Stabilization of sunflower and olive oils with savory (*Satureja kitaibelii*, Lamiaceae)

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Summary

The aim of the present study was chemical characterization of the savory (*Satureja kitaibelii*, Lamiaceae) extract, evaluation of its antioxidant potential and time-dependent monitoring of oxidative degradation parameters in sunflower and olive oil with added savory during storage and during the heating process. The savory extract was preliminary and in detail characterized for specific phenolic compounds, while the antioxidant potential was estimated in vitro through neutralization of 2,2'-diphenyl-1-picrylhydrazyl, hydroxyl and nitroso radicals, inhibition of lipid peroxidation and ferric reducing antioxidant power assay. The parameters of oxidative degradation of oils were estimated by peroxide value, specific extinction coefficients (K_{232}, K_{270}) and thiobarbituric acid reactive substances (TBARS) evaluation. Stabilization with savory showed a remarkable effect on oil oxidative degradation parameters. During storage at 4 °C for 70 days and heating at 180 °C, sunflower oil samples enriched with savory showed significantly lower peroxide values (constituting 17–49 % and 16–65 %, respectively, depending of the measurement time point), compared to control samples. The obtained results highlight the potential of savory as a novel valuable source of natural antioxidants, suggesting its potential to be used as a beneficial ingredient and make an impact on profitability of the functional food industry.

Keywords

savory; sunflower oil; olive oil; storage; heating process; oil oxidation

Edible oils include a large variety of products, representing an important source of essential fatty acids and some liposoluble vitamins. Oxidative stability of oils is the resistance to oxidation during processing and storage. Oxidation of edible oil produces off-flavour compounds and decreases the quality of oil. Oil oxidation is significant in terms of palatability, nutritional quality and toxicity. The oxidative stability of edible oils is affected by various factors such as fatty acid content, storage temperature, light, oxygen, metals, as well as the presence of antioxidants and pro-oxidants. Primary oxidation products are lipid hydroperoxides. Lipid peroxidation leads through formation of hydroperoxides to short-chain aldehydes, esters, acids, low-molecular-mass alcohols and other oxidation products. The time for secondary products formation from the primary oxidation products (hydroperoxides) varies with different oil types. In olive and rapeseed oils, secondary oxidation products are formed immediately upon the formation of the primary products. On the other hand, in sunflower oil, the threshold of primary oxidation products (in terms of concentration) required for the secondary oxidation products formation is significantly higher than in the olive and rapeseed oils [1–3].

The formation of lipid peroxides in edible oils can potentially lead to several negative effects on the human health, of which the most significant is reflected through their role in development of atherosclerosis. Aldehydes, as a class of secondary lipid oxidation products, are electrophiles with the ability to react with the nucleophilic functional

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groups present in lipids, proteins and DNA, which is the molecular basis of many pathophysiological processes. The toxicity of low-molecular-weight carbonyl compounds (aldehydes) to humans and animals is well known [4–6].

Deep frying is one of the most common procedures used in food preparation. During the frying process, chemical reactions such as oxidation, polymerization and hydrolysis develop in edible oils [7]. The formation of free radicals that rapidly react with the surrounding oxygen is frequently observed in edible oils, while isomerization and formation of *trans* isomers are also possible at high temperatures [8]. The resulting thermo-oxidative changes depend on the type of oil, temperature and heating time, while the oil oxidation degree during heating depends primarily on the polyunsaturated fatty acids (PUFA) content [9].

The early 1990s brought a new trend in the production of edible oils, which favours the production of so-called gourmet, flavoured or spicy oils. To improve the quality parameters of edible oils, different approaches have been implemented, such as blending different types of edible oils, investigations and utilization of new plant species as sources of natural antioxidants and using of synthetic or isolated antioxidants of natural origin [10]. The use of synthetic antioxidants in food is strictly regulated by legislation and very limited due to health concerns [11]. On the contrary, plants or plant extracts, as sources of natural antioxidants, are widely used for stabilization of edible oils. Plant chemical constituents such as carotenoids, tocopherols, phenolic acids and amides, phenolic mono- and diterpenes, flavonoids, gingerol-related compounds, diarylheptanoids and many other compounds can act as potent antioxidants and may have the potential to prevent lipid oxidation in food [12]. The benefits of antioxidants from natural sources were highlighted in recent years. Natural antioxidants' mechanisms of action in reducing the oxidation includes scavenging of free radicals and oxygen, electrophilic trapping, as well as decomposing or deoxidizing peroxides. The effectiveness of antioxidants depends on chemical reactivity of the antioxidant compound, environmental conditions, interaction with food components and physical location of the antioxidant in food systems [7]. The results of previous studies showed the presence of many antioxidant compounds in the species of Lamiaceae family. A very strong antioxidant potential has been demonstrated in the extracts of sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.). However, a large number of plant species are still not thoroughly studied regarding possible antioxidant properties that could be utilized in the food industry [13].

