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Commercial Cold Pressed Flaxseed Oils Quality and Oxidative Stability at the Beginning and the End of Their Shelf Life

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Abstract: The aim of the study was to determine quality and oxidative stability of selected cold pressed flaxseed oils, fresh (after producing, the beginning of shelf life) and stored at refrigerator temperature (after three months, the end of declared shelf life). The fresh oils were characterized by organoleptic assessment, fatty acids composition and bioactive compounds content (sterols, tocols, squalene, carotenoids, and phenols). For the fresh and stored oils oxidative stability in the Rancimat test, and the hydrolytic and oxidation degrees using standard methods were determined. It was found that fresh flaxseed oils were differentiated in fatty acid composition and content of bioactive compounds. Shares of saturated fatty acids, and content of squalene and phenolic compounds were most variable in the oils. At the end of shelf life flaxseed oils were characterized by 9-26% shorter induction time in compare to the initial state, and increased content of hydrolysis (acid value by 18-40%) and oxidation products (peroxide value by 16-37%, anisidine value by 13-41%, diene content by 10-21%, triene content by 23-42%) was detected.

Key words: flaxseed oils, oil quality, oxidative stability, bioactive compounds, fatty acid composition

1 INTRODUCTION

Flaxseed (*Linum usitatissium*) also known as linseed, is one of the most important world crops. It is thought to be one of the oldest cultivated crops with evidence of cultivation dating back thousands years ago. The plant is grown either as a fibre crop or as an oil crop, with fibre (linen) derived from the stem of fibre-type varieties and oil from seeds of oil-type varieties[1, 2]. According to the Food and Agriculture Organization of the United Nations Statistical Database (FAOSTAT)[3], about 0.6 mln ton of flaxseed oil was produced by China as the largest producer (25%), followed by Belgium (19%), United States (17%), Germany (8%), Ethiopia (7%) and India (7%). The oil is primarily used for industrial purposes, e.g. in the production of paints, floor covering (linoleum), anti-rust agent, additive in PVC plastics and agglomerating agent for coal[1, 4]. The interest in edible use of the oil is due to its high content of α-linolenic acid (ALA), belonging to the n-3 family. Especially low n-6/n-3 ratio makes it attractive for functional food and nutraceutical applications. Flaxseed oil is incorporated into milk, yoghurt, ice-cream and bread toppings, and marketed in the form of soft-gel capsules as dietary supplements[1, 5]. Unfortunately, the high degree of unsaturation makes this oil highly susceptible to oxidation during processing and storage, due to such factors as heat, light and oxygen[5]. As a result of the oxidation polyunsaturated fatty acids (PUFAs) and vitamins lose their biological properties and can form toxic products, sometimes with carcinogenic nature[6].

Edible flaxseed oil may be obtained by cold pressing alone or by a process of pre-pressing followed by solvent extraction and refining. The crude flaxseed oil produced by cold pressing consists primarily of triacylglycerols, but it also contains lesser amount of incomplete acylglycerols (mono- and diacylglycerols), sterols, tocopherols, phenolics, pigments (carotenoids and chlorophylls), phospholipids, and orbitides[4]. These bioactive compounds are so-called unsaponifiable fraction, wherein its content in flaxseed oils can be ranged from 0.39% to 0.78%[5, 7]. The content and composition of this fraction plays an important role in retarding oil deterioration. Previous studies have demonstrated difference in content of bioactive compounds in flaxseed oils resulting mainly from variety[2, 8, 9], climate[9], seed maturity[10], extraction method[4, 11], and

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storage time[12]. All these factors affect the heterogeneity of commercially available oils in terms of chemical composition and quality. For example, Choo *et al.*[5] who studied seven cold pressed flaxseed oils coming from New Zealand market found differences in fatty acid composition and content of tocopherols, chlorophylls and phenols. Only four of these oils possessed all the quality parameters below the maximum threshold specified in New Zealand Food Regulation and literature. Furthermore, they showed that fatty acid composition, chlorophyll, tocopherol and plastochromanol-8 contents may play significant role in the oil quality. It is important that chemical composition and quality of oils immediately after production determine their shelf life stability. For cold pressed oils it is usually set for 6 or 12 months[13], but for flaxseed oils it is even shorter, from 5 weeks to 3 months if they are kept in the refrigerator[5]. However, literature data show that significant oil deterioration can occur during long-term storage. Chowdhury *et al.*[14] have shown that acid and peroxide values of flaxseed oils extracted from seeds collected in Bangladesh increased extensively over 12 months of storage. In contrast, Prescha *et al.*[12] have shown that decrease of flaxseed oil quality followed after 6 months of storage. Moreover, the changes were larger in the case of oils from Linola seeds, a low ALA variety. Unfortunately, the influence of packaging and age of the oils were not taken into consideration on the stability of the oils.

Thus, in this study of fatty acid composition and bioactive compounds content in flaxseed oils available on the local market were determined. Additionally, the quality and oxidative stability of the oils were measured directly after production, and at the end of shelf life declared by the manufacturer.

2 MATERIALS AND METHODS

2.1 Oil samples and chemicals

The experimental materials included 6 samples of commercial flaxseed oil, typical for market in Poland. All oils were purchased directly from the manufacturers, and analyzed after opening and after 3 month of storage at refrigerator temperature (4 ± 2℃). The oils were stored in original package (simulation of domestic usage); a brown glass bottle (oils no 1-4), a brown plastic bottle (oil no 5) and transparent plastic bottle (oil no 6). According to declaration of manufacturer oil no 2 was enriched in mixture of tocopherols. Analytical-grade reagents (purity > 95%) were purchased from Sigma-Aldrich (St. Louis, MO, United States, supplier Poznań, Poland) and POCH (Gliwice, Poland), chromatography-grade solvents from Sigma-Aldrich, and analytical standards: fatty acids mixture from Supelco (Bellefonte, USA), lutein (95%), 5α-cholestane (97%) and D-catechin (98%) from Sigma-Aldrich, and tocopherols (95%) from Calbiochem (Nottingham, United Kingdom).

