

# The Effect of Selected Herb Extracts on Oxidative Stability of Vegetable Oils

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**Abstract:** The aim of this study was to investigate the effect of different herb extracts on the oxidative stability of hemp, flax, sesame and sunflower seed oils by means of Rancimat test. The dominant phenolic acids in herb extracts were detected by HPLC, while fatty acid profiles of oils, before and after oxidation, were determined by GC-FID. As expected, autoxidation increased the content of saturated fatty acids. The sesame oil showed the longest oxidative stability (3.43 h), while the shortest induction period was obtained for the flax oil. Lemon balm and oregano extracts accelerated the oxidation of hemp and flax oil, while in all other cases the addition of extracts had a positive effect on oil oxidative stability. The best results were obtained for lemon balm extract added to sesame and sunflower oil which resulted with OSI values of 1.33 and 1.48, respectively, while extremely prooxidative activity was obtained for lemon balm extract added to the hemp oil.

**Keywords:** Vegetable oils, Oxidation, Herb extracts, Rancimat, Fatty acids, GC-FID.

## INTRODUCTION

VEGETABLE oils intended for human consumption are very susceptible to oxidation processes (autoxidation and photosensitized oxidation), which could occur during food manufacturing, storage, distribution and final preparation. Oil oxidation is influenced by different factors such as fatty acid profile, processing technology, energy of heat or light, concentration and type of oxygen, presence of minor compounds such as metals, pigments, phospholipids, free fatty acids, antioxidants, etc.<sup>[1]</sup> The oil degradation processes generate *off*-flavour components making the oil unacceptable to the consumers, causing degradation of essential fatty acids and producing toxic compounds and oxidized polymers. All these changes result in oil rancidity and deterioration of its organoleptic and nutritional properties,<sup>[1–3]</sup> and oxidative stability of the oil is an important indicator to determine its quality, safety and shelf life. As lipid oxidation at ambient conditions occurs slowly, accelerated methods are employed in order to estimate the stability of fats and oils in a relatively short period.<sup>[3]</sup>

In the food industry antioxidants are substances used to prevent, delay and/or avoid oxidation processes.<sup>[4,5]</sup> As an alternative to synthetic food preservatives and antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole, etc., herbs and spices are promising sources of bioactive phytochemicals and secondary plant metabolites with good biological potential, especially antioxidant and antimicrobial effects. In the last few decades, studies have been focused on non-nutritive constituents of plants and showed that their biological activity is related to the presence of substances which usually occur in very small quantities. Among diverse groups of phytochemicals, their biological activity is usually attributed to the presence of phenolics. Species from the Lamiaceae plant family are well known for strong antioxidant activity which exceeds the effect of many currently used synthetic preservatives<sup>[6–10]</sup> so they are one of the most used and commercialized plants. Studies on chemical constituents of Lamiaceae plants are mainly focused on apolar compounds, as terpenoids from essential oils and hydrophilic components, like phenolic acids (exclusively caffeic acid derivatives).<sup>[11]</sup> This study was

designed to evaluate the effect, antioxidative or prooxidative, of the herb extract addition (lavender, lemon balm, oregano, and rosemary extract) on the oxidative stability of different vegetable oils (hemp, flax, sesame and sunflower seed oils) by the commonly accepted Rancimat method. Extracts were analysed for total phenolics and presence of dominant individual phenolic compounds, while in order to investigate the susceptibility of oils to oxidation, the fatty acid profile of the samples was also investigated.

## MATERIAL AND METHODS

### General

All used reagents and solvents were of analytical grade. Folin-Ciocalteu reagent and phosphoric acid were obtained from Kemika (Zagreb, Croatia), sodium carbonate from Merck (Darmstadt, Germany), acetonitrile and methanol from BDH Chemicals (London, UK) and heptane from Sigma-Aldrich (St. Louis, Missouri, USA).