Satureja genus belongs to Lamiaceae family, Nepetoideae subfamily, which is mainly spread in the eastern parts of the Mediterranean. Previous studies demonstrated clear and noticeable differences in the composition of essential oils between *S. montana* and *S. kitaibelii*. According to the latest taxonomic status revision, *Satureja montana* subsp. *kitaibelii* (Wierzb. ex Heuff.) P. W. Ball is classified as a subspecies of *Satureja montana*, although the name of *Satureja kitaibelii* Wierzb. ex Heuff. is accepted as a basionym. *Satureja kitaibelii* is a species endemic to the Balkan Peninsula. It is well known for its usage in traditional medicine as well as for various beneficial effects [14, 15].

Taking into account the overall worldwide utilization of various Lamiaceae species in the diet, it would be of great benefit to define their role not only as spices, but also as sources of bioactive compounds that can be easily administered through food. As such, they would act both as health-promoting agents and food stabilizers, leading to prolongation of products shelf life, as well as reducing of food industry costs.

To the best of our knowledge, there have been no studies on optimization of enrichment conditions of sunflower and olive oils with savory (*Satureja kitaibelii* Wierzb. ex Heuff., Lamiaceae), during storage and heat processing. Therefore, the aim of the present study was chemical characterization of the savory extract, evaluation of its in vitro antioxidant activity and time-dependent monitoring of oxidative degradation parameters of sunflower and olive oils with added savory during the storage and heating process.

MATERIALS AND METHODS

Reagents and chemicals

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical, sulphanilamide, *N*-(1-naphthyl)-ethylenediamine dihydrochloride, iron(II)-chloride tetrahydrate and iron(III)-chloride hexahydrate were obtained from Alfa Aesar (Haverhill, Massachusetts, USA). Folin-Ciocalteu (FC) reagent was obtained from Merck (Darmstadt, Germany). Ethanol, *n*-hexane, hydrochloric acid, ammonium thiocyanate, chloroform, trichloroacetic acid, hydrogen peroxide and sodium nitroprusside were obtained from Lach-Ner (Brno, Czech Republic). Sodium bicarbonate, iron(II)-sulphate heptahydrate, acetic acid and methanol were obtained from POCH (Gliwice, Poland). 1,1,3,3-Tetraetoxypropane, gallic acid, caffeic acid, *trans*-cinnamic

acid, *p*-coumaric acid, chlorogenic acid, rosmarinic acid, ferulic acid, quercetin, rutin, quercitrin, aluminium-chloride, 2-deoxy-D-ribose, 2-thiobarbituric acid, ethylenediaminetetraacetate dihydrate (EDTA) and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Sigma Aldrich (St. Louis, Missouri, USA). Cyclohexane was obtained from LGC Standards (Teddington, United Kingdom). All chemicals used in this study were of analytical grade, except for the reagents for high performance liquid chromatography (HPLC) analysis, which were of HPLC grade.

Materials

Refined sunflower oil (SO) and olive oil (OO) oils available on the market were purchased in Novi Sad, Serbia in 2017.

The aerial parts of *Satureja kitaibelii* in the stage of full blossom were collected in south-western part of Montenegro (locality: mount Orjen) in July 2017. The sample was identified at the Department of Biology and Ecology (Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia) and a voucher specimen of *S. kitaibelii* was deposited.

