Effects of roselle extract on the oxidative stability of hempseed oil

SIMONA OANCEA – OLGA DRĂGHICI – MIRABELA PERJU – FRANCISC V. DULF

Summary
Hempseed oil is exceptionally rich in valuable unsaturated fatty acids if adequate oxidative protection is ensured. The aim of this study was to investigate its stability at 60 °C in the presence of roselle (*Hibiscus sabdariffa)* extract rich in anthocyanins (7206.6 mg·kg^{-1} dry weight) and characterized by high antioxidant and antiradical activities. Thermal degradation of the oil during storage was evaluated on the basis of the fatty acid profile using analysis by gas chromatography-mass spectrometry and kinetic studies based on peroxide value and differential scanning calorimetry measurements. The evolution of the unsaturated fatty acids showed protective properties of the natural extract against lipid oxidation. Accumulation of peroxides in oil was reduced by 56 % in the presence of roselle extract compared to untreated oil, which was confirmed by kinetic analysis of the induction period. The thermal degradation kinetics using the Ozawa-Flynn-Wall method indicated that 0.2% (w/w) addition of roselle extract to hempseed oil improved its stability due to higher activation energy compared to other samples. The antioxidant effect of *Hibiscus* extract was also validated by Fourier-transform infrared spectroscopy. Our findings indicate that *H. sabdariffa* can be a safe and effective natural alternative to synthetic antioxidants applied to hempseed oil.

Keywords
antioxidant; oxidative stability; gas chromatography-mass spectrometry; thermal analysis; Fourier-transform infrared spectroscopy; oilseeds

Edible oils are important food items, not only because of their energy value, but also for their content of essential fatty acids, being consumed as such or used as ingredients in foodstuffs. A still underutilized exceptionally rich source of valuable fatty acids, in particular linoleic and linolenic acids, is represented by hempseed (*Cannabis sativa* L.) oil. The industrial hempseed oil, the type that contains less than 0.3 % Δ9-tetrahydrocannabinol (THC), has recently received much attention based on scientifically evidenced health benefits such as decrease of cholesterol levels in blood and of high blood pressure [1].

Oils rich in polyunsaturated fatty acids (PUFAs) are susceptible to oxidative degradation, as a result of complex free radical chain reactions between unsaturated fatty acids and oxygen, accelerated by various pro-oxidants. The formation of undesirable compounds affects the oil sensory characteristics and its shelf life as well as human health [2]. Antioxidants are required to delay the lipid oxidation. Four synthetic antioxidants, namely, butylated hydroxianisole (BHA, E320), butylated hydroxytoluene (BHT, E321), tert-butylhydroquinone (TBHQ, E319) and propyl gallate (E310) are widely used in fats and oils. However, certain synthetic antioxidants used in edible oils were found to be associated with adverse health effects [3]. Safety concerns directed the research towards their replacement with efficient and harmless natural antioxidants for controlling lipid oxidation. Among various essential oils tested for stabilization of hempseed oil, the oregano essential oil at 0.05% proved efficiency in protection against oxidation [4]. Other approved natural products, the tocopherol-rich extract (E306) and the rosemary extract (E392), are currently being used in fat-based food products. Despite the limited number

Simona Oancea, Olga Drăghici, Mirabela Perju, Department of Agricultural Sciences and Food Engineering, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, "Lucian Blaga" University of Sibiu, Ion Ratiu 7–9, 550024 Sibiu, Romania.
Francisc V. Dulf, Department of Environment and Plant Protection, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine, Mănăștur 3-5, 400372 Cluj-Napoca, Romania.

Correspondence author:
Simona Oancea, e-mail: simona.oancea@ulbsibiu.ro

of approved natural antioxidants, other bioactive compounds extracted from fruits (anthocyanins) were investigated to prevent lipid oxidation in several types of edible or dietary oils [5].

The assessment of quality and stability of edible oils is based on several techniques, such as sensory evaluation and volatile flavour, several common tests (peroxide value (*PV*), anisidine value, acid value, free fatty acid), accelerated testing procedures (active oxygen method, oil stability index, Schaal oven test) or other techniques (Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), nuclear magnetic resonance) [6]. Representation of the time variation of *PV* allows the calculation of several kinetic parameters, such as induction period, stabilization factor, oxidation rate ratio or antioxidant activity [7]. The thermal analysis by DSC under non-isothermal mode has often been used for investigation of lipid oxidation in various types of oils and for testing the antioxidant effectiveness of products, producing useful analytical and kinetic information. Other powerful techniques, such as FTIR spectroscopy, have successfully been applied for evaluation of peroxides in oils undergoing thermal degradation, the improved method with attenuated total reflectance (ATR) giving useful whole FTIR spectral data [8].