Fatty acid methyl esters (FAMES) were analysed by gas chromatograph (GC, model 3900; Varian Inc., Lake Forest, CA, USA) with flame-ionisation detection (FID), while phenolic acids in plant extracts were detected using high-performance liquid chromatography (HPLC) with UV detector (all of Series 200, Perkin-Elmer Inc., Shelton, CT, USA). Spectrophotometric measurements were performed on a SPECORD 200 Plus, Edition 2010 (Analytik Jena AG, Jena, Germany) and the oxidative stability of oils was determined using Rancimat model 743 (Metrohm, Herisau, Switzerland).

### Vegetable Oils

In this study, commercially available cold pressed vegetable oils, from flax, hemp and sunflower seeds (Ekozona, Biovega, Zagreb, Croatia), as well as sesame oil (Ekoplaza, Veghel, Nederland), were used as a lipid medium. All samples were stored at room temperature protected from light until analysis.

### Plant Extracts Preparation

Plant materials (Agristar, Zagreb, Croatia): lemon balm (*Melissa officinalis* L.), rosemary (*Rosmarinus officinalis* L.), lavender (*Lavandula spica* L.) and oregano (*Origanum vulgare* L.), were purchased from a local herbal pharmacy. Dry and homogenized plant material (5 g) was extracted with 100 mL of distilled water in an ultrasound bath for 2 hours at 60 °C. After cooling, samples were filtered. The extractions of each plant were performed in triplicate, and the obtained extracts were combined into the final extract that was used in further analyses.

### The Analysis of Fatty Acids

The fatty acid (FA) profile of vegetable oils was carried out by GC-FID using capillary column RTX 2330 (30 m × 0.25 mm

i.d., coating thickness 0.25 µm, Restek, Bellefonte, PA, USA). FAMES were prepared by dissolving 0.1 g of the oil samples in 2 mL of heptane and 0.2 mL of 2 M KOH in methanol. The tubes were capped and shaken vigorously for 30 seconds and then left to stratify until the upper layer (heptane solution containing FAMES) became clear. The injection volume was 1 µL while the split ratio was 1:40. Helium was used as a gas carrier with a flow rate of 3 mL min<sup>-1</sup>. The injector temperature was 225 °C and the detector temperature was 240 °C. The initial oven temperature was 140 °C while the final was 210 °C. The temperature was ramped at rate of 5 °C min<sup>-1</sup> for 16 minutes. The FAMES were identified by comparing the retention time of each component to a standard (Supelco 37, FAME Mix). The analyses were performed in duplicates and the results are expressed as FAMES percentages (%).

### HPLC Analysis of Phenolic Acids

The concentrations of rosmarinic, chlorogenic and carnosic acid in investigated Lamiaceae plant extract were determined by HPLC. The compounds were separated on an UltraAqueous, C18 column, 250 × 4.6 mm, 5 Å (Restek, USA) by gradient chromatography using mobile phases: 0.2% phosphoric acid (A), methanol (B) and acetonitrile (C). At 0 minutes, the gradient was 96% A, 2% B and 2% C. During the first 40 minutes, ratios were changed from the initial value to 50% A, 25% B and 25% C and were subsequently from 40 to 45 minute change to 40% A, 30% B and 30% C. From 45 to 60 min, the gradient was changed to 50% B and 50% C and kept at these values until 70 min. The obtained solvent ratio was kept for 10 min to achieve the stability of the column to the initial conditions. The flow rate of the mobile phase was 0.8 mL min<sup>-1</sup> and the injection volume of the samples was 20 µL. The compounds were detected at 280 nm. The identification was carried out by comparing their retention times with those obtained for pure standard compounds, while the quantification was carried out through the external calibration curves. The analyses were performed in duplicates and the results are expressed in mg of compound per liter of extract (mg L<sup>-1</sup>).

### Determination of Total Phenols

The content of total phenols in prepared plant extracts was determined by the Folin-Ciocalteu colorimetric method,<sup>[12]</sup> calibrated against gallic acid. All extracts were analysed in triplicate and the results are expressed as mg of gallic acid equivalents per litre of extract (mg GAE L<sup>-1</sup>).