Extract preparation

The extract was prepared by maceration with 70% (v/v) ethanol for 24 h at room temperature (25 °C), after which the extract was filtered and evaporated to dryness in a rotary evaporator. For further analysis, i.e. evaluation of the total phenolic and flavonoid contents as well as of antioxidant activity, 20% (w/w) aqueous solution of the extract was prepared and preserved at –20 °C. For the HPLC analysis, the dried extract was dissolved in 70% (v/v) methanol.

Sample preparation

Four aliquots (500 ml each) of sunflower and olive oils were prepared. In two aliquots, 5 g of grinded *S. kitaibelii* was added, while the two remaining aliquots were used as control samples, without the addition of the plant material. A total of eight types of samples were prepared and stored at two different temperatures. This resulted in sunflower oil with and without the added herb, stored at 25 °C or 4 °C (samples marked as SO25H, SO25, SO4H and SO4, where H designated oil sample with the addition of herb, i.e. savory) and olive oil (OO25H, OO25, OO4H and OO4). For the purpose of testing of the impact of oxidative stability during storage, sampling was carried out every 14 days, for 70 days starting from the day of oil preparation. Regarding the impact of heating on oxidative stability, after expiration of 70 days, all eight samples of oils were heated at the temperature of 180 °C and sampling was performed at time intervals of 45 min (i.e. after 0, 45, 90, 135, 180 and 225 min).

Quantification of total phenolics

Total phenolic content (*TPC*) was determined by the previously described Folin-Ciocalteu method with some modifications [16]. In the presence of certain reducing agents, such as phenolic compounds, Folin-Ciocalteu reagent forms a blue-coloured complex with a characteristic absorption maximum at 760 nm. Absorbance was measured using 1800 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). The content of total phenolics was determined using gallic acid as a reference standard and the result was expressed as grams of gallic acid equivalents (GAE) per kilogram of dry extract.

Quantification of flavonoids

The total flavonoid content (*TFC*) was determined spectrophotometrically, according to the previously described method, while the result was expressed as grams of quercetin equivalents (QE) per kilogram of dry extract [16]. The method was based on complexation of flavonoids with aluminum ion, and the absorbance maximum was measured using 1800 UV-Vis spectrophotometer.

HPLC analysis

Chemical characterization of the examined extract and quantification of the selected compounds was performed by a validated HPLC method. Phenolic compounds were separated using an Agilent Technologies 1100 liquid chromatographer equipped with a diode array detector (Agilent Technologies, Santa Clara, California, USA). The components were separated using a reversed-phase Nucleosil C18 column (250 mm × 4.6 mm, 5 μm particle size; Agilent Technologies) held at 30 °C. Solvent A was 0.1% (v/v) aqueous HCOOH with 10 mmol CH_3COONH_4, and solvent B was methanol. The mobile phase was delivered in the gradient mode comprising 0 min 10% B, 10 min 25% B, 20 min 45% B, 35 min 70% B, 40 min 100% B, 46 min 10% B. The HPLC mobile phase was prepared fresh daily and filtered through a nylon filter (pore size 0.45 μm). The injection volume was 10 μl and the run time was 48 min using a variable flow rate (0–10 min, 1 ml·min^{-1}; 10–20 min, 0.8 ml·min^{-1}; 20–30 min, 0.7 ml·min^{-1}; 30–46 min 1 ml·min^{-1}).

For quantification of the selected compounds, chemical standards of gallic acid (GA), caffeic acid (CA), *trans*-cinnamic acid (CNA), *p*-coumaric

acid (pQA), chlorogenic acid (CHA), rosmarinic acid (RA), ferulic acid (FA), quercetin (Qe), rutin (R) and quercitrin (Qt) were run under the same experimental conditions, using methanol as a solvent. The compounds of interest were monitored at 280 nm (GA, CA and CNA), 330 nm (pQA, CHA, RA, FA and Qe) and 350 nm (R and Qt). The experimental programs and data processes of quantification were both performed with the software Agilent OpenLAB Control Panel v.A.01.05 (Agilent Technologies). The results were expressed as milligrams per kilogram of dry extract.