2.2 Fatty acids composition

The fatty acid composition was evaluated by the standard gas chromatography method[15]. Methyl esters were prepared using a procedure described by Zadernowski and Sosulski[16]. The analysis was carried out by applying a GC 8000 series FISONS Instrument Gas Chromatograph equipped with a flame-ionization detector using a type DB-225 column (30 m × 0.25 mm × 0.15 μm) and helium as the carrier gas. Fatty acids were identified according to the retention time determined for fatty acid standards.

2.3 Bioactive compounds content

The content of sterols and squalene was determined by the GC coupled with MS (GC-MS) according to the method described by Roszkowska *et al.*[17]. The sample of oil was dissolved in hexane and 5α-cholestane solution (as an internal standard) was added, and the mixture was then saponified by adding 2 M KOH solution in methanol at temperature of 70℃ for 30 min. Unsaponifiable fraction was extracted with diethyl ether, which was subsequently evaporated under nitrogen conditions. The residues were re-dissolved in pyridine and N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), and heated at temperature of 60℃ for 1 h to complete derivatization. Mixture was re-dissolved in heptane and analysed using GC-MS QP2010 PLUS manufactured by Shimadzu Corporation (Kyoto, Japan). Separation of compounds was performed on a ZB-5ms capillary column (30 m × 0.25 mm × 0.25 μm) (Phenomenex, Torrance, CA, USA), and helium as a carrier gas was used with a 0.9 mL/min flow rate. The injector temperature was 230℃, and the column temperature was programmed in range from 70℃ to 310℃. The interface temperature of GC-MS was 240℃. The temperature of the ion source was 220℃ and the electron energy was 70 eV. The total ion current (TIC) mode was used for quantification (100–600 m/z range). The measurements were carried out using the internal standard method.

The content of tocols was determined by HPLC, according to the method described by Czaplicki *et al.*[7]. A sample of oil was diluted in hexane and subsequently centrifuged (25,000 g for 10 min) in a 5417R-type Eppendorf centrifuge (Eppendorf AG, Hamburg, Germany). The analysis was carried out using an Agilent Technologies 1200 RP-HPLC apparatus (Santa Clara, CA, USA), equipped with a fluorescent detector from the same manufacturer. Separation was performed on a LiChrospher Si60 column (250 mm × 4 mm × 5 μm) (Merck, Darmstadt, Germany). The mobile phase was a 0.7% 2-propanol in hexane solution with a 1 mL/min flow rate. The fluorescence detector was set at 296 nm of excitation and 330 nm of emission. Content of tocols was

calculated using external calibration curves.

The content of carotenoids was determined spectrophotometrically according to the method described by Craft[18]. The oil sample was dissolved in hexane and the absorbance was then measured at a wavelength of 454 nm (maximum of lutein absorption) using a UNICAM UV/Vis UV2 spectrophotometer (ATI Unicam, Cambridge, United Kingdom). Carotenoid content was calculated based on the molar absorptivity coefficient of lutein (equal to 147,300 L/mol·cm) and the molar mass of lutein (equal to 568.87 g/mol).

The content of total phenolic compounds was determined spectrophotometrically with a Folin–Ciocalteu phenol reagent according to the method described by Siger *et al.*[19]. The 4-fold extraction of phenolic compounds was carried out with 80% methanol v/v. Absorbance at 720 nm was measured using UNICAM UV/Vis UV2 spectrophotometer against a blank sample. The phenolic compounds content was calculated on the basis of the D-catechin calibration curve.

2.4 Oil oxidative stability and quality

The oxidative stability was determined on a Rancimat apparatus 743 (Methrom, Herisau, Switzerland) by measuring the induction period at 110℃[20].

The quality of oils was evaluated by descriptive organoleptic evaluation (colour, smell, taste), content of water and volatile compounds[21], acid value[22], peroxide value[23], anisidine value[24], and values of specific extinctions at 232 nm (K232) and 268 nm (K268) for conjugated dienes and trienes[25], respectively.

2.5 Statistical analysis

The obtained results of all analyses (performed in triplicate) were statistically analysed using Statistica 12.0 PL software (StatSoft, Kraków, Poland). The differences between the means were determined using analysis of variance (ANOVA) with Tukey test at $p \leq 0.05$ significance level. The obtained results of all analyses were higher than limits of quantification for used methods.

3 RESULTS AND DISCUSSION

3.1 Organoleptic characteristics of fresh flaxseed oils

Flaxseed oils organoleptic evaluation was carried out immediately after opening the packages. Only slight differences in the colour and the taste were detected. The colour of all samples was typical for cold pressed oils obtained from flaxseeds; from light yellow to yellow. In three of them (samples no 2-4) the presence of sediment at the bottom of the package was revealed. It could be the result of increased water content compared to other samples (Table 1) and a higher content of phospholipids. These compounds have a hydrophobic-hydrophilic structure, so while binding to water in the oil they make colloids released in the form of gums. Moreover, they cause a deterioration of the taste, odour, and colour of oils during long-term storage[26]. All tested oils had a characteristic smell of flaxseeds, without strange odour. The taste of the oils was specific, but in case of one of them nutty flavour was perceptible. It can be due to elevated temperatures, for example during seed drying or oil extraction by pressing. Wiesenborn *et al.*[27] studied the sensory quality laboratory pressed and commercial flaxseed oils. They found that nutty flavour was most noticeable in the commercial oil sample, which can also confirm that it was result of not controlled temperature changes. The taste may also derive from the presence of oxidized forms of cyclolinopeptides that occur naturally in flaxseeds. The amount and type of these compounds in oil depends on flax variety, quality of seeds, methods of extraction and purification of the oil, and its storage time[28, 29].

