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Chemical Composition and Oxidative Stability of Selected Plant Oils

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Abstract Scientific data on the oxidative stability of borage oil, *Camelina sativa* oil, linseed oil, evening primrose oil and pumpkin seed oil are scarce. Chemiluminescence (CL) methods most commonly used to determine the oxidative stability of oils include measurement of hydroperoxide, intensity of light emitted during the accelerated oxidation process performed at high (>100 °C) temperatures or assisted by forced flow of air/oxygen through the sample. The aim of this study was to investigate the chemical composition and oxidative stability of selected vegetable oils available on the Polish market. Oxidative stability was determined using a fast, novel chemiluminescence-based method, in which light emitted during oxidation process conducted at 70 °C in the presence of some catalyzing Fe^{2+} ions is measured. A reaction of the applied type has not been reported so far. High contents of tocopherols and phytosterols were found in the analyzed oil samples. Oxidative stability of the samples was in most cases higher than the stability of refined rapeseed oil, a relatively stable substance from the oxidation point of view.

Keywords Vegetable oils · Oxidative stability · Chemiluminescence

Abbreviations

ALA	n-3 α-Linolenic acid
CAO	Crude amaranth oil
CBO	Crude borage oil
CCSO	Crude *Camelina sativa* oil
CHD	Coronary heart diseases
CLSO	Crude linseed oils
CPO	Crude primrose oil
DAD	Diode-array detector
DHA	22:6, n-3 Docosahexaenoic acid
EFSA	European Food Safety Authority
EI	Electron ionization
EPA	20:5, n-3 Eicosapentaenoic acid
FLD	Fluorescent detector
GC–MS	Gas chromatography–mass spectrometry
HDL	High density lipoprotein
LDL	Low density lipoprotein
MUFA	Monounsaturated fatty acids
PSO	Pumpkin seed oil
PUFA	Polyunsaturated fatty acids
RRO	Refined rapeseed oil
TAG	Triacylglycerides
TIC	Total ion current
UV	Ultraviolet detector
WHO	World Health Organization

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Introduction

Scientific papers describing the chemical composition of various vegetable oils like amaranth oil, borage oil, *Camelina sativa* oil, linseed oils, evening primrose oil or pumpkin seed oil are abundant [1–15]. However, considerably less research has been done on vegetable oil, the oxidative stability of which determines the oil's durability, usefulness, and the stability of the biologically active compounds present in it. The shelf life of food products to a great extent depends on the oxidative stability of oils contained in them (apart from microbiological aspects). Oil types investigated in this study are commonly used to prepare dishes, as dressings for salads, or flavoring substances used to improve the taste/smell/nutritional value of fried/stewed food. Information on the oxidative stability of nutritionally valuable vegetable oils could help us to identify optimal parameters at which those oils should be stored and/or to determine their shelf lifetimes during which they can be used for food processing before undesirable oxidation significantly decreases their nutritional properties.

Khan and Shahidi [11–13] studied the oxidative stability of borage oil and evening primrose oil and found that it was low. Their results suggest that the geographical origin of an oil determines its oxidative stability. Low stabilities could be linked to high contributions of polyunsaturated fatty acids in the oil chemical composition [16, 17]. Pumpkin seed oils may also have low oxidative stability due to high amounts of unsaturated fatty acids (up to 78%) [18, 19], natural pigments (including carotenoids) [7], sterols [9] and ions of metals such as Ca, K, Mg, Mn, Fe, Zn [6, 20, 21].

Amaranth oil is considered as an antioxidant due to a rich contribution of squalene to its chemical composition [2], squalene also increases the oil oxidative stability. High oxidative stability of the oil also results from a low content of polyunsaturated fatty acids and a high content of monounsaturated fatty acids. It is also claimed that amaranth oil is more oxidation-resistant than refined rapeseed oil.

The well-studied linseed oil is not particularly oxidation-resistant due to a high content of C18:3 fatty acids (traditional flax specimens) [14, 15, 22, 23].

Camelina sativa oil is considered a good raw material for biodiesel production, and some literature data suggest that its oxidative stability is high [4, 5]. However, those data are sparse.

Some papers suggest that cold-pressed olive oil has a higher oxidative stability than refined rapeseed oil and soybean oil [24]. This could be explained by the fatty acid composition of the olive oil, and a high content of monounsaturated fatty acids that show better resistance to oxidation than fatty acids with higher number of unsaturated bonds.

Non-refined vegetable oils are commonly considered as oxidatively non-stable [25].

Methods routinely used to determine lipid oxidative stability include peroxide value determination, OSI (Oxidative Stability Index), determination of the induction time in the Rancimat test, or determination of the quantity of the aldehydes formed (method with TBA) as a secondary products of lipid oxidation [26]. There are also some chemiluminometry-based methods available in the literature. Chemical reactions of lipid hydroperoxides with luminol (catalyzed using a suitable catalyst) 27, 28], reactions with lucigenine, oxalate derivatives or decomposition of hydroperoxides with sodium hypochlorite might be effectively used to precisely determine the hydroperoxides. In several papers lipid oxidative stability was measured using chemiluminescence (CL) of oxidation of lipids caused by high temperature and/or air (oxygen) forced flow [14, 29, 30].

The aim of this study was to determine the oxidative stability of selected non-refined vegetable oils, and to compare them with the rapeseed oil considered as a fairly oxidation-resistant oil. Oxidative stability was determined using a novel method based on vestigial chemiluminescence resulting from an accelerated oxidation of tested samples at ramped temperature in the presence of known amounts of Fe^{2+} ions. The stability data obtained were associated with the results of the Rancimat test, peroxide value, tocopherols/sterols contents, and fatty acid composition of the analyzed oils.