Determination of antioxidant activity

Free radical-scavenging activity

The ability of the examined extract to neutralize DPPH, OH and NO radicals was determined using the previously described spectrophotometric methods [17]. Various concentrations of the examined extract were added to a solution of purple-coloured stable DPPH radical and disappearance of the colour was measured spectrophotometrically at 515 nm using 1800 UV-Vis spectrophotometer. Neutralization of OH radicals, which were generated in a Fenton reaction, was also monitored spectrophotometrically at 532 nm. The assay was based on degradation of 2-deoxy-D-ribose to malondialdehyde (MDA), whereby MDA forms a complex with 2-thiobarbituric acid (TBA). The ability of the extract to neutralize the generated nitric oxide (NO) radicals was measured spectrophotometrically at 546 nm, by the use of Griess reagent. Free radical-scavenging capacity (*RSC*) of various concentrations of extract was calculated by the Eq. 1 and expressed in percent:

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

where A_0 is absorbance of the control and A is absorbance of the extract.

The extract concentration that caused 50% neutralization of DPPH, NO and OH radicals (IC_{50}) was determined by regression analysis of the obtained *RSC* values.

Inhibition of lipid peroxidation

The potential of the extract to inhibit the lipid peroxidation process was determined spectrophotometrically by measuring the colour intensity of the complex formed in a reaction of TBA and MDA, at wavelength 532 nm using 1800 UV-Vis spectrophotometer [17]. The percentage of lipid peroxidation inhibition (I) was calculated by the Eq. 2:

$$I = 100 - 100 \times \frac{A}{A_0} \quad (2)$$

where A_0 is absorbance of the control and A is absorbance of the extract.

Ferric reducing antioxidant power assay

The capacity of the extract to reduce Fe^{3+} was evaluated by the procedure of BENZIE and STRAIN, with some modifications applied [18]. The absorbance was measured at 593 nm using 1800 UV-Vis spectrophotometer. The results were expressed as grams of ascorbic acid equivalents (AAE) per kilogram of dry extract, ascorbic acid being used as a reference standard.

Oxidative degradation parameters of sunflower and olive oils enriched with savory

The oxidative degradation parameters assessed were peroxide value (*PV*), levels of conjugated dienes and conjugated trienes (specific extinction coefficients at 232 nm and 270 nm – K_{232} and K_{270}) and thiobarbituric acid reactive substances (TBARS) value.

Determination of peroxide value

Determination of the peroxide value (*PV*) is one of the most commonly used methods for examining the primary oxidation products of vegetable oils. *PV* was determined using the published ferric thiocyanate method of International Dairy Federation with some modifications [19]. The method is based on the ability of hydroperoxides to oxidize ferrous to ferric ions in an acidic medium. Ferric ions react with thiocyanate and form a pink-coloured complex with a characteristic absorbance maximum at 500 nm, which was measured using 1800 UV-Vis spectrophotometer. A standard curve was constructed based on 0.1 mg·ml^{-1} Fe^{3+} standard work solution. *PV* was calculated by the Eq. 3:

$$PV = \frac{(A - A_0) \times L \times V}{55.845 \times S \times 0.1} \times 0.5 \quad (3)$$

where A is absorbance of the sample, A_0 is absorbance of the blank, L is slope of the standard curve constructed as an absorption (A) being a function of mass ($m(Fe^{3+})$), V is volume of n-hexane used to dissolve oil in millilitres, S is amount of oil sample in grams, number 55.845 represents molar weight of iron in grams per mole, number 0.1 represents volume of the sample dissolved in n-hexane added to the reaction mixture in millilitres and 0.5 is the correction factor. Results were expressed as milliequivalents of peroxide per kilogram of oil.

Determination of conjugated dienes and conjugated trienes

Conjugated dienes (CD) and conjugated trienes (CT) represent primary products of oil oxidation. The content of CD and CT was determined according to the standard ISO method using cyclohexane as a solvent [20]. Changes in the level of oxidation products were evaluated periodically by measuring the absorbance of oil sample solution at wavelength 232 nm for CD and 270 nm for CT using 1800 UV-Vis spectrophotometer. Results were expressed as specific extinction K_λ, being calculated according to the Eq. 4 and Eq. 5:

$$K_{232} = \frac{A_{232}}{c \times s} \quad (4)$$

$$K_{270} = \frac{A_{270}}{c \times s} \quad (5)$$

where A_{232} and A_{270} are the measured absorbances at wavelengths 232 nm and 270 nm, c is concentration of the solution in grams per 100 millilitres and s is the optical path length of the cuvette in centimetres.