3.2 Fatty acid composition of fresh flaxseed oils

The fatty acid composition of tested flaxseed oils is shown in Table 2. The most valuable, because it occurs in small amount in other vegetable oils, ALA accounted for 36.66% (sample no 3) to 53.19% (sample no 5) of the share of all fatty acids. The second polyunsaturated fatty acid in studied oils was linoleic acid, whose share did not exceed 18%. In total, the share of these essential fatty acids amounted to 52-71%. The values of n-3:n-6 fatty acid ratio calculated for the flaxseed oils were significantly differentiated, from 4.3:1 (sample no 4) to 2.3:1 (sample no 3).

Table 1 Organoleptic evaluation, and water and volatiles content of fresh flaxseed oils.

Feature	Oil number					
	1	2	3	4	5	6
Colour	light yellow	yellow, sediment at the bottle bottom			yellow	light yellow
Smell	characteristic of flaxseeds					
Taste	specific for flaxseeds					slightly nutty flavour
Water and volatiles (%)	0.34 ± 0.01	0.60 ± 0.01	0.75 ± 0.01	0.72 ± 0.00	0.24 ± 0.01	0.13 ± 0.00

Table 2 Fatty acids composition (%) in fresh flaxseed oils.

Fatty acids	Oil number						CV (%)
	1	2	3	4	5	6	
C14:0	0.34 ± 0.01^{b}	0.87 ± 0.03^{d}	0.97 ± 0.01^{e}	0.38 ± 0.03^{c}	0.29 ± 0.04^{b}	0.24 ± 0.03^{a}	61.9
C16:0	7.60 ± 0.04^{c}	8.25 ± 0.05^{e}	9.95 ± 0.04^{f}	6.95 ± 0.03^{a}	7.26 ± 0.07^{b}	8.07 ± 0.19^{d}	13.3
C18:0	5.21 ± 0.11^{c}	5.72 ± 0.12^{d}	7.89 ± 0.16^{e}	5.82 ± 0.03^{d}	4.20 ± 0.05^{a}	4.38 ± 0.23^{b}	24.1
C18:1	27.35 ± 0.28^{d}	29.66 ± 0.15^{f}	28.22 ± 0.06^{e}	24.31 ± 0.11^{b}	17.39 ± 0.05^{a}	25.01 ± 0.37^{c}	17.3
C18:2n-6	15.28 ± 0.05^{b}	15.32 ± 0.05^{b}	16.07 ± 0.07^{c}	11.78 ± 0.06^{a}	17.53 ± 0.16^{d}	15.09 ± 0.24^{b}	12.5
C18:3n-3	44.07 ± 0.26^{c}	39.93 ± 0.19^{b}	36.66 ± 0.02^{a}	50.53 ± 0.09^{e}	53.19 ± 0.11^{f}	47.01 ± 0.21^{d}	13.9
C20:0	0.17 ± 0.03^{a}	0.28 ± 0.05^{a}	0.25 ± 0.07^{a}	0.24 ± 0.10^{a}	0.16 ± 0.06^{a}	0.21 ± 0.01^{a}	21.7
PUFA	59.34 ± 0.31^{c}	55.24 ± 0.14^{b}	52.09 ± 0.30^{a}	62.31 ± 0.04^{d}	70.72 ± 0.28^{e}	62.10 ± 0.45^{d}	10.5
MUFA	27.35 ± 0.28^{d}	29.66 ± 0.15^{e}	28.22 ± 0.06^{e}	24.31 ± 0.11^{b}	17.39 ± 0.05^{a}	25.01 ± 0.37^{c}	17.3
SFA	13.32 ± 0.04^{d}	15.11 ± 0.01^{e}	19.06 ± 0.04^{f}	13.39 ± 0.07^{c}	11.90 ± 0.23^{a}	12.90 ± 0.08^{b}	17.9
OI (-)	1.04	0.96	0.90	1.13	1.24	1.10	11.7
n-3/n-6 (-)	2.88	2.61	2.28	4.29	3.04	3.12	22.6

a, b, c … – means in the same line followed by different letters are significantly different ($p \leq 0.05$).
O.I. – oxidizability index, it was calculated using the formula[17]: OI = (0.02 · C18:1 + 1 · C18:2 + 2 · C18:3) / 100.
PUFA – polyunsaturated fatty acids; MUFA – monounsaturated fatty acids; SFA – saturated fatty acids.
CV – coefficient of variation.

The share of the main monounsaturated fatty acid, oleic acid, ranged from 17.39% (sample no 9) to 29.66% (sample no 2). In turn, the saturated fatty acids: palmitic, stearic, arachidic and myristic averaged 8.01%, 5.54%, 0.51% and 0.22%, respectively (Table 2). Fatty acid composition of the oils is consistent with the results obtained by Bhatty[30], who said that with the increase of the share of ALA oleic acid share decreases in flaxseed oil.