Experimental

Materials

Refined rapeseed oil of the ZT Kruszwica (Kruszwica, Poland) brand was bought from a local food store. Crude plant oils (linseed, *Camelina sativa*, primrose, borage, pumpkin seed and amaranth seed) were bought from SZARŁAT (Warsaw, Poland). Potassium hydroxide (KOH), methanol (MeOH), hexane, acetonitrile (ACN), *tert*-butyl methyl ether (MtBE) of HPLC purity were bought from POCH (Gliwice, Poland). $FeCl_2$ >99% and NH_4SCN (ammonium thiocyanate) of HPLC purity were bought from Merck (Darmstadt, Germany). A high purity standard of 5α-cholestane was obtained from Sigma–Aldrich, (Bellefonte, PA, USA) while HCL 36%, chloroform ($CHCl_3$), H_2O_2 (30%) of analysis grade were obtained from CHEMPUR (Piekary Slaskie, Poland).

Fatty Acids Profile

A sample of 0.2 g of oil was dissolved in 2 ml of hexane. The mixture was submitted for saponification with 0.5 ml of sodium hydroxide solution in methanol (2 M) at room temperature for 2 h. Then 200 µl of the hexane layer was transferred into 1.5 ml autosampler vial and dissolved in 1 ml of hexane. After that, 1 µl of the sample was injected into a GC–MS system equipped with the BPX90 capillary column. Helium was used as the carrier gas at a constant flow rate of 0.9 ml min^{-1}. A split/splitless injector was operated at a temperature of 230 °C with a split rate set to 100:1. The GC's oven temperature was programmed as follows: 50 °C hold for 2 min, ramped to 230 °C at a rate of 2.5 °C min^{-1}, hold for 5 min. The GC–MS interface temperature was 220 °C, the ion source temperature was 200 °C. Electron ionization (EI) was used with the electron energy set to 70 eV. Full Scan (*m*/*z* ranged 50–500) Total Ion Current (TIC) was used for analyte quantification. Samples were analyzed in triplicate.

Tocopherols

A 0.2-g sample of the oil was dissolved in 5 ml of ACN/MtBE mixture (4:6 by vol.). The mixture was filtered through a micro syringe filter (titan PTFE 0.2 µm). A 5-µl sample was injected into a VP Shimadzu HPLC system equipped with the SPD-M10Avp Shimadzu DAD, the FLD RF-10ALxl Shimadzu fluorescence detector, reversed phase octadecyl silica C18 HPLC column Gemini C18 (150 mm × 2 mm × 3 µm) (Phenomenex Torrance, CA, USA), and a suitable guard column. The gradient flow rate was 0.15 ml min^{-1}, and the column oven temperature was 35 °C. ACN was the A phase and the ACN/MtBE mixture (4:6 by vol.) was the B phase. The gradient flow program was 100% of A until 12 min, then 100% of B for 30 min in order to elute triacylglycerides from the chromatographic system before the second injection. The chromatographic system was allowed to equilibrate to the initial conditions after every single analysis. Tocopherols were qualified using standard UV spectrum analysis (DAD 190–370 nm) and literature data for reference. Quantitative analysis of tocopherols was carried out using data from the fluorescence detector with excitation/emission wave lengths of 290/330 nm, respectively. Samples were analyzed in triplicate. The tocopherol:oil ratio was expressed in mg 100 g^{-1}. Under the chromatographic conditions used, β- and γ-tocopherols did not separate fully, so the sum of the β- and γ-tocopherols was determined.

Sterols

A 0.2-g oil sample was dissolved in 3 ml of hexane, then 50 µl of 5α-cholestane (0.4 mg ml^{-1}) internal standard was added. The mixture was saponified with 0.5 ml of sodium hydroxide solution in methanol (2 N) at room temperature for 1–2 h. Then, 200 µl of hexane layer was transferred into a 1.5-ml vial and diluted with 1 ml of hexane. Then 1 µl of the sample was injected into a GC–MS system (setup: DB-5 ms capillary column, A He carrier gas at 0.9 ml min^{-1} constant flow rate, split/splitless injector at 230 °C in the splitless mode, GC–MS interface temperature 240 °C, ion source temperature 220 °C, EI electron energy 70 eV, Full Scan *m*/*z* 100–600, TIC quantification). Oven temperature: 50 °C hold for 2 min, ramped to 230 °C at 15 °C min^{-1}, ramped to 310 °C at 3 °C min^{-1}, hold for 10 min. The internal standard method (5α-cholestane) was used for quantification. Samples were analyzed in triplicate.

Peroxide Value (PV)

A modified method originally proposed by Hornero-Mendeza et al. [31] was used to determine PV. Briefly, 200 mg of oil was mixed with 5 ml of hexane, 200 µl of the solution was mixed with 5 ml of methanol/chloroform/HCL solution (1:1:0.012, by vol.), then 100 µl of NH_4SCN (30% water solution) and 100 µl of 0.4% water solution of $FeCl_2$ were added. The reaction was conducted at room temperature. Five minutes later, the absorbance at the 480-nm wavelength was measured. Spectrophotometer readings were set to zero using a blank sample.