Thiobarbituric acid reactive substances assay

The content of secondary oxidation products was determined by TBARS assay previously described by Ke et al. with slight modifications [21]. The method is based on formation of a pink-coloured complex with an absorbance maximum at 535 nm, produced in the reaction of TBA and MDA, the oxidation products derived from unsaturated fatty acids. For calculations, a calibration curve based on known concentrations of 1,1,3,3-tetraethoxypropane (TEP) working solution was constructed. The TBARS value (*TBARSV*) was calculated by the Eq. 6:

$$TBARSV = \frac{(A - b)}{a \times m \times 1000} \quad (6)$$

where A represents absorbance of the oil sample, a is slope of the standard curve ($a = 0.0272$), b is intercept of the standard curve ($b = 0.0104$), m is the amount of sample oil in grams and 1000 is conversion factor to millimoles per kilogram. Results were expressed as millimoles of TBARS per kilogram of oil sample.

Statistical analysis

Microsoft Excel, v. 2010 software package (Microsoft, Redmond, Washington, USA) and Statistica v. 12.5 software (StatSoft, Tulsa, Oklahoma, USA) were used for the statistical analysis of experimental data. All values were expressed as mean of triplicate analyses with standard deviation (*SD*). The obtained dataset was analysed by methods of univariate statistics (Man-Whitney test with a level of significance $p < 0.05$), as well as by a multivariate statistical method of hierarchical cluster analysis (HCA), which was performed on squared Mahalanobis distances.

RESULTS AND DISCUSSION

Chemical characterization of *S. kitaibelii* extract

The quantified contents of total phenolics and flavonoids in the examined *S. kitaibelii* extract were 177.52 ± 3.45 g·kg^{-1} (expressed as GAE) and 28.20 ± 0.46 g·kg^{-1} (expressed as QE), respectively. Compared to the ethanolic extract of *S. montana*, a higher *TPC* was determined in the examined ethanolic extract of *S. kitaibelii*, but the highest *TPC* was previously found in a chloroform extract of *S. montana* [22]. However, the latter illogical result can be explained by the simultaneous extraction of aromatic monoterpenes (thymol and carvacrol), which are found as dominant components of *S. montana* essential oil. Generally, the extraction of phenolic compounds increases with the increase in solvent polarity, but variations in the content of secondary metabolites between samples of the same species originating from different geographical locations must not be neglected, due to the influence of abiotic (climatic, edaphic and orographic), as well as biotic factors [16].

The results of HPLC analysis are presented in Tab. 1. Caffeic acid, chlorogenic acid, rosmarinic acid, ferulic acid, rutin and quercetin were identified and quantified in the extract of *S. kitaibelii* (Fig. 1). HPLC analysis showed the presence of rosmarinic acid as a major constituent. It is the most common phenolic acid occurring in Nepetoidae subfamily of Lamiaceae family [23]. Other phenolic acids found in *S. kitaibelii* extract were hydroxycinnamic acids, namely, caffeic acid, chlorogenic (3-caffeoylquinic acid isomer) and ferulic acid. Our results are in concordance with previous studies in which *S. kitaibelii* extract was analysed and over 40 compounds were identified and quantified, including phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and flavonoids (specifically flavones and flavonols). A flavonol glycoside, rutin (quercetin-3-O-rutinoside), was also detected, and this compound had previously been identified in other *Satureja* species such as *S. cilicica*, *S. corulea* and *S. icerica* [15]. Quercetin and rutin are widely used in the food industry. However, the precise mechanisms by which flavonoids exert their antioxidant properties are not completely understood. Antioxidant poten-

Tab. 1. Contents of selected phenolic compounds in *S. kitaibelii* extract.

