03
Fatty acids	[area %]				
palmitic acid	(16:0)	5.8	5.4	5.5	5.7
stearic acid	(18:0)	3.9	2.7	2.6	2.6
arachidic acid	(20:0)	0.2	0.5	0.6	0.5
behenic acid	(22:0)	0.3	0.4	0.4	0.4
palmitoleic acid	(16:1-9c)	0.1	0.5	0.5	0.3
oleic acid	(18:1-9c)	20.5	48.8	48.7	46.2
gondoic acid	(20:1-11c)	0.2	4.1	1.0	1.1
linoleic acid	(18:2-9c.12c)	31.3	25.6	27.8	27.9
α-linolenic acid	(18:3-9.12.15c)	36.1	10.1	12.3	14.7
others		1.6	1.9	0.6	0.6

of linseed oil is expressed by iodine value 156 g I_2 100 g^{-1} of fat. The iodine value of lipids from soya spread (S2) without addition of linseeds was 116 g I_2 100 g^{-1} of fat. The lipids in spreads S1 and S2, have the iodine values in the range from 127 to 134 g I_2 100 g^{-1} of fat.

Storage experiment of linseed oil and soya spreads

During storage of industrially produced soya spreads (S1), their lipids resist oxidation longer than the linseed oil, containing 67 % polyunsaturated fatty acids. The experiment has determined the oxidation stability (induction period and peroxide value), acid value and *p*-anisidine value in linseed oil and the oxidation stability of lipids from industrially produced soya spreads (S1) during storage (see Fig. 1 and Table 3.).

After 14 and 28 storage days the induction period of linseed oil was decreased by 17 % and 34 %, compared with the induction period of linseed oil at zero time. After 14 storage days the induction period of lipids in industrially produced soya spreads with the added 5, 7 and 10 % linseeds decreased by 3, 4 and 10 % respectively and after 28 storage days the induction period of lipids decreased by 19, 26 and 32 % respectively (compared with the induction period of soya spread lipids at zero time).

After 28 storage days the highest peroxide value in linseed oil was determined, namely peroxide value 10 mmol 0.5 O_2 kg^{-1} of fat whereas the lipids of soya spread with 10 % addition of linseeds had the peroxide value 3 mmol 0.5 O_2 kg^{-1} of fat. The storage experiments confirmed a significant increase of peroxide value accompanied by a decrease in induction period.

Influence of microwave heating on soya spread lipids

Microwave heating is routinely used for heating and food preparation. The microwave, but also conventional heating, lead to oxidative degradation of lipids and especially vitamins (Schmidt, 2010).