The oils presented in this work demonstrated the greatest variation in the percentage share of myristic, stearic and arachidic acids (CV > 20%). In turn, the variation coefficients of share of polyunsaturated fatty acids were 12.5% and 13.9% for linoleic and linolenic acids, respectively (Table 2). Such a different fatty acid composition of the oils may be due to the use for the oil production seeds of different varieties, or their mixtures. Zhang *et al.*[2] evaluated characteristics of flaxseed oils from two different varieties, namely fibre-flax and oil-flax seeds. They stated that fatty acids of the oil obtained from seeds of oil-type flax contained more linolenic acid (58.03%) than the oil from seeds of fibre-type flax (47.37%). Recently, a significant interest in new varieties of flax seeds with altered proportions of fatty acids has been observed. In traditional varieties ALA content is in the range of 34.1-64.6%, whereas in modified from 1.6% to 39.1%[6]. According to research of Gambuś *et al.*[31], fatty acid composition in oils from seed of traditional flax varieties, Hungarian Gold and Opal, cultivated in Poland as follows: palmitic acid accounted for 5.8% and 9.2%, stearic 2.9% and 5.2%, oleic 18.5% and 27.9%, linoleic 12.3% and 16.0%, and ALA 44.6% and 59.7%, respectively. One of the modified varieties is variety named 'Linola' with a relative low level of linolenic acid (up to 2% of total fatty acid content) and a very high level of linoleic acid (up to 71% of total fatty acid content)[32]. This modification significantly contributed to improving the stability of the oil. Moreover, climate in northern Europe is highly suitable for the production of Linola seeds[8]. Diversity in the composition of fatty acids in the oils can also be caused by environmental and growing conditions[33]. In practice, the most important is the atmosphere; the colder the climate, the higher the content of unsaturated fatty acids[34]. This is evidenced by the results obtained by Choo *et al.*[5], who studied among others the fatty acid composition of oils from flax originating from England, Australia and New Zealand. Oils from Australia and New Zealand have in their composition lowest shares of ALA compared to those from England. In turn, Kasote *et al.*[11] demonstrated that the fatty acid composition also changes depending on the number of pressings, e.g. content of ALA is the highest in oil after double-pressing. In addition to the methods of obtaining, a method of cleaning can be important for fatty acid composition of flaxseed oils. Prescha *et al.*[12] reported that ALA share increased by approx. 5% in the refined oil in comparison to the unrefined cold-pressed. However, the proportion of saturated fatty acids was similar in both oils. Fatty acid composition is the main factor determining susceptibility of oils to oxidation. Calculated in the work oxidizability index (OI) shows that oil no 3 is potentially the most resistant to oxidation (OI = 0.90), whereas oil no 5 the least (OI = 1.24)

Table 3 Bioactive compounds content in fresh flaxseed oils.

Compounds	Oil number						CV (%)
	1	2	3	4	5	6	
Sterols (mg/100 g)							
total	409.40 ± 5.81^{a}	455.74 ± 4.71^{b}	512.54 ± 1.06^{c}	521.12 ± 3.55^{d}	533.77 ± 1.92^{e}	538.83 ± 3.36^{e}	10.4
β-sitosterol	156.23 ± 2.82^{a}	194.70 ± 0.79^{c}	195.74 ± 1.03^{c}	199.79 ± 0.95^{d}	196.74 ± 0.72^{c}	188.42 ± 1.35^{b}	8.6
cycloartenol	127.40 ± 1.03^{b}	119.01 ± 1.33^{a}	140.48 ± 1.55^{c}	143.05 ± 1.22^{c}	173.98 ± 0.90^{d}	179.98 ± 1.39^{e}	16.7
campesterol	66.79 ± 0.78^{a}	76.47 ± 0.68^{b}	82.30 ± 0.97^{c}	84.81 ± 0.42^{d}	98.03 ± 0.63^{f}	86.63 ± 0.92^{e}	12.7
Δ5-avenasterol	29.52 ± 0.38^{a}	42.93 ± 0.85^{b}	60.56 ± 0.22^{e}	52.94 ± 0.49^{d}	30.09 ± 0.45^{a}	45.69 ± 0.81^{c}	28.3
stigmasterol	22.52 ± 0.76^{b}	13.34 ± 0.62^{a}	23.91 ± 0.82^{b}	27.25 ± 0.74^{c}	27.05 ± 0.55^{c}	28.46 ± 0.44^{d}	23.4
others	6.94 ± 0.05^{a}	9.29 ± 0.45^{c}	9.55 ± 0.22^{c}	13.28 ± 0.28^{d}	7.89 ± 0.23^{b}	9.65 ± 0.29^{c}	23.0
Tocols (mg/100 g)							
total	61.09 ± 6.02^{c}	85.93 ± 1.12^{d}	55.87 ± 1.55^{b}	49.64 ± 1.02^{a}	55.41 ± 1.78^{b}	48.88 ± 0.79^{a}	23.1
α-tocopherol	nd	6.93 ± 0.17	nd	nd	nd	nd	–
pl-8	25.80 ± 1.14^{c}	25.98 ± 0.44^{c}	25.57 ± 1.43^{c}	14.87 ± 0.38^{a}	20.43 ± 1.64^{b}	22.16 ± 0.38^{b}	19.4
γ -tocopherol	35.29 ± 0.89^{c}	49.39 ± 0.79^{d}	30.30 ± 1.12^{b}	34.77 ± 0.64^{c}	34.98 ± 0.85^{c}	26.72 ± 0.41^{a}	21.9
δ-tocopherol	nd	3.64 ± 0.06	nd	nd	nd	nd	–
Squalene (mg/100 g)							
total	4.29 ± 0.04^{c}	1.90 ± 0.14^{b}	1.01 ± 0.13^{a}	1.93 ± 0.11^{b}	1.04 ± 0.08^{a}	1.80 ± 0.14^{b}	60.1
Carotenoids (mg lutein/100 g)							
total	1.52 ± 0.01^{b}	2.04 ± 0.00^{c}	1.94 ± 0.32^{c}	2.66 ± 0.23^{d}	2.95 ± 0.03^{e}	1.21 ± 0.10^{a}	32.3
Phenols (mg catechin/100 g)							
total	2.19 ± 0.78^{c}	0.62 ± 0.11^{b}	0.36 ± 0.04^{a}	0.44 ± 0.10^{a}	0.66 ± 0.03^{b}	0.39 ± 0.07^{a}	90.9

a, b, c … – means in the same line followed by different letters are significantly different ($p \leq 0.05$).
nd – not detected; CV – coefficient of variation; pl-8 – plastochromanol-8 .