Class of phenols	Subclass	Phenolic compound	Content [mg·kg^{-1}]
Phenolic acids	Hydroxybenzoic acid derivates	Gallic acid	ND
	Hydroxycinnamic acid derivates	Caffeic acid	478.80 ± 23.94
		trans-Cinnamic acid	ND
		p-Coumaric acid	ND
		Chlorogenic acid	11.03 ± 0.55
		Rosmarinic acid	11 999.44 ± 719.97
		Ferulic acid	26.43 ± 1.58
Flavonoids	Flavonols	Quercetin	892.47 ± 62.47
	Glycosides	Rutin	485.88 ± 38.87
		Quercitrin	ND

Values represent mean ± standard deviation of triplicate analysis (the content is expressed per kilogram of dry extract).
ND – not detected.

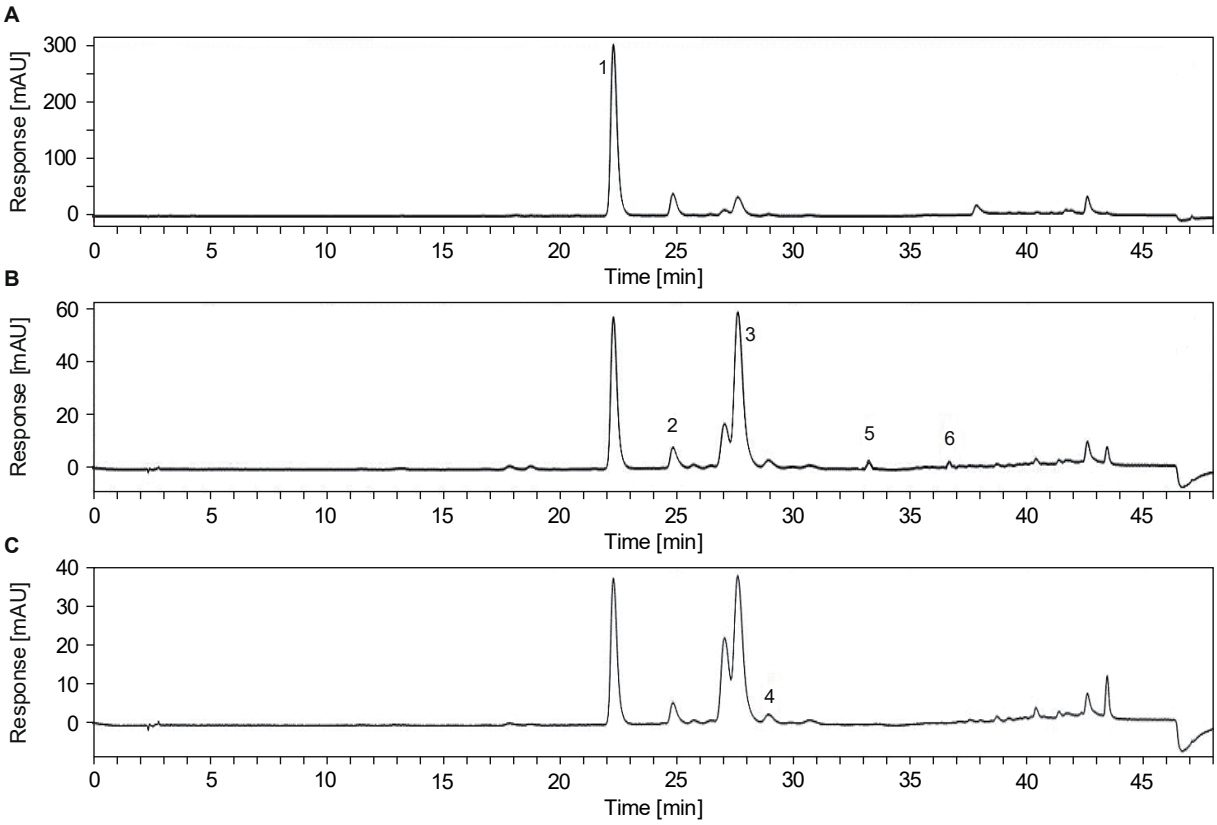

Fig. 1. HPLC with diode array detector chromatogram of *S. kitaibelii* extract.