Calibration Curve

A 2-ml sample of 30% H_2O_2 water solution titrated iodometrically [32] was dissolved in 100 ml of distilled water. Then, 90 ml of the mixture was diluted to 1,000 ml with distilled water. Calibration solutions were prepared by dilution of 5, 10, 20, 30, 40, 50, 60, 70 and 80 ml of the solution to 100 ml with distilled water. A 200-µl sample of each calibration solution was submitted to the H_2O_2 concentration calibration test, using the method described in "Peroxide Value". The calibration curve obtained ($A = 0.1057\,X_{H_2O_2}, R = 0.993$) was used to calculate the oxygen concentration in the oxidized oil samples. Results in mg $O_2 \cdot kg^{-1}$ were calculated using the following equation:

$$X = \left(\frac{A}{106}\right) \cdot \varepsilon \cdot R \quad (1)$$

where

X oxygen concentration in mg O_2 kg^{-1}
A absorbance
ε fraction of O_2 in H_2O_2, $\varepsilon = 0.94$
R dissolution of oil (in order to calculate PV per kg of oil)

The above equation holds for absorbance values within the 0.049–0.998 range.

Rancimat Test

Conductometric analysis of the total volatile compounds formed during lipid hydroperoxide degradation was carried out on a Methrom Rancimat device (Herisau Switzerland) [33–35]. Briefly, 2.5 g of oil was placed in a reaction cell held at 100 °C and oxidized with an air stream at a flow rate of 10 l h^{-1}. Samples were analyzed in triplicate.

Chemiluminescence

Chemiluminescence was used to analyze the oxidative stability of the oils. Oxidation of lipids is accompanied by a very weak light emission that may be used to study the dynamics/progress of the process provided some particular conditions are met. Lipid luminescence is a consequence of their oxidation with free radicals [36–39]. Mechanisms of this reaction are shown schematically below [39]:

$$\left.\begin{array}{ll} \text{I} & R^{\bullet} + R^{\bullet} \\ \text{II} & R^{\bullet} + RO^{\bullet} \\ \text{III} & R^{\bullet} + RO_2^{\bullet} \\ \text{IV} & RO^{\bullet} + RO^{\bullet} \\ \text{V} & RO^{\bullet} + RO_2^{\bullet} \\ \text{VI} & RO_2^{\bullet} + RO_2^{\bullet} \end{array}\right\} \xrightarrow{k_i} p^* \rightarrow p + h\nu \qquad (2)$$

p^* Reaction product in the excited state
p Reaction product in the ground state

Quantum efficiency of reaction VI is higher than for other reactions. Recombination of $RO_2^{\bullet}$ (peroxide radical) gives electronically excited carbonylic compounds, mainly in triplet states like $R = O^*$and oxygen. The reaction runs as follows [39]:

$$RO_2^{\bullet} + RO_2^{\bullet} \xrightarrow{k} R = O^* + RO^{\bullet} + O_2 \rightarrow R = O + h\nu (\geq 300 \text{kJ/mol}) \qquad (3)$$

Reactions of this type occur in the tissues of living organism. Production and decomposition of peroxide radicals during non-enzymatic reactions of gelatinous film oxidation (e.g. unsaturated fatty acids) plays a particular role in ultra-low chemiluminescence. The chemiluminescence method used in this study was based on measurement of the intensity of light emitted during decomposition of the peroxide radicals. The rate of peroxide radical decomposition was increased by means of an increased reaction temperature and added Fe^{2+} catalyst ions. All the oils studied were ranked in respect to their oxidative stability on the basis of the time required to reach their maximum chemiluminescence intensity. The chemiluminescence method used showed a good correlation with two other traditional methods used to determine the oxidative stability of oils.

Chemiluminescence of the analyzed oil samples at 80 °C in the presence of Fe^{2+} ions was measured by means of a chemiluminometer operated in the Single Photon Counting mode. A 2.5-g sample of the analyzed oil at room temperature was pumped by HPLC pump via a capillary into 50-ml glass tube containing 20 ± 0.5 mg of $FeCl_2$ catalyst and inserted into the cell of the chemiluminometer. The oil was pumped for 25 s following 15 s of pre-analysis. The Hamamatsu R1527p photomultiplier tube used as the detector was operated at −10 °C and 1 s gate time.

Results

Fatty acid profiles of the analyzed oil samples are shown in Table 1. The oils turned out to be strongly diversified in

Table 1 Fatty acid profile and their concentration in % of various vegetable oils

Fatty acids	RRO	CLSO	CCSO	CPO	CBO	PSO	CAO
C16:0	2,5	3,5	4,5	3,4	5,7	6,24	6,5
C17:0	–	–	–	–		–	0,8
C18:0	0,7	1,3	1,3	0,8	1,4	1,95	1,4
C18:1 *cis*-9	71,8	17,5	11,1	3.0	16,1	32,64	24,0
C18:1 *cis*-11	2,2	0,5	0,7	0,3	0,5	0,59	0,9
C18:2 *cis*-9,12	15,4	12,2	21,6	86,0	46,3	58,17	65,4
C18:3 *cis*-6,9,12	0,1	65	39,7	0,1	23,1	–	–
C18:3 *cis*-9,12,15	5,9	–	–	6,2	0,1	0,17	0,6
C20:0	0,2	–	0,6	0,1	0,1	0,13	0,2
C20:1 *cis*-11	0,8	–	14,6	–	3,6	0,13	–
C20:2 *cis*-11,14	–	–	1,7	–	0,1	–	–
C20:3 *cis*-11,14,17	–	–	1,2	–	–	–	
C22:0	–	–	0,1	–	–	–	–
C22:1 *cis*-13	0,2	–	2,5	0,1	2,0	–	0,1
C22:2 *cis*-13,16	–	–	0,1	–	–	–	–
C24:1 *cis*-15	–	–	0,3	–	1,0	–	–

RRO refined rapeseed oil, *CLSO* crude linseed oils, *CCSO* crude *Camelina sativa* oil, *CPO* crude primrose oil, *CBO* crude borage oil, *PSO* pumpkin seed oil, *CAO* crude amaranth oil

their fatty acid profiles, as well as for SFA, MUFA or PUFA.