In search of natural antioxidants as candidates for stabilization of hempseed oil, our research group focused on edible flower extracts, which have been less studied for such application. Flowers of roselle (*Hibiscus sabdariffa* L.) have been used as medicinal and culinary herb, showing health benefits due to its bioactive composition, mainly phenolic antioxidant compounds, known as bio-antioxidants based on their both biological and antioxidant effects [9]. Regarding the roselle anthocyanins, most of the published research reported delphinidin-3-sambubioside and cyanidin-3-sambubioside as major anthocyanins of *H. sabdariffa* conventional extracts [10].

The antioxidant properties of *H. sabdariffa* extract can add value to cold-pressed hempseed oil, a polyunsaturated oil prone to oxidation. Thus, this paper presents an approach of using several analytical methods (gas chromatography-mass spectrometry (GC-MS), *PV*, DSC, ATR-FTIR) to investigate the influence of natural antioxidants (*Hibiscus* extract, α-tocopherol) on the oxidative stability of hempseed oil during accelerated oxidation (Schaal oven test). Kinetic parameters were determined for the first stage of oxidation using *PV* and DSC analyses. Comparative studies with untreated oil and oil with α-tocopherol were carried out.

MATERIALS AND METHODS

Plant material and chemical reagents

Dried commercial roselle flowers were obtained from a Romanian food supplements producer. The moisture content was determined at 105 °C using the moisture analyser MAC 210/NP (Radwag, Radom, Poland). Cold-pressed hempseed oil was obtained from a local store in Sibiu, Romania. Tocopherols were not removed from the oil samples. Chemical reagents of analytical grade without further purification were used.

Extraction, assay of total anthocyanins and total antioxidant capacity

Anthocyanins from *Hibiscus* flowers were extracted using 80% (v/v) ethanol at 4 °C during 3 days (cold maceration with occasional shaking). The extract was filtered through Whatman 1 filter paper (pore size 0.7 μm, Whatman, Maidstone, United Kingdom) and centrifuged at 4 °C, 7400 ×g for 10 min (centrifuge Universal 320 R; Hettich Lab Technology, Tuttlingen, Germany). The content of anthocyanins was determined spectrophotometrically by the pH differential method [11] using Specord 200Plus UV-Vis spectrophotometer (Analytik Jena, Jena, Germany). Total anthocyanins were expressed as milligrams of cyanidin-3-O-glucoside equivalents (CGE) per kilogram of dry weight (DW). The extract was further concentrated using a rotational vacuum concentrator RVC 2-18 CDplus (Christ, Osterode am Harz, Germany).

The total antioxidant capacity of the *Hibiscus* extract was determined using the ferric reducing ability power (FRAP) as described by Benzie and Strain [12] and the radical-scavenging activity (*RSA*) of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as described by Brand-Williams et al. [13]. The results of FRAP were expressed as milligrams of ascorbic acid equivalents (AAE) per kilogram DW. The results by DPPH were expressed as reduction index of *RSA* in percent calculated according to Eq. 1:

$$RSA = \frac{A_0 - A}{A_0} \times 100 \quad (1)$$

where A_0 is absorbance of the control (DPPH only) and A is aborbance in the presence of the extract.

Preparation of the hempseed oil-*Hibiscus* extract system

The oil-natural extract system was prepared using 0.5 g soya lecithin as emulsifier per 100 g oil and 0.2 g *Hibiscus* anthocyanins extract per 100 g

oil, under magnetic stirring in the dark. The antioxidant effectiveness of the extract was studied versus one of the strongest natural antioxidant, α-tocopherol (Alfa Aesar, Kandel, Germany) added at 0.1 g per 100 g oil and compared to control (untreated oil). An oil sample with lecithin (0.5% w/w) was evaluated as well.

Oxidation conditions of hempseed oil samples

The resistance to oxidation of either control hempseed oil (untreated) or oils with added antioxidants (*Hibiscus* extract, α-tocopherol) was evaluated using the Schaal oven test, which accelerates the oxidation process, being normally conducted at 60 °C [14]. Oil samples distributed in glass containers in layers of 2 cm were stored in a forced-draft air oven (model UFE 400; Memmert, Schwabach, Germany) at 60 °C over a 9-day period. The heating period was limited by the evolution of *PV* of the oil with *Hibiscus* extract until the level of 40 meq·kg^{-1} [15]. Oil samples were removed from the oven after 2, 4, 7 and 9 days and analysed for *PV*, fatty acid composition, by FTIR and DSC.