### Determination of Oil Oxidative Stability by Rancimat Method

The resistance of vegetable oils (with and without the addition of herb extracts) to autoxidation was determined by the Rancimat method. In order to investigate the

influence of the added extract on oil oxidative stability, volumes of 100  $\mu\text{L}$  the herb extracts (diluted in proportion 1:10) were added to the vegetable oils (3.0 g). After the extract addition, final concentrations of the added phenolics in oil samples were 16.7  $\mu\text{g GAE g}^{-1}$  for lemon balm extract, 7.1  $\mu\text{g GAE g}^{-1}$  for rosemary extract, 18.7  $\mu\text{g GAE g}^{-1}$  for oregano extract and 11.3  $\mu\text{g GAE g}^{-1}$  for lavender extract, respectively. The analyses were performed at 120°C while the airflow was 20 L h<sup>-1</sup>. The results for oil oxidative stability were expressed as induction periods (IPs) in hours (h) and as oxidative stability index (OSI) which was calculated as:<sup>[5,13]</sup>

$$\text{OSI} = \frac{\text{Induction time of oil with antioxidant}}{\text{Induction time of pure oil}}$$

Values OSI > 1 testify of the antioxidative potential of the added antioxidants (extracts), while OSI < 1 of their prooxidative activity. All measurements were performed in triplicate.

### Statistical Analysis

All results are expressed as mean value  $\pm$  standard deviation. The statistical analysis was performed by STATGRAPHICS® Centurion XVI (StatPoint Technologies, Inc., The Plains, Virginia, USA) software. The differences between total phenols and induction periods were

analysed by one-way ANOVA, while Pearson's correlation coefficient ( $p < 0.05$ ) was used to determine the relations between all investigated variables.

## RESULTS AND DISCUSSION

The FA compositions of investigated oils, before and after the oxidation, are given in Table 1. In fresh oil samples, polyunsaturated fatty acids (PUFAs) were the most abundant (from 45.39 to 74.19%), followed by monounsaturated fatty acids (MUFAs, from 14.72 to 40.16%) and saturated fatty acids (SFAs, from 10.88 to 14.45%). The content of PUFAs in hemp, flax and sunflower oil was around 3-fold higher than the content of MUFAs, while in sesame oil the share of PUFAs and MUFAs were relatively close in range, 45.39 and 40.16%, respectively. In sesame oil the highest amount of SFAs (14.45%) was also detected. The most abundant fatty acids in fresh oil samples were palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3), and this trend was also apparent in the oxidised oils.<sup>[14–16]</sup> In all samples the dominant PUFA was linoleic acid except in flax oil where it was linolenic acid. The high content of linoleic acid was also found in hemp oil, while in sesame and sunflower oil its content was almost negligible (lower than

**Table 1.** Fatty acid composition (%) of fresh and oxidised hemp, flax, sesame and sunflower oil determined by gas chromatography with flame-ionisation detection (GC-FID).