(Table 2).

3.3 Content of bioactive compounds in fresh flaxseed oils

Bioactive compounds in studied oils (Table 3) accounted for 478-594 mg/100 g and were composed in order of mass share of sterols (83-91%), tocopherols (8-16%), squalene (0.2-0.9%), carotenoids (0.2-0.5%), and phenolic compounds (0.1-0.5%).

The content of sterols in the oils ranged from 409.40 mg/100 g for sample no 1 to 538.83 mg/100 g for sample no 6 (Table 3). In this regard, the tested oils were found to be the least differentiated (CV = 10.4%). The main sterols in the oils were β-sitosterol, cycloartenol and campesterol, together making ca. 85% of total sterols. The highest content of β-sitosterol (199.79 m/·100 g) was found in oil no 4, cycloartenol (179.98 mg/100 g) in oil no 6, while campesterol (98.03 mg/100 g) in oil no 5 (Table 3). In smaller amount occurred Δ5-avenasterol and stigmasterol, the amount of which in the oils did not exceed 61 mg/100 g and 29 mg/100 g, respectively. It was stated that Δ5-avenasterol most differentiated studied samples (CV = 28.3%). Extremely different in the content of the sterol turned out to be sample no 1 and 3, and the difference between them was double (Table 3). The results of sterols analysis in our work are in accordance with results presented by Ciftci *et al.*[35]. However, they are twice higher than those obtained by Czaplicki *et al.*[7].

Tocols in studied flaxseed oils accounted for an average of 59.47 mg/100 g. The oil no 2 characterized by the highest content of the sum of tocols, equal to 85.93 mg/100 g (Table 3). This oil, according to information provided by the manufacturer, was enriched with mixture of tocopherols. It included two forms of tocopherols, α- and δ-tocopherols, which are not presented or are presented in small amount in flaxseed oils. The presence of α-tocopherol found in flaxseed oil, among others, Choo *et al.*[5] and Shim *et al.*[4], and this amount does not exceed 4 mg/100 g. The lowest content of tocols, approx. 49 mg/100 g, has been shown for oils no 4 and 6. The dominant form of tocols in tested oils was γ-tocopherol, which accounted for 54-70% of all tocols. Its average content in not enriched oils (without sample no 2) was 32.41 mg/100 g (Table 3). According to the literature data, the content of the compound in flaxseed oil is in the range from approx. 10 mg/100 g[5, 36, 37]

to approx. 50 mg/100 g[4, 38]. A characteristic feature of the flaxseed oils is also high content of plastochromanol-8[39]. This compound in the studied oils was in an amount from 14.87 mg/100 g (sample no 4) to 25.98 mg/100 g (sample no 2) (Table 3). These values are close to results of Olejnik *et al.*[40] and Obranović *et al.*[9], but a four-fold higher than in the work Choo *et al.*[5]. Such big variations in content of all tocols could be the result of different harvesting locations and varieties, extraction technologies or storage time of flaxseed oil[5, 9, 37]. Tocols play an important protective function in oils. Their task is to protect PUFA against oxidation, by interrupting the oxidation reaction[41]. Nogala-Kałucka *et al.*[42] found that γ-tocopherol has the best protective properties at temperature of 4℃ in comparison to other tocopherols. The most effective antioxidant activity with respect to triacylglycerols (TAG) this homologue exhibits at concentration of 0.01% (w/w)[42, 43]. In turn, α-tocopherol has strong antioxidant activity in the human body, while the activity of β, γ and Δ homologues is limited because they are immediately metabolized in the liver and excreted in the bile or urine[44]. However, it was shown that plastochromanol-8 has activity even 1.5 times greater than α-tocopherol[40].

Squalene in the flaxseed oils appeared in amount from 1.01 mg/100 g (sample no 2) to 4.29 mg/100 g (sample no 1) (Table 3). Due to such a low content this compound is omitted in most studies of bioactive compounds in flaxseed oils. The results of tests carried out by Herchi *et al.*[45] shown that the content of the compound in flaxseed oil depends on the variety and maturity of seeds. They found that the level of squalene decreases during ripening of seeds, from 3.36-27.24 mg/100 g of oil at 7 days after flowering (varietal differentiation) to approx. 0.5 mg/100 g of oil at full maturity.

The content of carotenoids in oils presented in the work varied from 1.21 to 2.95 mg/100 g (Table 3). According to Daun *et al.*[36] the content of carotenoids in flaxseed oils is in the range 2-11.5 mg/100 g, with the greatest amount of lutein (to about 7 mg/100 g). In turn, the results obtained by other researchers were much lower. For example Tuberoso *et al.*[46] in their study obtained carotenoid pigment content of 0.7 mg/100 g. In contrast, content of these compounds in flaxseed oils analysed by Teh and Birch[47] was 0.06 mg/100 g, and it was expressed as β-carotene. Another study, conducted by Franke *et al.*[48] shows that cold pressed flaxseed oil contains only (*all-E*)-lutein in the amount of 0.37 mg/100 g. Carotenoids inhibit the photosensibilized oxidation of oil by physical or chemical quenching of singlet oxygen. During the physical singlet oxygen quenching their structure is not destroyed. However, in the case of chemical quenching degradation of their structure occurs, followed by breaking of double bonds and conversion to the epoxide or carbonyl derivatives, which appears as discoloration or colour brightening[49].