Gas chromatography-mass spectrometry

An aliquot (10–15 mg) of each lipid extract was transesterified into fatty acid methyl esters (FAME) using the acid-catalysed method [16] and analysed by GC–MS. A PerkinElmer Clarus 600 T chromatograph (PerkinElmer, Waltham, Massachusetts, USA) equipped with a Supelcowax 10 capillary column (60 m × 0.25 mm inner diameter, 0.25μm film thickness; Supelco, Bellefonte, Pennsylvania, USA) was used. The column temperature was set from 140 °C to 220 °C at a rate of 7 °C·min^{-1} and held for 23 min. Helium was used as carrier gas at a constant flow rate of 0.8 ml·min^{-1}. The mass spectra were recorded in positive ion electron impact mode. The mass scans were performed from *m/z* 22 to 395. Identification of fatty acids was carried out by comparing their retention times with those of known standards and the generated mass spectral data with those of the NIST library (National Institute of Standards and Technology, USA). The relative concentration of each fatty acid was expressed as peak area percentage of total fatty acids.

The oxidizability value (*Cox*) of the investigated oil samples was calculated according to Eq. 2 based on the percentage of unsaturated C18 fatty acids, applying the formula proposed by Fatemi and Hammond [17]:

$$Cox = \frac{1x_1 + 10.3x_2 + 21.6x_3}{100} \quad (2)$$

where x_1, x_2 and x_3 is the percentage of C18:1, C18:2 and C18:3 of the total fatty acids.

Kinetic parameters from peroxide value

PV of oil samples was determined iodometrically and expressed as milliequivalents of O_2 per kilogram of oil [18]. From the plot of *PV* vs time, the X-coordinate of the intersection point of the two linear variations drawn for the initiation and propagation stages of lipid oxidation was determined [19]. This allowed the calculation of the main kinetic parameters, namely, stabilization factor (*F*), oxidation rate ratio (*ORR*) and antioxidant efficiency (*A*).

F was determined as the ratio of the induction period of the oil with anthocyanins extract and the induction period of the control (untreated oil).

ORR was calculated as the ratio of the slopes of the regression equations for the initiation stage, in the presence and in the absence of anthocyanins extract.

Antioxidant efficiency *A* was calculated by dividing the *F* value by *ORR* [20].

Kinetic studies using differential scanning calorimetry

The thermal stability of hempseed oil in the presence and absence of antioxidants was studied by using the DSC technique under non-isothermal conditions. The SDT-Q600 calorimeter (TA Instruments, New Castle, Delaware, USA) calibrated against zinc and indium was used. Samples of 3.5 ± 0.2 g, placed in platinum cups, were heated at 2.5, 5, 7.5, 10, 15 and 20 °C·min^{-1} according to the recommendations of the International confederation for thermal analysis and calorimetry (ICTAC) [21]. Heating was performed under nitrogen flow of 50 ml·min^{-1} up to 35 °C in the first step, followed by the second step of continuous heating under oxygen flow of 50 ml·min^{-1} up to 300 °C. The oxidation onset temperature (T_{on}), which corresponds to the temperature at which an inflection appears in the DSC curve, was determined using the Universal Analysis 2000 software supplied by TA Instruments.

DSC kinetic studies were performed using the Ozawa-Flynn-Wall method following the theoretical basis as previously described [5]. The heating rate (β) can be expressed in terms of temperature (*T*) according to Eq. 3:

$$\log\beta = \log\left(\frac{AE_a}{R}\right) - 2.315 - 0.4567\frac{E_a}{R} \times \frac{1}{T} \quad (3)$$

where *A* is the pre-exponential factor, E_a is the activation energy and *R* is the universal gas constant.

Following the graphical representation of

the Eq. 3 ($\log\beta$ against $1/T$), the slope value (a) allowed the calculation of the activation energy (E_a) according to Eq. 4:

$$a = -0.4567\frac{E_a}{R} \quad (4)$$

Attenuated total reflectance Fourier-transform infrared spectroscopy

The ATR-FTIR spectroscopic experiments were carried out using the ALPHA-E spectrometer with Eco-ZnSe-sampling module (Bruker, Billerica, Massachusetts, USA) run under Opus/Mentor spectroscopy software (Bruker). ATR spectra of treated and untreated hempseed oil samples at time 0 and various stages of oxidation were collected periodically (32 scans at a resolution of 4 cm^{-1}) in the range 600–4000 cm^{-1}.