The C16:0 fatty acids content in the analyzed samples ranged from 2.5 to 6.5%, with the highest contributions observed for CAO and PSO. C18:0 fatty acid was found at the highest concentration in PSO, other oils contained less of that acid. RRO had the highest content of C18:1 (about 82% *cis*-9 and 2.2% *cis*-11) while in CPO C18:1 contributed only 3% (*cis*-11) and 0.3% (*cis*-9). The highest content of linoleic acid (C18:2) was found in CPO (86%) and the lowest content in CLSO (12.2%). Margaric acid was found only in CAO (0.8%). Saturated/unsaturated long chain fatty acids also contributed negligibly, except for eicozenic acid C20:1 *cis*-11 present in high amounts in CCSO (14.6%) and CBO (3.6%).

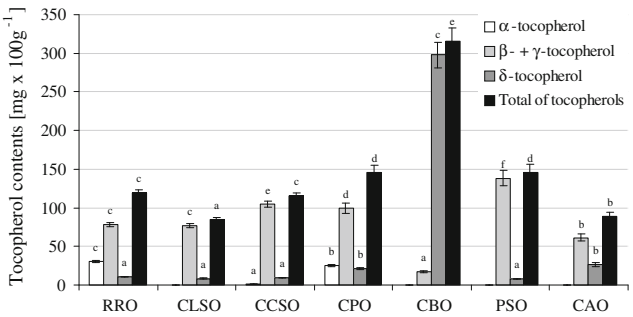

Fig. 1 Tocopherol profile of various vegetable oils and their concentrations in mg 100 g^{-1} . *RRO* refined rapeseed oil, *CLSO* crude linseed oils, *CCSO* crude *Camelina sativa* oil, *CPO* crude primrose oil, *CBO* crude borage oil, *PSO* pumpkin seed oil, *CAO* crude amaranth oil

The tocopherols content in the analyzed vegetable oil samples is shown in Fig. 1. Concentrations of α-tocopherol in RRO, CPO and CCSO samples were 30.9, 23.9 and 1.3 mg 100 g^{-1}, respectively. Both δ-tocopherol and β- + γ-tocopherols were found in all studied oil samples.

Phytosterols results are summarized in Table 2. The total amount of phytosterols in CPO, RRO, CCSO and CLSO varied between 720.4 and 513.2 mg 100 g^{-1}. The highest amount (1,249.0 mg 100 g^{-1}) was found in CAO, the lowest amount–in CBO and PSO (365.5 and 345.0 mg 100 g^{-1}, respectively). Vegetable stanols were also found in the analyzed oil samples.

Cholesterol (a sterol typical for animal fat) was found in only two oils at concentrations ranging from 29.2 to 33.7 mg 100 g^{-1}. Sterol composition varied among the studied CAO, PSO and CBO oils attaining a maximum concentration of 1249.0 mg 100 g^{-1} in the case of phytosterols in CAO. Several other sterols (7Δ-avenasterol, 7Δ-sitosterol, citrostadienol, campesterol) were found. Spinasterol, derivatives of stigmasta-7,25-dienol and stigmasta-7,22,25-trienol were found in CPSO. 25-Hydroxy-24-methylcholesterol, lupeol, gramisterol, citrostadienol, cycloartenol were uniquely found only in CBO. No significant differences in composition and amounts of phytosterols

Table 2 Phytosterol profile and their concentration in mg 100 g^{-1} of various vegetable oils

Phytosterols and stanols	RRO	CLSO	CCSO	CPO	CBO	PSO	CAO
Brassicasterol	$69,6^{(0,2)}$	–	$33,9^{(3,0)}$	–	–	–	$21,4^{(1,5)}$
Campesterol	$232,5^{(13,2)}$	$97,5^{(1,8)}$	$109,6^{(9,3)}$	$35,3^{(4,6)}$	$82,6^{(4,8)}$	–	$198,0^{(24,8)}$
Stigmasterol	$2,4^{(0,3)}$	$31,4^{(1,2)}$	$9,4^{(0,1)}$	$6,5^{(1,1)}$		–	$18,5^{(3,0)}$
β-Sitosterol	$324,7^{(2,8)}$	$162,5^{(6,9)}$	$361,3^{(32,5)}$	$645,8^{(0,5)}$	$80,7^{(12,6)}$	–	$559,5^{(9,1)}$
Delta 5-avenasterol	$8,7^{(0,5)}$	$38,8^{(5,0)}$	$25,7^{(0,4)}$	$24,8^{(0,1)}$	$87,1^{(10,1)}$	–	$2,2^{(0,8)}$
Delta-7-avenasterol	–	–	–	–	–	70,4(10,2)	153,9(1,7)
25-hydroxy-24-methylcholesterol	–	–	–	–	$53,1^{(6,4)}$	–	–
Lupeol + gramisterol	–	–	–	–	$10,4^{(2,4)}$	–	–
Delta-7-sitosterol	–	–	–	–	–	–	$256,0^{(42,0)}$
Cholesterol	–	–	$33,7^{(4,1)}$	–	–	–	$29,2^{(0,2)}$
Spinasterol + β-Sitosterol	–	–	–	–	–	$187,9^{(22,8)}$	–
Stigmasta-7,25-dienol	–	–	–	–	–	$74,1^{(18,3)}$	–
Stigmasta-7,22,25-trienol	–	–	–	–	–	$16,7^{(5,6)}$	–
Citrostadienol	–	–	–	$8,0^{(0,1)}$	$16,5^{(4,7)}$	–	$7,0^{(0,9)}$
Campestanol	–	–	–	–	–	–	$3,4^{(0,4)}$
Cycloartenol	–	$183,0^{(6,0)}$	$18,5^{(1,6)}$	–	$35,2^{(3,7)}$	–	–
Σ	$637,9^{(9,3)c}$	$513,2^{(14,9)d}$	$592,2^{(47,0)c}$	$720,4^{(6,2)b}$	$365,5^{(44,5)e}$	$349,0^{(45,6)e}$	$1249,0^{(62,8)a}$