In terms of the content of phenolic compounds oil no 1 was distinguished. It contained 2.19 mg/100 g, and the value was almost four times higher than for other oils (Table 3). Abuzaytoun and Shahidi[50] obtained similar reducing power of Folin-Ciocalteu by the compounds extracted with 80% methanol from flaxseed oil. In contrast, Siger *et al.*[51] in their study received almost 50% lower total phenolic compounds content. The method with using Folin-Ciocalteu reagent measures the ability of phosphomolybdic and phosphotungstic acids reducing to the blue complexes, and it may otherwise the phenols react with other compounds also, e.g. amino acids and vitamins[52]. Phenolic compounds are considered as antioxidants, but their antioxidant activity depends on the content of hydroxyl groups and a methyl substituent at the position ortho or para, as well as polarity, solubility and stability during oil processing[46, 53, 54]. The study conducted by Siger *et al.*[51] showed that p-hydroxybenzoic, vanillic and ferulic acids are the major phenolic acids that are presented in the flaxseed oil, while Tuberoso *et al.*[46] found only vanillic acid. On the other hand, Hasiewicz-Derkacz *et al.*[8] found that vanillin is the most abundant phenolic compound in flaxseed oil. The other components are non-hydrolysable (proanthocyanidins) and hydrolysable tannins, p-coumaric acid, ferulic acid, caffeic acid, coniferyl and syringic aldehyde, and small amount of flavonoids. Moreover, this study showed that seeds of transgenic flax accumulate more water soluble compounds such as phenolic compounds, which are released to the oil and highly increase its stability.

3.4 Oxidative stability and quality of flaxseed oils at the beginning of shelf life

The Rancimat test showed that the most oxidative stable oil was oil no 2 (the induction time equal to 4.35 h), while the lowest stability showed oil no 1 (2.00 h) (Table 4). The high stability of oil no 2 may be the result of increased content of tocopherols, which explains a high correlation coefficient of 0.76 designated for this relationship. In addition, the enhanced stability could also affect relatively low, as for flaxseed oils, ALA share (ca. 40%). In study of Szterk *et al.*[54] induction time for flaxseed oil heated at a lower temperature (100℃) and 2-fold slower air flow (10 L/h) was 5.85 h. In contrast, Bozan and Temelli[37] obtained lower induction time (1.57 h) using the temperature of 110℃. The diversity of the stability of cold pressed flaxseed oils from retail outlets was indicated by research of Raczyk *et al.*[55]. The induction time of oils tested by them shaped in the range 3.47-5.63 hours. In regard to cold pressed rapeseed, oil flaxseed oil is approx. 2-fold less stable, and compared to the most commonly consumed refined rapeseed oil is 4-fold more susceptible to oxidation[17].

All tested oils in the fresh state were characterized by

Table 4 Oxidative stability and quality of flaxseed oils.

Oil number	Induction time (h)	Acid value (mg KOH/g)	Peroxide value (mEq O_2/kg)	Anisidine value (-)	Dienes (K232)	Trienes (K268)
At the beginning of shelf life						
1	2.00 ± 0.04^{aB}	0.50 ± 0.03^{aA}	0.80 ± 0.00^{aA}	0.96 ± 0.06^{aA}	1.83 ± 0.03^{aA}	0.17 ± 0.02^{aA}
2	4.33 ± 0.07^{dB}	0.85 ± 0.07^{cA}	0.75 ± 0.07^{bA}	0.78 ± 0.04^{aA}	1.86 ± 0.05^{aA}	0.26 ± 0.07^{aA}
3	2.58 ± 0.03^{bB}	1.25 ± 0.07^{dA}	1.00 ± 0.02^{cA}	0.82 ± 0.02^{aA}	1.86 ± 0.03^{aA}	0.20 ± 0.01^{aA}
4	2.73 ± 0.04^{cB}	0.95 ± 0.07^{cA}	0.75 ± 0.07^{bA}	1.07 ± 0.06^{aA}	1.83 ± 0.05^{aA}	0.26 ± 0.03^{aA}
5	2.09 ± 0.06^{aB}	0.50 ± 0.00^{aA}	0.95 ± 0.07^{cA}	0.56 ± 0.05^{aA}	1.69 ± 0.04^{aA}	0.19 ± 0.01^{aA}
6	2.78 ± 0.08^{cB}	0.70 ± 0.00^{bA}	0.80 ± 0.03^{bA}	0.76 ± 0.03^{aA}	1.78 ± 0.11^{aA}	0.17 ± 0.01^{aA}
At the end of shelf life						
1	1.56 ± 0.03^{aA}	0.63 ± 0.00^{aB}	1.08 ± 0.01^{aB}	1.23 ± 0.08^{aB}	2.01 ± 0.06^{aB}	0.21 ± 0.05^{aA}
2	3.74 ± 0.06^{aA}	1.11 ± 0.03^{aB}	0.89 ± 0.00^{aB}	0.88 ± 0.01^{aB}	2.25 ± 0.06^{aB}	0.32 ± 0.02^{aA}
3	2.35 ± 0.35^{aA}	1.75 ± 0.00^{aB}	1.23 ± 0.00^{aB}	1.04 ± 0.05^{aB}	2.11 ± 0.07^{aB}	0.27 ± 0.02^{aB}
4	2.10 ± 0.01^{aA}	1.25 ± 0.01^{aB}	0.86 ± 0.04^{aB}	1.22 ± 0.07^{aB}	2.15 ± 0.01^{aB}	0.33 ± 0.02^{aB}
5	1.67 ± 0.13^{aA}	0.59 ± 0.06^{aB}	1.30 ± 0.03^{aB}	0.78 ± 0.01^{aB}	1.99 ± 0.14^{aB}	0.27 ± 0.02^{aB}
6	2.05 ± 0.06^{aA}	0.89 ± 0.06^{aB}	0.94 ± 0.03^{aB}	0.99 ± 0.08^{aB}	1.96 ± 0.02^{aB}	0.24 ± 0.01^{aB}

a, b, c … - means in the same column separately for fresh and stored oils followed by different letters are significantly different ($p \leq 0.05$).