Statistical analysis

All experiments were performed in duplicate. The results are presented as mean ± standard deviation. The statistical analysis was carried out using the Student's *t*-test and one-way ANOVA. Differences at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Total anthocyanins and antioxidant activities of *H. sabdariffa* extract

The investigated ethanolic extract prepared by maceration from dried roselle flowers showed a total anthocyanins content of 7206.6 $mg \cdot kg^{-1}$ DW. Anthocyanins represent the main phenolic compounds of roselle being responsible for its strong antioxidant effects [22]. The in vitro antioxidant activity of the Hibiscus extract, as measured by FRAP assay, was 51462.4 $mg \cdot kg^{-1}$ DW, indicating its potential to reduce Fe^{3+} to Fe^{2+}. The reduction index of the radical-scavenging activity by DPPH was 87.8 % showing good abilities of the natural extract to scavenge free radicals and to be further used in lipid systems as a chain-breaking antioxidant. Consequently, the concentrated crude extract was tested for its antioxidant effect in hempseed oil. The effectiveness was evaluated using several analytical tests in comparison with untreated oil and oil with added α-tocopherol. The latter antioxidant was chosen on the basis of its similar mechanism of protection against lipid oxidation to that of natural compounds of polyphenolic structure [23]. Both types of compounds terminate the free radicals chain reactions by donating hydrogen to peroxyl radicals (LOO•) or act as singlet oxygen ($^1O_2$) quenchers. In addition, anthocyanins may work as metal chelators being efficient in scavenging various reactive oxygen species [24].

Changes in the fatty acid composition, peroxide accumulation as well as DSC and ATR-FTIR analysis of oil samples subjected to accelerated oxidation were monitored during 9-day heating at 60 °C.

Changes in fatty acid composition of hempseed oil during accelerated oxidation

Modifications in the fatty acid content of hempseed oils in the presence and absence of natural antioxidants during heating at 60 °C were investigated by GC-MS analysis, a useful method previously applied, in addition to other modern techniques, to characterize various hemp products [25]. The fatty acid composition of the hempseed oil used in the present study is given in Tab. 1.

The hereby investigated oil was highly unsaturated (> 90 %), containing large amounts of linoleic acid (with the relative percentage of 86.5 % of total fatty acids) as the main component, followed by oleic and α-linolenic acids. γ-Linolenic acid represented 0.4 % of the total fatty acids. Low amounts of C20:1 (*n*-9) were found. The determined content of PUFAs was higher than other reported values ranging from 76.3 % to 82.8 % [26],

Tab. 1. Fatty acid composition of the cold-pressed hempseed oil.

Fatty acid		Peak area [%]
C16:0	Palmitic acid	2.6 ± 0.1
C18:0	Stearic acid	0.9 ± 0.0
C18:1 *n*-9	Oleic acid	4.9 ± 0.2
C18:1 *n*-7	Vaccenic acid	0.2 ± 0.0
C18:2 *n*-6	Linoleic acid	86.5 ± 4.1
C18:3 *n*-6	γ-Linolenic acid	0.4 ± 0.0
C18:3 *n*-3	α-Linolenic acid	4.1 ± 0.2
C20:0	Arachidic acid	0.2 ± 0.0
C20:1 *n*-9	Gondoic acid	0.1 ± 0.0
C22:0	Behenic acid	0.1 ± 0.0
Groups of fatty acids		
SFA		3.8 ± 0.2
MUFA		5.2 ± 0.2
PUFA		91.0 ± 4.4
n-3 PUFAs		4.1
n-6 PUFAs		86.9
Ratio PUFAs/SFAs		23.7

Relative concentration of fatty acids is expressed as peak area percentage of total fatty acids. Values represent mean ± standard deviation ($N = 3$).
SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

but relatively close to those reported for cv. Finola (sum of PUFAs 84 %) [27]. The differences might be related to hemp varieties, crop ripening stage or the way of oil production. However, the high content in PUFAs makes hempseed oil prone to oxidation. Saturated fatty acids were found in lower amounts (< 4 %), which recommend hempseed oil as a good product for „healthy" diets. The total content of SFAs was lower than previously reported [27], which may be linked to the oil variety, as shown by the study of DEFERNE and PATE [28] with an indication that „temperate variety oils are less saturated.