The induction period of lipids in commercial soya

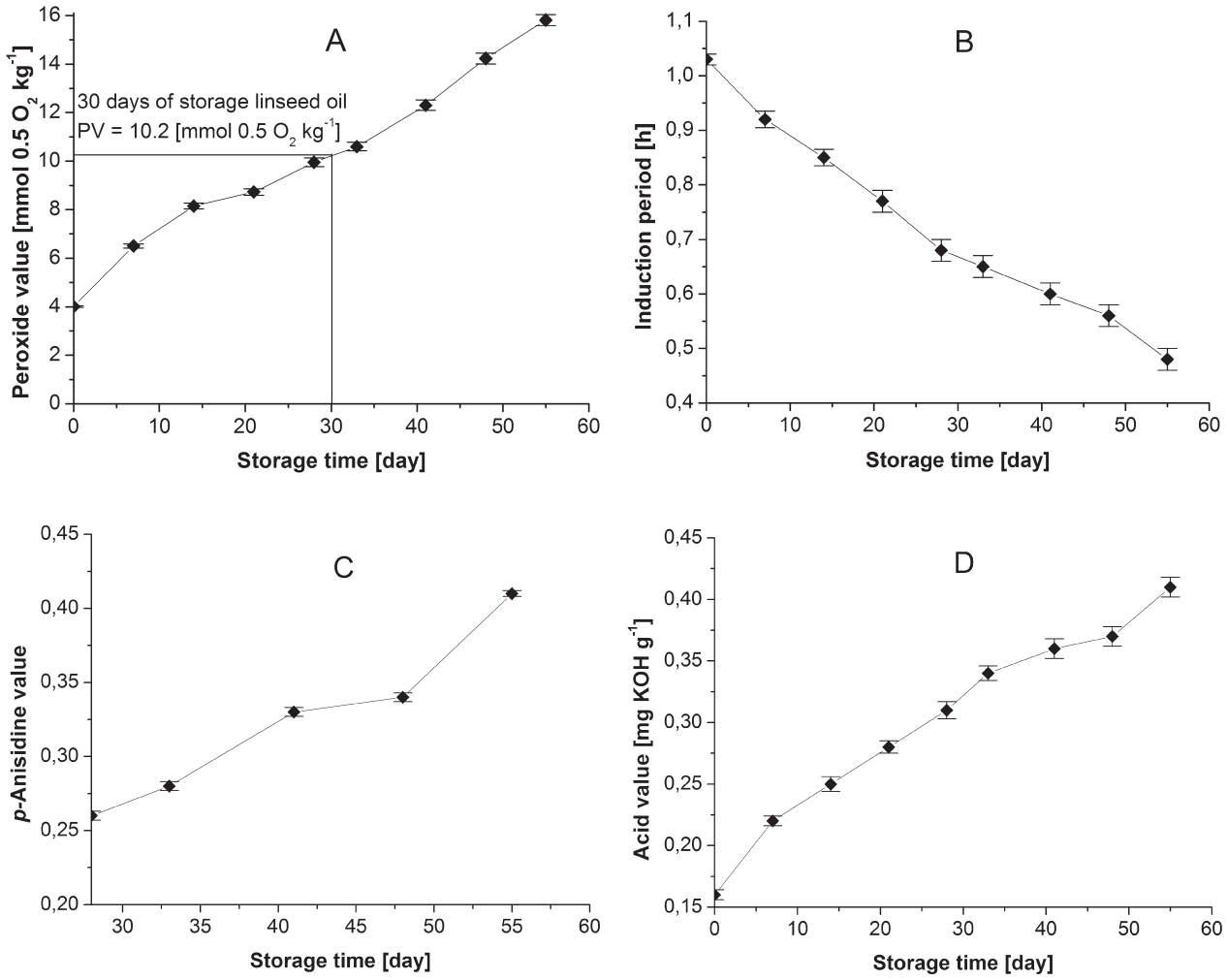

Fig. 1. The influence of storage on the oxidative stability (A-B), p-anisidine (C) and acid value (D) of linseed oil (stored in clear glass bottle without protective atmosphere in a refrigerator at 5 °C).

Tab. 3. The influence of storage on peroxide value and induction period of lipids in industrially produced soya spreads (S1) with added crushed linseeds (stored in sample flask without protective atmosphere in a refrigerator at 5 °C).

Storage life [days]	Lipids of soya spreads (S1) with addition of linseeds:					
	5 %		7 %		10 %	
	PV	IP	PV	IP	PV	IP
0	1.78 ± 0.05	2.77 ± 0.02	1.91 ± 0.06	2.15 ± 0.01	2.00 ± 0.05	1.89 ± 0.02
14	2.08 ± 0.13	2.69 ± 0.02	2.31 ± 0.12	2.07 ± 0.02	2.40 ± 0.11	1.70 ± 0.03
28	2.71 ± 0.15	2.24 ± 0.03	2.91 ± 0.14	1.59 ± 0.02	3.03 ± 0.15	1.28 ± 0.03

PV: Peroxide value [mmol 0.5 O_2 kg^{-1} of fat]. IP: Induction period [h].

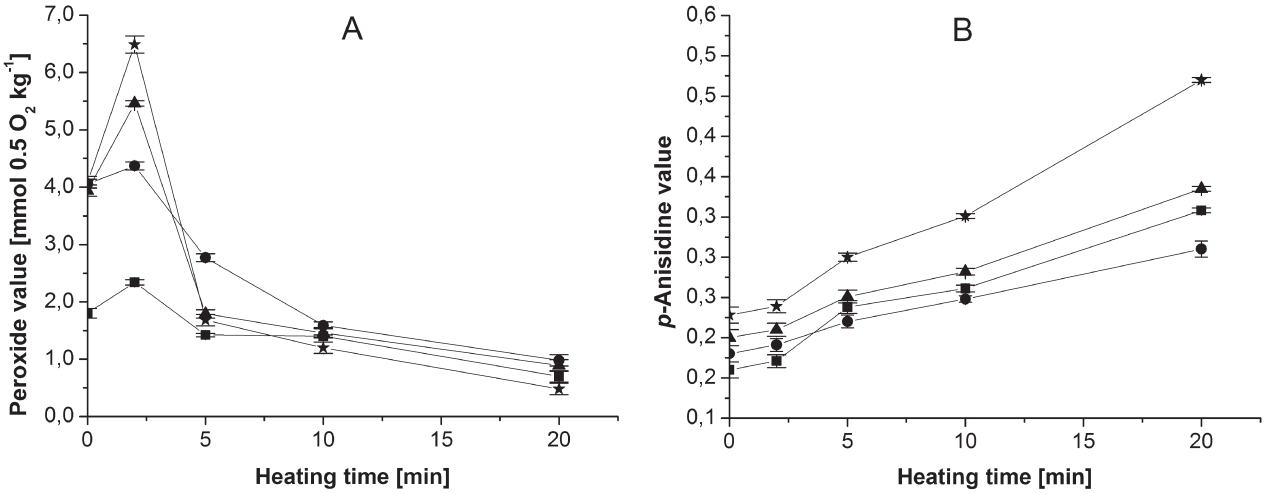

Fig. 2. Influence of microwave heating on peroxide value (A) and p-anisidine value (B) of lipids in commercial soya spreads (S2) enriched with the addition of (■) 0 %, (●) 2 %, (▲) 5 % and (★) 10 % ground linseeds.