Fatty acid	Fresh oil				Oxidised oil			
	Hemp	Flax	Sesame	Sunflower	Hemp	Flax	Sesame	Sunflower
16:0	6.98 $\pm$ 0.14	6.17 $\pm$ 0.01	8.25 $\pm$ 0.12	6.43 $\pm$ 0.08	8.67 $\pm$ 0.11	7.55 $\pm$ 0.50	8.90 $\pm$ 0.23	8.50 $\pm$ 0.14
17:0	0.05 $\pm$ 0.00	0.06 $\pm$ 0.00	0.04 $\pm$ 0.01	0.04 $\pm$ 0.00	0.07 $\pm$ 0.00	0.08 $\pm$ 0.01	0.05 $\pm$ 0.00	0.06 $\pm$ 0.00
18:0	2.82 $\pm$ 0.03	4.73 $\pm$ 0.00	5.44 $\pm$ 0.03	3.24 $\pm$ 0.02	3.66 $\pm$ 0.02	5.96 $\pm$ 0.30	6.01 $\pm$ 0.07	4.27 $\pm$ 0.04
20:0	0.80 $\pm$ 0.04	0.06 $\pm$ 0.00	0.53 $\pm$ 0.01	0.21 $\pm$ 0.01	1.03 $\pm$ 0.06	0.09 $\pm$ 0.00	0.60 $\pm$ 0.03	0.29 $\pm$ 0.01
22:0	0.32 $\pm$ 0.03	0.17 $\pm$ 0.00	0.12 $\pm$ 0.00	0.73 $\pm$ 0.06	0.45 $\pm$ 0.01	0.21 $\pm$ 0.00	0.13 $\pm$ 0.01	0.96 $\pm$ 0.06
24:0	0.12 $\pm$ 0.01	0.09 $\pm$ 0.00	0.07 $\pm$ 0.00	0.23 $\pm$ 0.03	0.17 $\pm$ 0.00	0.12 $\pm$ 0.00	0.09 $\pm$ 0.01	0.29 $\pm$ 0.02
<b><math>\Sigma</math>SFA</b>	<b>11.09</b>	<b>11.41</b>	<b>14.45</b>	<b>10.88</b>	<b>14.05</b>	<b>14.11</b>	<b>15.78</b>	<b>14.37</b>
16:1 n9	0.03 $\pm$ 0.00	0.02 $\pm$ 0.00	0.03 $\pm$ 0.00	0.02 $\pm$ 0.00	0.03 $\pm$ 0.00	0.03 $\pm$ 0.00	0.03 $\pm$ 0.00	0.02 $\pm$ 0.00
16:1 n7	0.14 $\pm$ 0.01	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00	0.16 $\pm$ 0.00	0.11 $\pm$ 0.01	0.11 $\pm$ 0.01	0.11 $\pm$ 0.00
17:1	0.02 $\pm$ 0.00	0.05 $\pm$ 0.00	0.03 $\pm$ 0.01	0.02 $\pm$ 0.00	0.03 $\pm$ 0.01	0.11 $\pm$ 0.01	0.05 $\pm$ 0.00	0.02 $\pm$ 0.00
18:1 t n9	0.01 $\pm$ 0.00	-	-	-	-	-	-	-
18:1 n9	14.09 $\pm$ 0.27	23.45 $\pm$ 0.02	39.80 $\pm$ 0.05	23.57 $\pm$ 0.08	16.7 $\pm$ 0.07	27.60 $\pm$ 1.40	42.51 $\pm$ 0.11	28.80 $\pm$ 0.08
20:1	0.44 $\pm$ 0.02	0.14 $\pm$ 0.00	0.19 $\pm$ 0.00	0.17 $\pm$ 0.00	0.54 $\pm$ 0.00	0.18 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.00
<b><math>\Sigma</math>MUFA</b>	<b>14.72</b>	<b>23.76</b>	<b>40.16</b>	<b>23.88</b>	<b>17.48</b>	<b>28.13</b>	<b>42.92</b>	<b>29.17</b>
18:2 ct n6	0.10 $\pm$ 0.00	0.05 $\pm$ 0.00	-	-	0.11 $\pm$ 0.00	-	-	-
18:2 tc n6	-	0.06 $\pm$ 0.00	-	-	-	0.09 $\pm$ 0.00	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00
18:2 n6	57.95 $\pm$ 0.22	15.04 $\pm$ 0.03	44.96 $\pm$ 0.12	65.10 $\pm$ 0.05	56.0 $\pm$ 0.38	14.50 $\pm$ 0.77	40.96 $\pm$ 0.01	56.30 $\pm$ 0.07
18:3 n3	16.14 $\pm$ 0.07	49.68 $\pm$ 0.00	0.42 $\pm$ 0.00	0.10 $\pm$ 0.01	12.2 $\pm$ 0.12	39.50 $\pm$ 2.00	0.34 $\pm$ 0.00	0.09 $\pm$ 0.00
<b><math>\Sigma</math>PUFA</b>	<b>74.19</b>	<b>64.83</b>	<b>45.39</b>	<b>65.24</b>	<b>68.47</b>	<b>54.20</b>	<b>41.30</b>	<b>56.45</b>

\*GAE – gallic acid equivalents. Values for total phenolics with different letters denote significant differences among samples ( $F$ -ratio 1738.9,  $p < 0.01$ ).