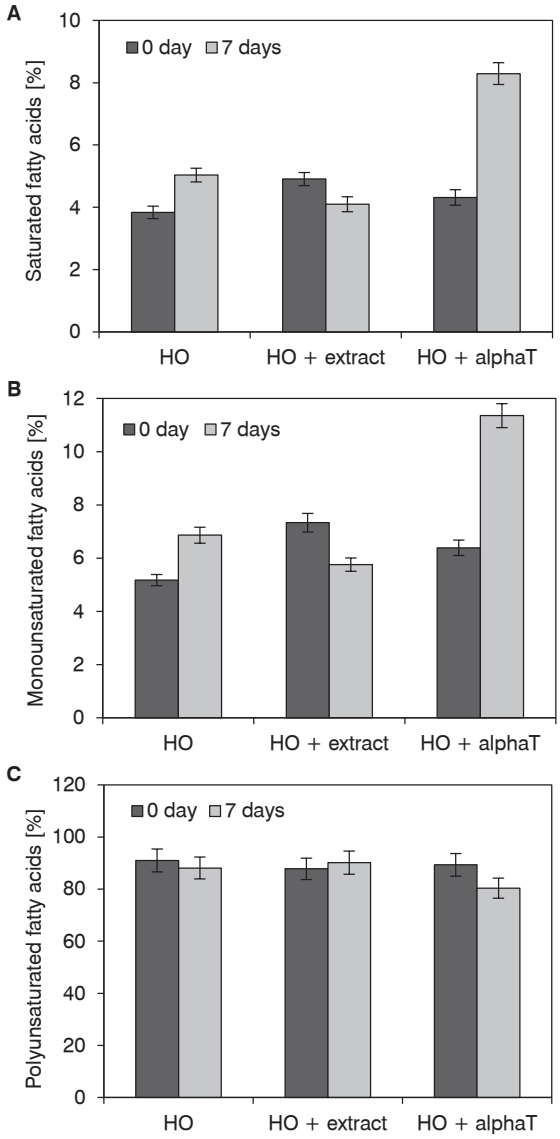

Fig. 1. Fatty acid composition of hempseed oil during storage at 60 °C.

A – saturated fatty acids, B – monounsaturated fatty acids, C – polyunsaturated fatty acids.
Relative concentration of fatty acids is expressed as peak area percentage of total fatty acids. Values represent mean ± standard deviation (N = 3).
HO – hempseed oil, HO + extract – hempseed oil with *Hibiscus* extract, HO + alphaT – hempseed oil with α-tocopherol.

The changes in major fatty acid composition during oil heating at 60 °C registered in the first 7 days are shown in Fig. 1.

The level of saturated fatty acids (SFAs), palmitic and stearic acids, increased by 31 % in control and by 92 % in oil with α-tocopherol, while it decreased by 17 % in oil with added anthocyanins extract. The more pronounced increase in oil with α-tocopherol demonstrated a pro-oxidant effect of the added α-tocopherol, as also noticed by JERZYKIEWICZ et al. [29], and correlated with our further *PV* investigation.

The level of PUFAs decreased by 3 % in control and by 10 % in oil with added α-tocopherol after 7 days of storage at 60 °C, being mainly attributed to the decrease of linoleic acid, correlated with its concomitant increase in monounsaturated fatty acids (MUFAs) level, largely of oleic acid.

The relative percentages of total unsaturated fatty acids decreased after 7 days at 60 °C in control and in oil with added α-tocopherol, while remained similar in the sample with added anthocyanins extract, showing the protective effect of roselle extract against oxidative oil degradation. The results are in agreement with those reported by other authors on edible oils subjected to heating processes [30].

The oxidative stability of the oil with added anthocyanins was demonstrated by *Cox* value, which remained unchanged (increasing slightly from 9.81 to 9.89) after heating for 7 days. The *Cox* value decreased by 2 % in control (from 9.93 to 9.73) and by 5 % in oil with added α-tocopherol (from 9.84 to 9.33).

Kinetic parameters of inhibited oil oxidation

The Schaal oven test at 60 °C was selected as a useful accelerated oxidative method for testing resistance to oxidation of hempseed oils (control, oil with *Hibiscus* extract and oil with α-tocopherol). During the test, the hereby investigated lipid system containing high amounts of PUFAs was exposed to oxidation over a 9-day period due to heat, consequently generating primary oxidation products (hydroperoxides, peroxides). The heating period was limited by the evolution of *PV* of the oil with *Hibiscus* extract until the level of 40 meq·kg^{-1} [15]. Because the oil sample with lecithin showed *PV* evolution with an increasing trend situated between control and sample with *Hibiscus* extract over the 9-day storage period, the kinetic study

was done for other samples. At the end period, *PV* of oil with lecithin was by 34 % higher than that of oil with *Hibiscus* extract but by 3 % lower than control.