Numbers in brackets are standard deviations (SD). Letters from a to e are homogeneous groups

RRO refined rapeseed oil, *CLSO* crude linseed oils, *CCSO* crude *Camelina sativa* oil, *CPO* crude primrose oil, *CBO* crude borage oil, *PSO* pumpkin seed oil, *CAO* crude amaranth oil

were found in the rest of the analyzed oil samples. β-Sitosterol and campesterol were the dominant phytosterols found in these oils.

Figure 2 shows changes of peroxide values in the studied oils at 60 °C. The rate of change observed in CLSO and CCSO was very dynamic. From the PV rate change point of view, all the analyzed oils can be ranked as follows: CPO > CAO > RRO > CBO > PSO.

Figure 3 shows the results of the Rancimat tests. It was found that PSO had the longest induction time 13.63 h (SD = 0.15). CLSO was the least stable oil—induction time for that oil amounted merely to 5.85 h (SD = 0.16).

Figure 4a and b show the results of chemiluminescence measurements performed at 80 °C with the addition of $FeCl_2$ used to speed up the oxidation process. Oils heated in the presence of the catalyst exhibited varying chemiluminescence. The time to reach the maximum chemiluminescence intensity was specific for each tested oil. The time was the longest for PSO (234.1 min, SD = 28.3) and the shortest for CLSO (24.8 min, SD = 1.2)

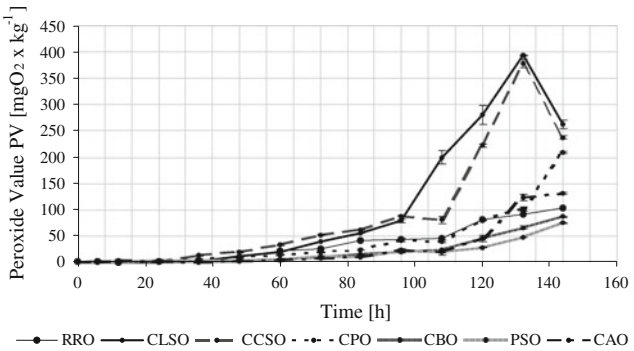

Fig. 2 Peroxide Value (PV) changes in various vegetable oils in 60 °C. *RRO* refined rapeseed oil, *CLSO* crude linseed oils, *CCSO* crude *Camelina sativa* oil, *CPO* crude primrose oil, *CBO* crude borage oil, *PSO* pumpkin seed oil, *CAO* crude amaranth oil

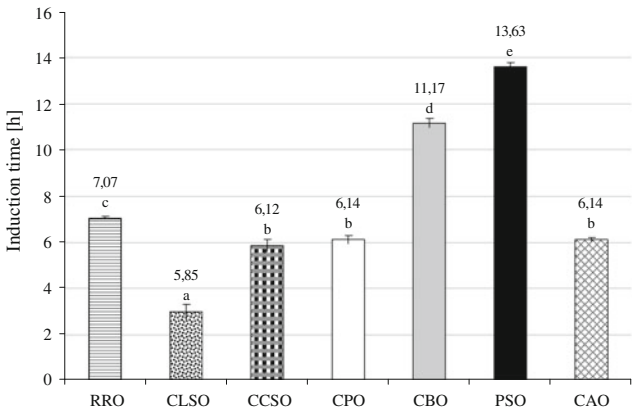

Fig. 3 Rancimat test of various vegetable oils in 100 °C and air flow 10 l h^{-1}. *RRO* refined rapeseed oil, *CLSO* crude linseed oils, *CCSO* crude *Camelina sativa* oil, *CPO* crude primrose oil, *CBO* crude borage oil, *PSO* pumpkin seed oil, *CAO* crude amaranth oil