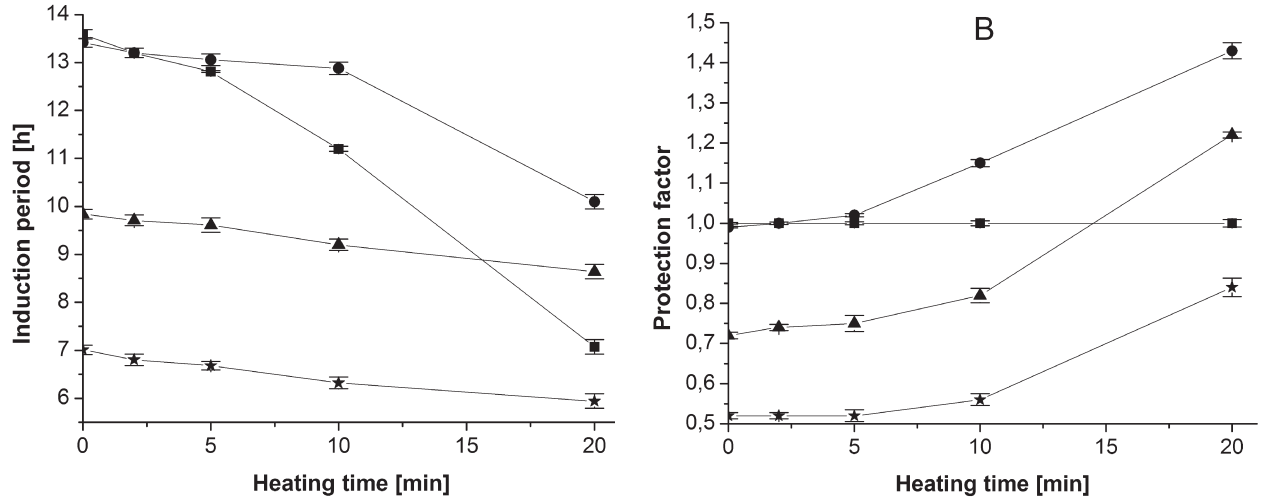

Fig. 3. Influence of microwave heating on the induction period (A) and the protection factor (B) of lipids in commercial soya spreads (S2) enriched with (■) 0 %, (●) 2 %, (▲) 5 % and (★) 10 % ground linseeds.

spreads (S2) enriched with the ground linseeds was higher than the induction period of lipids in industrially produced soya spreads (S1) with added crushed linseeds. On the basis of these results, we decided to carry out the microwave heating of lipids in commercial soya spreads (S2) enriched with the ground linseeds. The influence of microwave heating on chemical parameters and stability of commercial soya spread lipids (S2) is described in Fig. 2 and Fig. 3.

The microwave heating of lipids from soya spreads

(S2) enriched with 2, 5 and 10 % ground linseeds for 2 minutes increased the peroxide value by 1.01, 1.39 and 1.59 times respectively, when compared with the peroxide number of soya spread lipids (S2) at zero time.

The 5 minute microwave heating of lipids from soya spreads (S2) enriched with 2, 5 and 10 % ground linseeds caused a break of hydroperoxides and decreased the peroxide value from 4 mmol 0.5 O_2 kg^{-1} of fat, witch is the average PV of soya spread lipids in zero time, to 2.8, 1.8 and 1.7 mmol 0.5 O_2 kg^{-1} of fat respectively. In the same time the content of secondary oxidation products in soya spread lipids (S2) expressed as *p*-anisidine value increased (see Fig. 2B).

The induction period is an important evaluation parameter of the influence of microwave heating on the stability of soya spread lipids. The microwave-heated lipids (5 min) of soya spreads (S2) enriched with 2 % linseeds were more stable than lipids of soya spread (S2) without enrichment of linseeds (Fig. 3). In the lipids from commercially produced soya spreads enriched with 5 and 10 % ground linseeds the influence of unsaturation on stability of lipids was dominant.

Conclusions

From the experiment it can be concluded that storing industrially produced soya spreads (S1) with addition 5, 7 and 10 % of linseeds have proved by 2—3 times (in average) higher stability in comparison to stability of linseed oil. This oil stored in the refrigerator should be (in agreement with the Food Codex of SR) consumed up to 30 days from the date of opening of its bottle at the latest (peroxide value up to 10 mmol 0.5 O_2 kg^{-1} of fat). Compared with the lipids of soya spread (S2) without enriching of linseeds, the microwave-heated lipids of commercial soya spread (S2) enriched with 2 % ground linseeds have the protective factor with the values 1.02 (5 min heating), 1.15 (10 min) and 1.43 (20 min). The addition of linseeds (2 %) to the soya spreads increase the content of alpha-linolenic fatty acid in the lipids from 7 to 10 %. In the lipids from commercial soya spreads (S2) enriched with 5 and 10 % ground linseeds the influence of their unsaturation on stability of lipids is dominant.

The presence of lignans, increased alpha-linolenic and linoleic acid content introduced by a suitable addition of linseeds into soya spreads make spreads more stable and nutritionally very valuable.

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