Primary oxidation of oil samples to peroxides and hydroperoxides was estimated from *PV*. According to the Codex Alimentarius Commission [31], *PV* of refined and cold-pressed edible oils should not exceed 10 meq·kg^{-1} and 15 meq·kg^{-1}, respectively. As shown in Fig. 2, *PV* of all investigated samples constantly increased with time of storage at 60 °C. During the heating period, sample with *Hibiscus* extract showed high efficiency by 56% inhibition of the formation of primary oxidation products compared to that of the control sample. A pro-oxidant effect of the added α-tocopherol was noticed after 2 days, while the antioxidant effect of the *Hibiscus* extract was still recorded after 7 days. However, it was shown that tocopherols may act as pro-oxidants, in particular when present at high concentrations, but also in relation to the character of the lipid system and duration of oxidation [29].

As can be noticed from Fig. 2, the induction and propagation periods identified for control and oil with anthocyanins extract allowed the determination of two important kinetic characteristics of the antioxidant potential, the effectiveness (through the *F* factor) and the strength (through the *ORR* ratio). The calculated kinetic parameters are presented in Tab. 2. The obtained *ORR* value was less than 1 indicating a far slower progress of oxidation in the presence of *Hibiscus* extract. The stabilization factor (*F*) indicates the antioxidant effect as followed: pro-oxidant effect for $F < 1$, no effect at $F = 1$ and antioxidant effect for $F > 1$. The obtained *F* value (1.67) points out the ability of the *Hibiscus* extract to scavenge the generated peroxides. The antioxidant activity calculated as *F*/*ORR* describes the efficiency of the natural product to end the auto-oxidation process and its ability to decrease the oxidation rate during the induction period. The *A* values vary within a wide range, depending on the type of oil, temperature and exogenous antioxidants. Yanishlieva-Maslarova and Marinova [20] showed

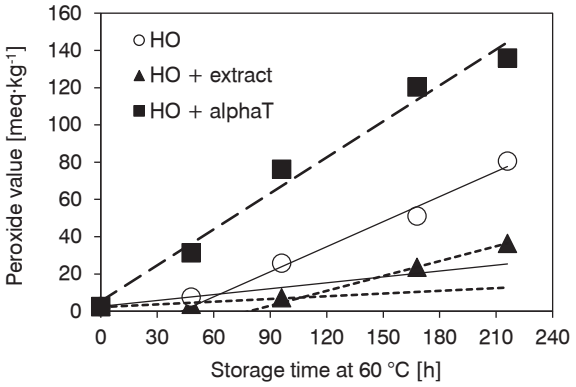

Fig. 2. Peroxide accumulation in hempseed oils stored at 60 °C.

HO – hempseed oil, HO + extract – hempseed oil with *Hibiscus* extract, HO + alphaT – hempseed oil with α-tocopherol.

that 1.0–10.3 × 10^{-3} mol·l^{-1} ferulic acid, a polyphenolic acid, added to sunflower oil triacylglycerols, led to antioxidant activity (*A*) ranging from 4.3 to 9.0 during oxidation at 100 °C. In our previous research, we reported greater *A* values for a powerful red onion skin extract, which was added to parsley oil for its oxidative stabilization [5].

Kinetic studies of inhibited oil oxidation

The DSC method was used to comparatively evaluate the thermo-oxidative degradation of treated and untreated oils, under oxygen flow of 50 ml·min^{-1} up to 300 °C. The temperature at which peroxide formation starts (T_{on}), as determined from the DSC curve using a heating rate of 2.5 °C·min^{-1}, ranged between 157.1 °C and 159.7 °C for initial samples with *Hibiscus* extract and control, respectively. After 9-day storage period at 60 °C, the oxidative processes progressed at lower values of T_{on}. However, hempseed oil with added anthocyanins showed superior values compared to control and sample with added α-tocopherol, suggesting an improvement of stability given by the selected bio-antioxidant.

The kinetic parameters of thermo-oxidation

Tab. 2. Main kinetic parameters of peroxide accumulation in hempseed oils during heating at 60 °C.