A, B - means in the same column separately for each kind of oil followed by different letters are significantly different ($p \leq 0.05$).

good quality, as indicated by their low degree of hydrolysis and oxidation (Table 4). According to the Codex Standard[56] for edible fats and oils cold pressed oils should have acid value less than 4.0 mg KOH/g oil. In the studied oils this discriminant ranged 0.5-1.25 mg KOH/g (Table 4). The content of free fatty acids, which is a measure of the acid value, was the highest in the case of oil no 4. Herchi *et al.*[10] found that the content of free fatty acids depends on the maturity of the raw material, and the smaller it is the more mature the raw material used to the oil production is. Cited authors showed more than 4-fold lower content of the hydrolysis products in the oil from mature raw material (seeds collected after 56 days of flowering) compared to the oil from immature raw material (seeds collected after 7 days of flowering). The differences in the degree of hydrolysis of commercial flaxseed oils were also indicated by Choo *et al.*[5]. The acid value for studied flaxseed oils that were sold in New Zealand were in the range of 0.5-2.5 mg KOH/g.

In our work oils were characterized by a very low content of peroxides (primary oxidation products), forming in the range 0.6-1.0 mEq O_2/kg (Table 4). The maximum permissible value of the discriminant for cold pressed oils is 15 mEq O_2/kg[56]. According to Herchi *et al.*[10] a peroxide value varies, depending on the degree of maturity of the raw material. Generally, oils from flaxseeds at early stages of maturity showed lower peroxide value (1.3 mEq O_2/kg), but it increased slightly during ripening (3.2 mEq O_2/kg), and then decreased to the initial state. However, Kasote *et al.*[11] demonstrated the impact of the number of pressings on the quality of flaxseed oil. The smallest peroxide value they received for the oil pressed twice, probably due to greater amount of phenolic antioxidants that were extracted with the oil. The anisidine value, indicator of secondary oxidation products content (mainly non-volatile 2-alkenals[57], of fresh studied oils were in the range of 0.56-1.07 (Table 4). Unfortunately, the limit of this quality discriminant of oils is not established, and the health safety of the oils is difficult to define. Anisidine values of the flaxseed oils analysed in this work are in accordance with results reported by Choo *et al.*[5] and Prescha *et al.*[12].

3.5 Oxidative stability and quality of flaxseed oils at the end of shelf life

Deterioration of oxidative stability and quality of studied flaxseed oils after 3 months of storage was observed. The induction time of oils was in range of 1.56-3.74 h (Table 4). The highest change (26%) in stability was noted for oil no 6, and the lowest (9%) for oil no 4 (Fig. 1).

The highest hydrolytic degree change occurred in oil no 3 (40% increase in acid value), whereas oxidative degree change was dependent on the type of oil and type of oxidation products. Generally, during storage at refrigerator peroxides and aldehydes were formed more rapidly in oil no 5; change of peroxide and anisidine values were ca. 37% and 40%, respectively (Fig. 1).

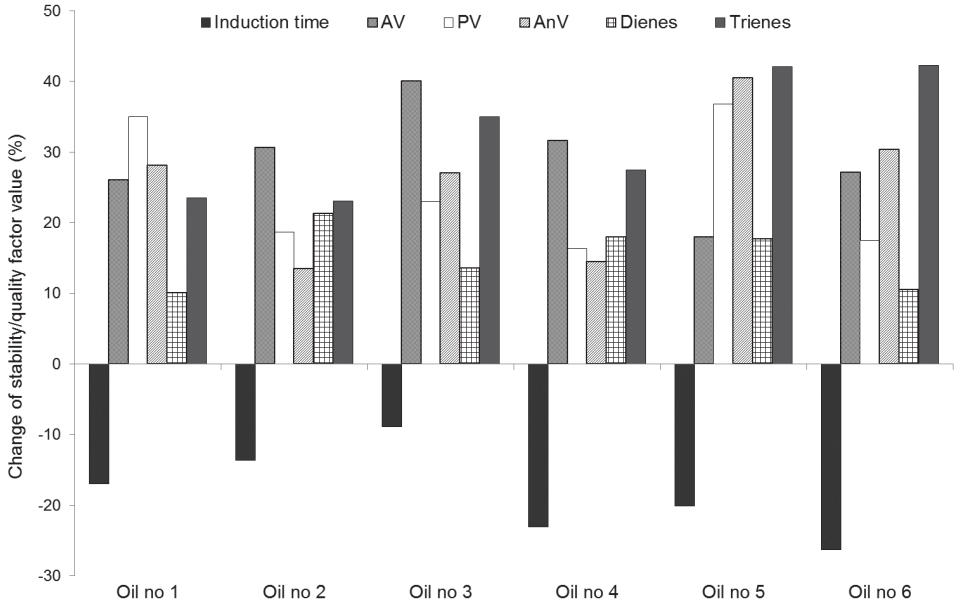

Fig. 1 Percentage changes of stability and quality factors of flaxseed oils after storage at refrigerator temperature.