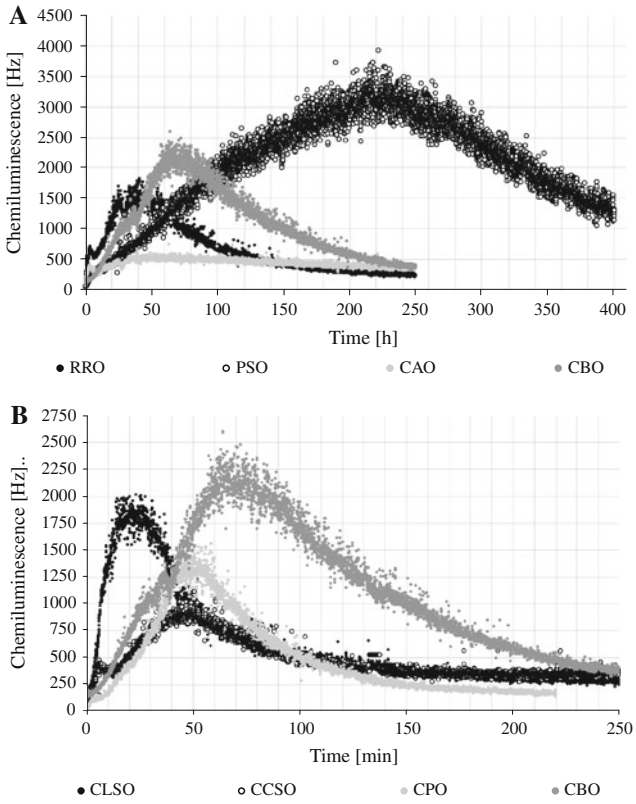

Fig. 4 Chemiluminescence of various vegetable oils in 80 °C with 20 mg $FeCl_2$ added. *RRO* refined rapeseed oil, *PSO* pumpkin seed oil, *CAO* crude amaranth oil, *CBO* crude borage oil, *CLSO* crude linseed oils, *CCSO* crude *Camelina sativa* oil, *CPO* crude primrose oil

Discussion

The fatty acid composition of the analyzed oil samples was generally in accordance with literature data reported for those types of oil [7–9, 11, 15, 40, 51]. The composition is one of the most important factors determining the oxidative stability of the oils. It is known that unsaturated lipids are easily oxidized and this phenomenon comes down to the oxidation of (bonded with glycerol fatty acid) unsaturated hydrocarbon chains, especially polyunsaturated ones [26, 42]. Consequently, a higher contribution of unsaturated fatty acids should result in lower oxidative stability. The rate of the oxidation reaction increases with the degree of unsaturation. The rate of C18:2 acid oxidation is 10–40 times higher than that of C18:1, and the rate of C18:3 oxidation is 2–4 times faster than that of C18:2 [42].

The fatty acid composition determined in the investigated oils (see Table 1) might suggest that, from the point of view of their oxidative stability, the analyzed oils should

be ranked in the following order: RRO, PSO, CAO, CBO, CCSO, CPO and finally CLSO. However, the results of direct tests of the stability (PV, Rancimat test, CL catalyzed with Fe^{+2} ions) do not quite agree with the fatty acid composition: refined rapeseed oil did not exhibit the highest oxidative stability, whereas borage oil and pumpkinseed oil were oxidatively very stable despite a less favorable composition of fatty acids as compared to RRO. CBO showed the highest content of tocopherol, which might explain a high oxidative stability of this oil. What is more, only the CBO tocopherol fraction was rich in δ-tocopherol (suspected to be an antioxidant). Mortensen and Skibsted (1997) [43] demonstrated that δ-tocopherol had the highest reactivity against free radicals, which might also contribute to the high stability of this oil.

PSO contained significantly less tocopherol than CBO, however, it was the most oxidation-resistant oil. This could be explained by the chemical composition of the oil: PSO had an oxidatively favorable fatty acid composition. It did not contain significant amounts of C18:3 but contained considerable amounts of C18:1, which strongly increased its oxidative stability. Both PSO and CBO had the lowest contents of sterols. This fact also indicates that oxidative stability of an oil might be related to the content of plant origin sterols. Sterols are polycyclic alcohols classified as steroids. Sterols are usually found in vegetable oils as fatty acid esters. The autoxidation process is accompanied by hydrolysis of the esters, which leads to the formation of free fatty acids that act favorably on the autoxidation process and increase its rate [26, 42]. Since the sterol content in PSO and CBO was low, the rate of increase in the free fatty acid content was reduced and the oxidation process proceeded slower than in other oils.

This hypothesis could also explain the results obtained for CAO. The fatty acid composition of CAO might suggest that this oil is highly stable; however, its stability as determined directly, turned out to be relatively low. The high phytosterol content might significantly hasten the oxidation process despite the high contribution of tocopherols and squalene, which act as antioxidants [44, 45]. CAO is regarded as a very precious source of dietary phytosterol, it can contain up to 2 g 100 g^{-1} of phytosterols [46]. Results of this study showed that CAO was the richest source of phytosterols among all the oils examined.

Another aspect is the chemical structure of plant-origin sterols. Even if sterols (like brassicasterol, stigmasterol, ergosterol or spinasterol) have two carbon–carbon double bonds in the β ring, they also contain unsaturated double bonds in the aliphatic-side chains. The latter may be easily oxidized. The analyzed oils did not contain any practically significant quantities of this type of sterol. Consequently the sterol pro-oxidative action in the analyzed oils might be explained by the effect of the TAG's ester bond hydrolysis rather than the oxidation of sterol molecules in the aliphatic-side chains.

The average phytosterol content agreed with the literature data reported for oils of the studied types [9, 46–48]. The phytosterol profile observed by us was slightly different to that published in the literature, but that can probably be traced down to different plant varieties, different climatic conditions, etc. Campesterol and sitosterol found in the oil samples analyzed by us are the most important and the most frequently identified phytosterols in vegetable oils.

The polyphenol contents is also another important issue. Determination of polyphenol was out of the scope of this study, however, comprehensive information about polyphenols in vegetable oils may be found in the literature. In general, non-refined vegetable oils contain vast amounts of polyphenols that may improve oxidative stability being antioxidants [24, 49–51]. Both CBO and PSO contain high amounts of polyphenols, which most probably contributes to the high oxidative stability of those oils. However, the relationship between oxidative stability and polyphenol/sterol content is not very well known and further research into this subject is needed.