Sample	Regression equations		Induction period *IP* [h]	Stabilization factor *F*	Oxidation rate ratio *ORR*	Antioxidant efficiency *A*
	Initiation	Propagation				
Hempseed oil (control)	$0.1054x + 2.5785$	$0.4485x - 19.206$	63.49			
Hempseed oil with *Hibiscus* extract	$0.0486x + 2.1808$	$0.2662x - 21.026$	106.42	1.67	0.46	3.63

of oils stored in dark at 60 °C were calculated using the Ozawa-Flynn-Wall method based on the linear variation of heating rate ($\log\beta$) with $1/T$. The linear regression R^2 ranged between 0.89 and 0.98. The values of E_a of the thermal decomposition reaction in control and oils with natural antioxidants were calculated on the first day and after 9 days of accelerated oxidation at 60 °C using Eq. 4. The results are shown in Fig. 3. Initially, the E_a values were very similar for the three investigated oil samples. Sample with *Hibiscus* extract and that with α-tocopherol showed slightly lower values compared to control. Significant effects regarding the oil stability were noticed during the accelerated oxidation. The protective properties of exogenous antioxidants may be rated in terms of E_a: the higher the E_a value at final monitored oxidation period, the greater the antioxidant efficiency. As can be seen in Fig. 3, at the end point of the test, E_a drastically decreased by 88.3 % in control as compared to the initial stage. Instead, a much lower degradative effect was observed for the other two samples. The most significant protective effect against oxidation was noticed in the sample with *Hibiscus* extract, at the end stage E_a being decreased only by 23.6 %. In case of hempseed oil with added α-tocopherol, E_a decreased by 28.6 % compared to the first day.

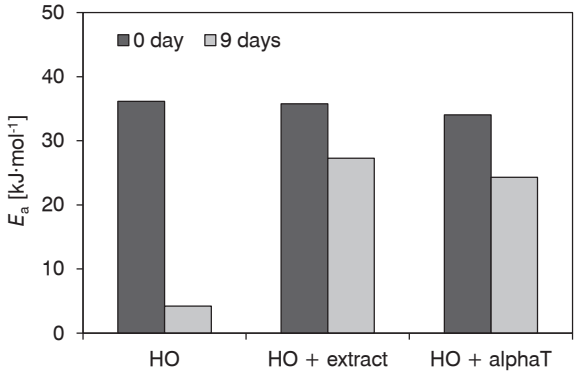

Fig. 3. Activation energy of oil samples following 9 days storage at 60 °C.

HO – hempseed oil, HO + extract – hempseed oil with *Hibiscus* extract, HO + alphaT – hempseed oil with α-tocopherol.

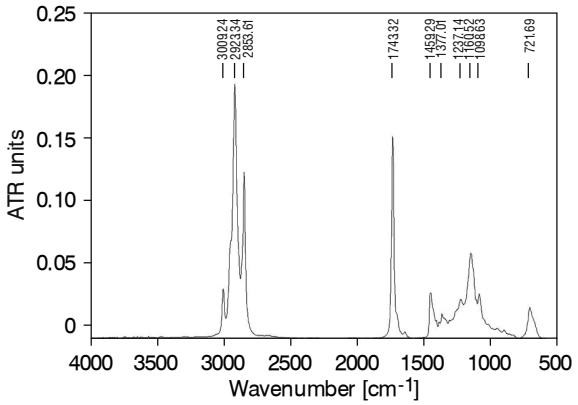

Fig. 4. Attenuated total reflectance Fourier-transform infrared spectrum of hempseed oil.

The obtained results revealed that the addition of roselle extract to hempseed oil improved its thermal stability at 60 °C due to higher E_a of thermal decomposition. According to our knowledge, no such studies regarding the thermal degradation kinetics using Ozawa-Flynn-Wall method have been reported for hempseed oil.

Attenuated total reflectance Fourier-transform infrared spectroscopy

FTIR spectroscopy represents a powerful technique which may be used to investigate edible oils oxidation, either qualitatively or quantitatively [8]. Hereby, the ATR-FTIR spectroscopic experiments were performed to monitor spectral changes in functional groups taking place during oxidative degradation at 60 °C. Hydroperoxides formed during the first stage of oxidation give a „traditional“ peak of 3444 cm^{-1} in the FTIR spectra. This peak can shift toward lower wavenumbers due to their accumulation in high amounts under oxidation [8].