0.5%). Among MUFAs, the dominant acid was oleic acid, with more than 2-fold higher concentration in sesame oil than in others. Furthermore, relatively high amounts of SFAs, palmitic and stearic acid were also found in sesame oil.

Fatty acids respond differently to heating processes. Generally, SFAs are relatively thermostable but at high temperatures and in the presence of high concentrations of oxygen, the generation of oxidation products could occur. Unsaturated fatty acids are more heat-labile, and with the increase of unsaturation degree, they usually become less stable, making PUFAs the most unstable forms.<sup>[17]</sup> As expected, fresh and oxidised oils had notable differences in PUFAs due to their degradation during the oxidation. The final result is reduced amount of linolenic acid in hemp (degradation of 24.4%) and flax oil (degradation of 20.5%) as well as a reduced amount of linoleic acid in sesame (degradation of 8.9%) and sunflower oil (degradation of 13.5%). Oleic acid was dominant MUFA in fresh and oxidised oils, with the higher concentrations in oxidised oil, probably as it is a product PUFAs oxidation. Similarly, the proportions of palmitic and stearic acids were higher in oxidised than in fresh oils. *Trans*- geometrical isomers of oleic and linoleic acid have been identified both in fresh and oxidized oils, but in the amounts that were barely detectable or in most cases not detectable although it was expected that their content would be significantly higher after the oxidation.<sup>[18]</sup>

The aim of this study was to investigate the effect of herb extract addition on the oxidative stability of hemp, flax, sesame and sunflower seed oils. There is a large number of researches on the use of herb extracts, phenolics and other antioxidants for the control of lipid oxidation of fats and oils.<sup>[4,5,19–22]</sup> For that purpose, in the present study, four well-known herbs and spices; lavender, lemon balm, oregano, and rosemary, were used. The phenolic content was determined by the Folin-Ciocalteu method, and the results are presented in Table 2. The highest content of phenolics was detected in oregano extract (5624 mg GAE L<sup>-1</sup>), while the lowest was found in rosemary extract (2124 mg GAE L<sup>-1</sup>).

Generalić Mekinić *et al.*<sup>[11]</sup> reported that Lamiaceae plants contain high levels of phenolics, mainly rosmarinic acid that has a great influence on antioxidant activity of the samples. Carnosic acid, on the other hand, is a phenolic diterpene found in the leaves of Lamiaceae plants. It has also been widely used as a food additive due to its strong antioxidant and antimicrobial properties.<sup>[23]</sup> Finally, chlorogenic acid, an ester of caffeic acid and (–)-quinic acid, was also investigated in numerous studies due to positive biological properties including antioxidant.<sup>[24]</sup> In this study extremely high content of chlorogenic acid was detected in oregano extract (107 mg L<sup>-1</sup>) while in other extracts it ranged from 3.85 to 10.77 mg L<sup>-1</sup>. Other extracts were rich in rosmarinic acid; rosemary with 172.25 mg L<sup>-1</sup>, lemon balm with 173.58 mg L<sup>-1</sup> and lavender with 94.67 mg L<sup>-1</sup>. The concentrations of carnosic acid in extracts were around 30 mg L<sup>-1</sup> (Table 2).