Results of this study show that the proposed CL method is well-suited and useful for determining the oil oxidative stability. The addition of Fe^{2+} ions allowed us to reduce the analysis time and provided very good reproducibility of the results when compared to tests conducted at an increased temperature or temperature/air flow only. Application of Fe^{2+} ions increased the rate of lipid hydroperoxide decomposition according to the Fenton reaction scheme:

$$\mathrm{ROOH} + \mathrm{Fe}^{2+} \rightarrow \mathrm{RO}^{\bullet} + \mathrm{Fe}^{3+} + \mathrm{HO}^{-}$$

$$\mathrm{ROOH} + \mathrm{Fe}^{3+} \rightarrow \mathrm{ROO}^{\bullet} + \mathrm{Fe}^{2+} + \mathrm{H}^{+}$$

which in turn caused the CL signal was produced mainly by lipid hydroperoxide reactions rather than by other possible reactions described in the literature (Eq. 2). Photoemission intensity at type IV and type VI reactions (Eq. 2) was multiplied in the proposed conditions. Correlation of results produced by the proposed CL method with results of the Rancimat test or the PV method can be easily proven.

The proposed CL method is faster and needs lower temperatures than Rancimat tests (temperatures in excess of 100 °C are needed for the Rancimat test, since at lower temperatures, the analysis time is very long and the water in which the electrodes used to measure conductivity are immersed evaporates). Moreover, the Rancimat tests need special gases (air, oxygen, nitrogen or argon) to increase the oxidation process rate, or as a medium to transfer volatile compounds into the cylinder with water where the

conductivity is measured. The CL method is free of such shortcomings. The Rancimat test detects secondary products of lipid oxidation, whereas the chemiluminescence methods detect transitional products. Using the CL methods it is also possible to determine when the termination stage begins and the stage of oxidation propagation begins to dominate the reaction medium.

Conclusions

- Various chemiluminescent-based methods could be an alternative to Rancimat tests.
- Fatty acid composition does not provide sufficient information to predict oil oxidative stability.
- Combination of chemical composition results with direct oxidative stability test results gives a much better image of stability of an analyzed oil.
- Contents of triacylglyceride-soluble substances strongly influence oil oxidative stability.

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References

1. León-Camacho M, García-González DL, Aparicio R (2001) A detailed and comprehensive study of amaranth (*Amaranthus cruentus* L.) oil fatty profile. Eur Food Res Technol 213:349–355
2. Sun H, Wiesenborn D, Tostenson K, Gillespie J, Rayas-Duarte P (1997) Fractionation of squalene from amaranth seed oil. JAOCS 74:413–418
3. Angelini LG, Moscheni E, Colonna G, Belloni P, Bonari E (1997) Variation on agronomic characteristic and seed oil composition of new oilseed crops in central Italy. Ind Crops Prod 6:313–323
4. Abramovic H, Butinar B, Nikoli V (2007) Changes occurring in phenolic content, tocopherol composition and oxidative stability of *Camelina sativa* oil during storage. Food Chem 104:903–909
5. Bernardo A, Howard-Hildige R, O'Connell A, Nichol R, Ryan J, Rice B, Roche E, Leahy JJ (2003) Camelina oil as a fuel for diesel transport engines. Ind Crops Prod 17:191–197
6. Kreft I, Stibilj V, Trkov Z (2002) Iodine and selenium contents in pumpkin (*Cucurbita pepo* L.) oil and oil-cake. Eur Food Res Technol 215:279–281
7. Parry J, Hao Z, Luther M, Su L, Zhou K, Yu LL (2006) Characterization of cold pressed onion, parsley, cardamom, mullein, roasted pumpkin, and milk thistle seed oils. JAOCS 83:847–854
8. Parry JW, Cheng Z, Moore J, Yu LL (2008) Fatty acid composition, antioxidant properties, and antiproliferative capacity of selected cold-pressed seed flours. JAOCS 85:457–464
9. Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM (2007) Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. Plant Foods Hum Nutr 62:85–91
10. Fukushima M, Matsuda T, Yamagishi K, Nakano M (1997) Comparative hypocholesterolemic effects of six dietary oils in cholesterol-fed rats after long-term feeding. Lipids 32:1069–1074
11. Khan MA, Shahidi F (2000) Oxidative stability of stripped and nonstripped borage and evening primrose oils and their emulsions in water. JAOCS 77:963–969
12. Khan MA, Shahidi F (2001) Effects of natural and synthetic antioxidants on the oxidative stability of borage and evening primrose triacylglycerols. Food Chem 75:431–437
13. Khan MA, Shahidi F (2002) Photooxidative stability of stripped and non-stripped borage and evening primrose oils and their emulsions in water. Food Chem 79:47–53
14. Stenberg C, Svensson M, Johansson M (2005) A study of the drying of linseed oils with different fatty acid patterns using RTIR-spectroscopy and chemiluminescence (CL). Ind Crops Prod 21:263–272
15. Lazzari M, Chiantor O (1999) Drying and oxidative degradation of linseed oil. Polym Degrad Stab 65:303–313
16. Sensidoni A, Bortolussi G, Orlando C, Lognay G, Fantozzi P, Paquot M (1995) Composition and oxidative stability of borage (*Borago officinalis*) and borage-virgin olive oil blends. Lebensm Wiss Technol 28:343–346
17. Hudson BJF (1984) Evening primrose (Oenothera spp.) oil and seed. JAOCS 61:540–543
18. Younis YMH, Ghirmay S, Al-Shihry SS (2000) African *Cucurbita pepo* L.: properties of seed and variability in fatty acid composition of seed oil. Phytochemistry 54:71–75
19. El-Adawy TA, Taha KM (2001) Characteristics and composition of different seed oils and flours. Food Chem 74:47–54
20. Mansour EH, Dworschak E, Lugasi A, Barna E, Gergely A (1993) J Sci Food Agric 61:73–78
21. El-Adawy TA, Taha KM (2001) Characteristics and composition of different seed oils and flours. J Agric Food Chem 49:1253–1259
22. Rudnik E, Szczucinska A, Gwardiak H, Szulc A, Winiarska A (2001) Comparative studies of oxidative stability of linseed oil. Thermochim Acta 370:135–140
23. Marciniak-Łukasiak K, Bikowska A, Krygier K (2006) Influence of use of nitrogen into oxidative stability of mixtures of rapeseed and linseed oils. ŻYWNOŚĆ Nauka Technologia Jakość 47:206–215
24. Koski A, Psomiadou E, Tsimidou M, Hopia A, Kefalas P, Heinonen KWM (2002) Oxidative stability and minor constituents of virgin olive oil and cold-pressed rapeseed oil. Eur Food Res Technol 214:294–298
25. Isbell TA, Abbott TP, Carlson KD (1999) Oxidative stability index of vegetable oils in binary mixtures with meadowfoam oil. Ind Crops Prod 9:115–123
26. Fox NJ, Stachowiak GW (2007) Vegetable oil-based lubricants: a review of oxidation. Tribol Int 40:1035–1046
27. Yang GC, Qiang W, Morehouse KM, Rosenthal I, Ku Y, Yuraweczs P (1991) Determination of hydroperoxides in edible oils by electron spin resonance, thiobarbituric acid assay, and liquid chromatography-chemiluminescence techniques. J Agric Food Chem 39:896–898
28. Jimenez AM, Navas MJ (2002) Chemiluminescence methods (present and future). Grasas y Aceites 53:64–75
29. Lorance G, Fridovich I, Handler P (1962) Chemiluminescence induced by operation of iron-flavoproteins. Biochemistry 5:779–783
30. Ilgvarr J, Hedenburg SJF (1985) Chemiluminescence in autoxidation of hydrocarbons. A method for fingerprinting and evaluation of oxidative stability. Ind Eng Chem Prod Res Dev 24:442–447
31. Hornero-Mendez D, Perez-Galvez A, Minguez-Mosquera MIA (2001) Rapid spectrophotometric method for the determination of