The ATR-FTIR spectrum of the control showed the following characteristics (Fig. 4): the absorption band at 3009.24 cm^{-1} indicating the C-H stretching of *cis*-double bond, the two intensive bands at 2923.34 cm^{-1} and 2853.61 cm^{-1} assigned to the asymmetric and symmetric stretching vibration of the aliphatic CH_2 functional group, and the sharp band at 1743.32 cm^{-1} indicating the C=O stretching vibration of the triglyceride ester group. The absorption band at 1459.29 cm^{-1} is assigned to C=H scissors deformation vibration, the band at 1377.01 cm^{-1} to the bending vibration of CH_2 functional group, while the bands at 1160.52 cm^{-1} and 1237.14 cm^{-1} indicate the vibration of the C-O ester and CH_2 groups.

By subtracting the time 0 spectrum from all of the other samples recorded at different stages of oxidation during the 9-day period at 60 °C, the differences of the ROOH band determined as maximum in the region 3422–3469 cm^{-1} were investigated. The results showed a sharp increase in absorption assigned to ROOH in the first two days of oxidation at 60 °C for all samples, with the highest one for the control sample, which formed hydroperoxides in great amounts due to the lack

of antioxidant protection. After 9 days, the absorbance differences decreased as follows: oil with α-tocopherol (0.0078) > control oil (0.0051) > oil with *Hibiscus* extract (0.0035). This indicated the antioxidant effect of the natural extract.

The present investigation of the oxidative stability of inhibited hempseed oil through GC-MS, FTIR and kinetic studies by *PV* and DSC analyses essentially showed the protective effect of the added *Hibiscus* extract. The crude hydroethanolic extract contains high amounts of bioactive constituents, mainly anthocyanins (flavonoids). Natural flavonoids (catechins, quercetin, anthocyanidins) are known for their antioxidant properties acting as chain-breakers, metal chelators or free radical scavengers [9, 23]. Delphinidin and cyanidin, which constitute the predominant anthocyanidins in *Hibiscus,* are efficient superoxide scavengers [24].

In the present investigation, the *Hibiscus* extract showed reasonable stability under the Schaal oven test. The pH value of the investigated hempseed oil was 5. Previous studies showed that thermal degradation of *Hibiscus* anthocyanins is pH-dependent, good recovery being still registered during heating at 70 °C or at higher temperatures in acidic environment (below pH 5) [32]. Anthocyanins stability under acidic conditions is associated with their presence in the form of flavylium cation. In accordance with these findings, efficient protection of hempseed oil provided by *Hibiscus* extract indicates that the extract in heated oil at 60 °C under pH 5 environment still contains antioxidant anthocyanins. We consider that no drastic anthocyanins degradation occurred. Other factors, such as glycosylation and acylation patterns of anthocyanins and the physical-chemical environment, e.g. the presence of other polyphenolic compounds [10] may enhance the stability of anthocyanins from *Hibiscus* extract. Despite that *Hibiscus* anthocyanins hydrolysed to some extent with increasing heating time, as shown by *PV* plot and FTIR analysis results, it is possible that the resulting degradation products, gallic and protocatechuic acids known for their own antioxidants properties confirmed in some edible oil [33–34], compensated for the loss of anthocyanins. This may explain the low oxidation rate of the sample with added anthocyanins extract compared to the others.

CONCLUSIONS

This study reports the efficiency of the roselle crude extract in thermo-oxidative stabilization of hempseed oil during 9-day period at 60 °C compared to control and oil with added α-tocopherol. The changes in total unsaturated fatty acids determined by GC-MS analysis and *Cox* value demonstrated the protective effect of anthocyanins extracted from *H. sabdariffa* against oxidation of hempseed oil. The peroxides formation was inhibited by 56 % in hempseed oil with *Hibiscus* extract compared to control. The kinetic parameters determined from *PV* plots showed that oil oxidation evolved at lower rates in the presence of the natural extract. *PV* significantly increased in oil with α-tocopherol due to the progress of prooxidant effects. The results of the thermal degradation kinetics by DSC using Ozawa-Flynn-Wall method indicated that 0.2% (w/w) addition of *Hibiscus* extract to hempseed oil improved its thermal stability at 60 °C due to the increased E_a of thermal decomposition compared to the other investigated samples. The FTIR analysis showed an increase in hydroperoxides in the first two days under oxidative conditions, in particular for control sample. At the end of the oxidation period, the oil sample with *Hibiscus* extract exhibited improved stability. The physical-chemical, spectroscopic, chromatographic and thermal experiments suggested that *Hibiscus sabdariffa* anthocyanins have, to some extent, heat resistance at 60 °C and pH 5 within the investigated lipid system and confirmed our hypothesis of oxidative stabilization of hempseed oil. The roselle extract can compete with other natural antioxidants, being safe and effective.

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