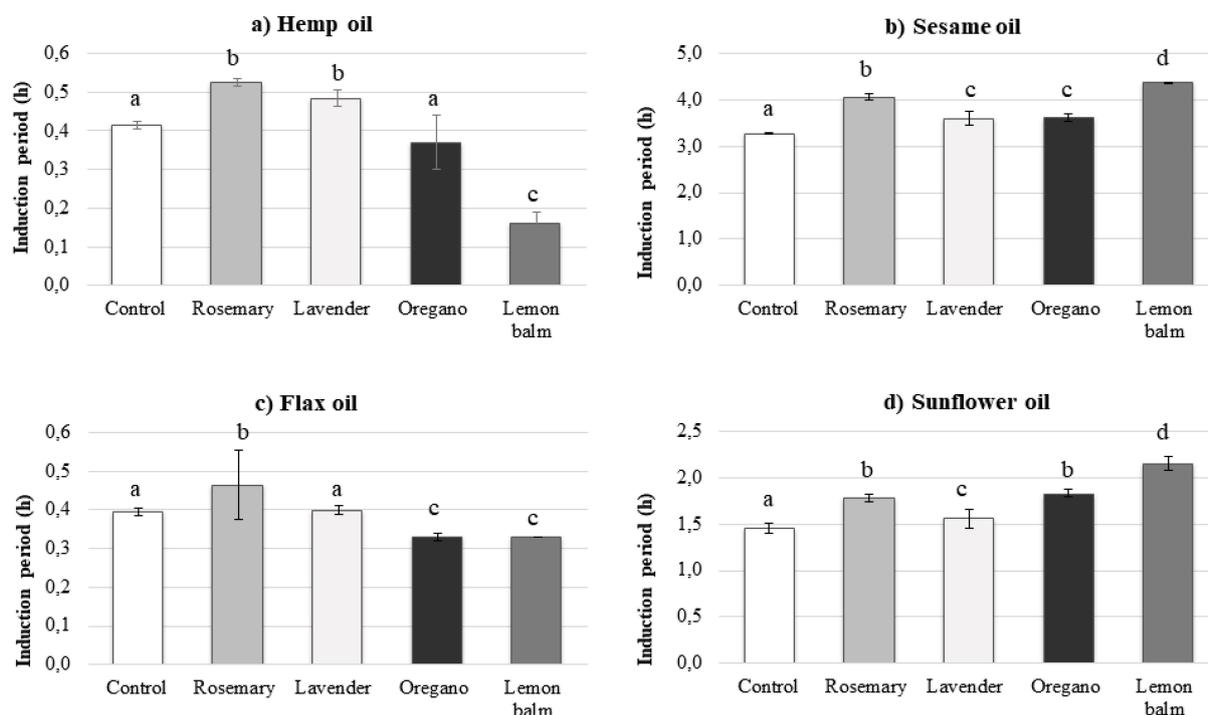
The results of oxidative stability of oils without and with herbal extract addition, tested using the Rancimat method, are presented in Figure 1. Rancimat assay evaluates the rate of secondary oxidation product formation, as the assessment of the ability of antioxidants to break the free radical chain reaction.<sup>[5]</sup> This accelerated method employs high temperatures and air-flow supply to estimate the oil oxidative stability and shelf life in a relatively short time.<sup>[11]</sup> The results, expressed as IPs, are defined as the time (in h) required to reach the end-point of oxidation.<sup>[21]</sup> The detected IPs for fresh oils were in a range from 0.42 h for hemp oil to 3.28 h for sesame oil which was the most stable one among tested oils. It has been proved that IP depends primarily on oil fatty acid composition, and as can be seen from the Table 1, the sesame oil had the lowest content of PUFAs, but the highest content of MUFAs and SFAs. Also, as previously reported,<sup>[21]</sup> PUFA/SFA ratio can be used as a measure of oil tendency to undergo autoxidation. This is in correlation with the obtained results as sesame oil has the lowest PUFA/SFA ratio (3.14).

In comparison to the results obtained for pure vegetable oils, it can be concluded that the addition of herb extracts has an impact on oil stability (Figure 1, Table 3).

**Table 2.** Content of (a) total phenols (in mg GAE L<sup>-1</sup>, *n* = 3) and phenolic acids (in mg L<sup>-1</sup>, *n* = 2); (b) chlorogenic acid; (c) rosmarinic acid; and (d) carnosic acid in plant water extracts expressed as means ± standard deviations.