peroxide value in food lipids with high carotenoid content. JAOCS 78:1151–1155
32. Yamamoto Y, Niki E, Tanimura R, Kamiya Y (1985) Study of oxidation by chemiluminescence. IV. Detection of low levels of lipid hydroperoxides by chemiluminescence. JAOCS 62:1248–1250
33. Płatek T (1996) Badania nad wpływem procesów rafinacyjnych na stabilność oksydatywną oleju rzepakowego. Wydawnictwo SGGW 45-74
34. Wan PJ Accelerated stability methods, vol 67. AOCS, Champaign, pp 299–306
35. Laübli W, Bruttel AP (1986) Determination of the oxidative stability of fats and oils: comparison between the active oxygen method (AOCS Cd 1257) and the Rancimat method. JAOCS 63:792–794
36. Catala A (2006) An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. IJBCN 38:1482–1495
37. Dodeigne C, Thunus L, Lejeune R (2000) Chemiluminescence as diagnostic tool. A review. Talanta 51:415–439
38. Navas MJ, Jimenez AM (1996) Review of chemiluminescence methods in food analysis. Rapid Commun 55:7–15
39. (1989) Biospektroskopia 3. In: Twardowski J (ed) PWN, Warszawa, pp 107–205
40. Karvonen HM, Aro A, Tapola NS, Salminen I, Uusitupa MIJ, Sarkkinen ES (2002) Effect of alpha-linolenic acid rich *Camelina sativa* oil on serum fatty acid composition and serum lipids in hypercholesterolemic subjects. Metabolism 51:1253–1260
41. Christie WW (1999) The analysis of evening primrose oil. Ind Crops Prod 10:73–83
42. Choe E, Min DB (2006) Mechanisms and factors for edible oil oxidation. Compr Rev Food Sci Food Saf 5:169–186
43. Mortensen A, Skibsted LH (1997) Real time detection of reactions between radicals of lycopene and tocopherol homologues. Free Radic Res 27:229–234
44. Rao MKG, Achaya KT (1968) Antioxidant activity of squalene. JAOCS 45:296
45. Ko Ting-Fu, Weng Yih-Ming, Robin Y-YChiou (2002) Squalene content and antioxidant activity of *Terminalia catappa* leaves and seeds. J Agric Food Chem 50:5343–5348
46. Berger A, Monnard I, Dionisi F, Gumy D, Hayes KC, Lambelet P (2003) Cholesterol lowering properties of amaranth flakes, crude and refined oils in hamsters. Food Chem 81:119–124
47. Phillips KM, Ruggio DM, Toivo JI, Swank MA, Simpkins AH (2002) Free and esterified sterol composition of edible oils and fats. J Food Compost Anal 15:123–142
48. Mandl A, Reich G, Lindner W (1999) Detection of adulteration of pumpkin seed oil by analysis of content and composition of specific D7-phytosterols. Eur Food Res Technol 209:400–406
49. Ninfali P, Aluigi G, Bacchiocca M, Magnani M (2003) Antioxidant capacity of extra-virgin olive oils. JAOCS 78:243–247
50. Khattab R, Eskin M, Aliani M, Thiyam U (2009) Determination of sinapic acid derivatives in canola extracts using high-performance liquid chromatography. JAOCS published online 30 October
51. Koski A, Pekkarinen S, Hopia A, Wahala K, Heinonen M (2003) Processing of rapeseed oil: effects on sinapic acid derivative content and oxidative stability. Eur Food Res Technol 217:110–114