The formation of peroxides from PUFA in the early stage of oxidation may result in double bond isomerization. It is observed in increase of absorption value measured at 233 nm, employed to monitor the formation of conjugated dienes. These compounds are related to the primary oxidation products, whereas absorption value measured at 270 nm indicates the presence of secondary oxidation products, i.e. aldehydes and ketones[10]. In studied flaxseed oils values of absorbance at 233 nm and 270 nm increased by an average of 16% and 30%, respectively. Higher change of triene content than diene content during flaxseed oil storage was also confirmed by Prescha *et al.*[12] who suggest that it may be related to high concentration of ALA.

Analysing the effect of chemical composition of oils on their stability and quality changes during storage, it was found that the stability deterioration was higher if the share of linolenic acid was higher ($r = 0.81$). Among the bioactive compounds, plastochromanol-8 had the greatest impact on improving the stability of oils ($r = -0.67$). In turn, change of oxidation degree of oils was dependent on the stearic acid and oleic acid shares for peroxide ($r = -0.69$) and anisidine ($r = -0.68$) values, respectively. Furthermore, it was shown that the formation of peroxides was slower in oils with higher content of Δ5-avenasterol ($r = -0.98$), whereas the formation of aldehydes was slower in oils contained more tocopherols ($r = -0.55$). Protective effect of avenasterol was observed by Małecka[58] during study of antioxidant properties of unsaponifiable matter isolated from tomato seeds, oat grains and wheat germ oils. The 0.3% addition of the unsaponifiables isolated from tomato seeds oil, containing the highest amount of Δ5-avenasterol, to refined rapeseed oil showed a higher antioxidative activity than the other isolates. The protective factor which was calculated based on changes of peroxide values in rapeseed oil during storage at 60℃ for tomato seed oil unsaponifiables was tree times higher than in case of the 0.02% addition of butylhydroxyanizole (BHA). This sterol also has been reported to reduce frying oil deterioration. Its effectiveness to protect from oxidation was studied during heating at 180℃ of soybean and cottonseed oils. All heated oils with added extract from oat, rice or olive oil containing Δ5-avenasterol deteriorated more slowly than the control samples[59-61]. Gordon and Magos[62] explained this phenomenon that lipid free-radicals react rapidly with sterols containing unhindered allylic carbon atoms such as in the ethylidene group and then allylic free radicals at C29 are formed. In isomerization process are produced stable allylic tertiary free-radicals at the C atom in the 24-position and they interrupts the oxidation chain. Moreover, study conducted by Rudzińska *et al.*[63] showed that phytosterol containing an additional double bond in the side chain, such as avenasterol, were more stable than phytostanols. In this study avenasterol and brassicasterol degradation rates in margarines enriched in plant sterols during storage for 18 weeks at two typical temperatures (4℃ and 20℃) appeared to be at the lowest level among all assessed phytosterols.

Finally, a type of packaging also plays an important role in protection the quality of flaxseed oils. We found, that despite the similarity of oils no 6 (packed in a transparent plastic bottle) and no 4 (packed in a brown glass bottle) in terms of the initial state, the quality and stability changes were greater in oil no 6. The effect of packaging materials on quality and stability of oils has been considered by many researchers. Méndez and Falqué[64] studied influence of container type on extra-virgin olive oil quality. Four com-

mercial extra-virgin olive oils were placed into 5 different containers: plastic bottle (transparent), opaque plastic bottle (covered with Al foil), glass bottle (transparent), tin, and Tetra-brik Aseptic®. Results of the study indicate that transparent plastic bottles are not suitable for storage longer than 3 moths. It is due to its oxygen permeability and oil exposition to light. These factors are able to promote hydrolysis of TAG and oxidation of fatty acids. Covering plastic bottles with aluminium foil reduces light transmission through container. Transparent glass bottles provided no oxygen permeability but oil was more exposed to light. It was shown that better types of package are plastic-coated paperboard laminate, tin containers and Tetra-brik Aseptic®. Pristouri *et al*.[65] also investigated effect of packaging on quality characteristics of extra-virgin olive oil. Basing on their results the most appropriate packages are glass, dark coloured containers. In turn, Tawfik and Huyghebaert[66] studied interactions between stability of vegetable oils and packaging materials. Their results show that the permeability to oxygen of different bottles clearly affects the oxidation degree of vegetable oils.

The results of our study showed that rapid deterioration of quality, due to high sensitivity to oxidation, is connected with high content of α-linoleic acid in flaxseed oils. Study followed by El-Gharbawi *et al*.[67] shows that increasing temperature and storage time causes an increase of acid, peroxide and TBA values. The major volatiles produced in autoxidation process of flaxseed oil are propanal and hexanal. Propanal is an oxidation product of α-linolenic acid, whereas hexanal formation takes place in oxidation of linoleic acid[50]. It is known that more unsaturated fatty acids are more prone to oxidation. Polyunsaturated fatty acids, linolenic and linoleic, undergo oxidation rapidly even at room temperature, while oleic acid can be oxidised only at elevated temperatures. Furthermore, the reaction rate of oxygen with linolenic acid is about twice as fast as that of linoleic acid[68, 69].

4 CONCLUSIONS

Based on the obtained results, it can be concluded that deterioration of flaxseed oil stability and quality depends on their chemical composition, initial hydrolysis and oxidation degrees, and the type of package. Flaxseed oils available on the market are varied in terms of fatty acid composition and content of bioactive compounds. These differences may result from use for the oil production seeds of different varieties, or their mixtures. However, buying of oil in the fresh state provides its good quality for a far longer period than declared by the manufacturer shelf life. It would be preferable to store flaxseed oil at refrigerator temperature, both at home and in shop. Using a dark-colored glass bottle to package the oil provides additional protection before its quality deterioration.

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