	Total phenols / mg GAE* L <sup>-1</sup>	Chlorogenic acid / mg L <sup>-1</sup>	Rosmarinic acid / mg L <sup>-1</sup>	Carnosic acid / mg L <sup>-1</sup>
Lemon balm	5036 ± 45 <sup>(a)</sup>	10.8 ± 0.0	173.6 ± 0.3	29.1 ± 0.3
Rosemary	2124 ± 50 <sup>(b)</sup>	3.9 ± 0.1	172.3 ± 1.5	30.9 ± 1.3
Oregano	5624 ± 28 <sup>(c)</sup>	107.1 ± 0.1	3.7 ± 0.1	30.7 ± 1.8
Lavender	3382 ± 110 <sup>(d)</sup>	10.3 ± 0.1	94.7 ± 0.0	29.7 ± 0.5

\*GAE – gallic acid equivalents. Values for total phenolics with different letters denote significant differences among samples (*F*-ratio 1738.9, *p* < 0.01).



**Figure 1.** Induction periods (in h) of vegetable oils detected by Rancimat method, before (control sample of pure oil) and after herb water extract addition, expressed as means  $\pm$  standard deviations ( $n = 3$ ). Different letters above the bars denote samples that have significantly different induction periods ( $p < 0.05$ ).

The addition of extracts to sesame and sunflower oils prolonged their IPs in all cases, and the best results were obtained after addition of lemon balm extract, 1.33 and 1.48, respectively. The rosemary extract addition provided good results (OSIs 1.24 and 1.22). Rosemary extract showed antioxidant effect also in hemp and flax oil where induction periods were prolonged to 0.53 and 0.47 h, respectively. The total phenolic content could not be a referent measure to predict the activity of the extracts as the presence of the individual compounds also has significant influence. Furthermore, previous studies on antioxidant activity of rosmarinic acid confirmed its

prooxidant activity<sup>[21,25,26]</sup> but the overall antioxidant effect of extracts is a result of interactions of all present phenolic compounds. Besides phenolics, some other compounds from the extracts (or from the oils), e.g. pigments, terpenes, vitamins, sterols, minerals, could affect positively or negatively oxidative stability of oils. It is interesting to notice that lemon balm extract had the opposite effect on hemp and flax oil where its prooxidative activity was detected; IP of hemp oil was reduced from 0.42 to 0.16 h (OSI 0.39) and of flax oil from 0.40 to 0.33 (OSI 0.84). The prooxidative activity of oregano extract in hemp and flax oil was also detected.

**Table 3.** Oxidative stability index (OSI) of investigated vegetable oils with the addition of herb water extracts (100  $\mu$ L/3.0 g of oil)

	OSI <sup>(a)</sup>			
	Hemp	Flax	Sesame	Sunflower
Lemon balm	0.39	0.84	1.33	1.48
Rosemary	1.27	1.18	1.24	1.22
Oregano	0.89	0.84	1.10	1.36
Lavender	1.17	1.01	1.10	1.07

<sup>(a)</sup> OSI < 1 – prooxidative activity, OSI > 1 – antioxidative activity.

## CONCLUSION

Food lipids are components susceptible to oxidation processes and their deterioration is one of the major spoilage issues in lipid-containing foods. The tendency to use natural compounds as food additives instead of synthetic ones is supported by the results of this study and opens new possibilities of preventing lipid oxidation using plant extracts. However, according to the obtained results, it can be concluded that the chemical composition of oil (fatty acid composition, PUFA/SFA ratio, the content of antioxidants, etc.) is a basic parameter that affects the oil

oxidative stability. Furthermore, the herb extract phenolic profile is also important, especially the effect of dominant phenolic compounds. Although the presence and influence of other compounds that also contribute to the overall extract activity should not be neglected, the application of investigated herbs and/or pure phenolic compounds, (e.g. rosmarinic acid and chlorogenic acid), in different concentrations and/or in some real food systems should be the objective of future research.

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