A – detection at 280 nm, B – detection at 330 nm, C – detection at 350 nm
1 – caffeic acid, 2 – chlorogenic acid, 3 – rosmarinic acid, 4 – rutin, 5 – quercetin, 6 – ferulic acid.

tial depends on the chemical structure, such as hydroxyl groups in 3- and 5-position, 3',4'-*ortho*-dihydroxy orientation of hydroxyl groups and substitution of hydroxyl groups with sugars. Several studies [24, 25] reported that rutin has been less effective in inhibition of PUFA and has a less free radical-scavenging activity than its aglycone quercetin, due to the presence of sugar moiety at 3-O-position. Therefore, chemical characterization of *S. kitaibelii* extract suggests that quercetin and rutin may contribute to stabilization of sunflower and olive oils.

Antioxidant activity of *S. kitaibelii* extract

Based on the obtained values for neutralization of DPPH, OH and NO radicals, as well as inhibition of lipid peroxidation process, IC_{50} values were calculated (Tab. 2). The strongest antioxidant potential was recorded in DPPH test system, while a higher IC_{50} value was observed in NO test system. IC_{50} value for OH radical was not determined in the examined range. Furthermore, the potential of the examined extract to inhibit lipid peroxidation process was relatively modest, in contrast to the strong reduction potential demonstrated in FRAP assay (244.93 ± 12.91 g·kg^{-1}, expressed as AAE). The results obtained for free radical-scavenging capacity of *S. kitaibelii* extract (DPPH radical and FRAP assay) were lower than the results obtained for synthetic antioxidants, such as butylated hydroxyanisole (BHA), under the same experimental conditions [26]. The results indicated that *S. kitaibelii* extract has pronounced antioxidant activities against free radicals, which is in correlation with the high content of phenolic compounds, in particular rosmarinic acid [26].

As far as the antioxidant activity is concerned, only a few studies were published dealing with *Satureja kitaibelii*, more data are available on other species from *Satureja* genus. The aquatic extract of *S. montana* showed a lower potential of DPPH neutralization, compared to the examined ethanolic extract, while the ethanolic extract of *S. hortensis* showed the weakest ability to neutralize DPPH radicals [27]. In the tested concentration range, *S. kitaibelii* extract showed no significant potential for neutralization of OH radicals. According to the literature data [28], *n*-butanol extract showed the highest antioxidant activity in the OH test system, while the aqueous extract showed the lowest activity. IC_{50} determined for the NO radical neutralization, according to the available literature [26, 28], was the first description of the *S. kitaibelii* extract antioxidant potential in the NO test system. It was noticed that *n*-butanol and ethyl acetate extract of *S. kitaibelii* significantly inhibited the lipid peroxidation process, but the analyses were not carried out under the same experimental conditions, and it was not possible to compare the antioxidant potentials of these extracts [28]. Furthermore, the results obtained by FRAP assay in our study were similar to the previous results obtained for the ethanolic extract of *S. montana* [27].

Oxidative degradation parameters of sunflower and olive oils enriched with savory

The results of previous studies [10, 12, 13, 29–34] suggested the benefits of antioxidants use in preventing lipid oxidation in edible oils. *PV* is a significant indicator of the oxidation degree in the initial stages of lipid oxidation. Changes in *PV* during the storage time and subsequent heating process are shown in Fig. 2. The samples of SO with added *S. kitaibelii* stored at 25 °C (SO25H) showed a *PV* decrease followed by a *PV* increase. This trend in *PV* dynamics can be explained by the fact that the concentration of antioxidant compounds was sufficient to maintain *PV* in a satisfactory range, after which *PV* began to increase due to the higher production of various oxidation products. The same samples stored at 4 °C (SO4H) showed statistically significantly lower *PV* ($p < 0.05$) relative to the control samples, which was conditioned by slowing down the formation of hydroperoxides at lower temperatures. However, *PV* did not decrease linearly with the decrease in storage temperature, as the temperature is only one of the factors affecting oil oxidation. A previous study of adding various aromatic Lamiaceae species (lemon balm – *Melissa officinalis* L., peppermint – *Mentha piperita* L., spearmint – *Mentha spicata* L., basil – *Ocimum basilicum* L., oregano – *Origanum vulgare* L. and summer savory – *Satureja hortensis* L.) pointed to the initial decrease in *PV* as a result of the antioxidant activities of plants added to the examined oil samples [29]. The results obtained in our study

Tab. 2. Antioxidant activity of *S. kitaibelii* extract.

	Inhibition of free radicals			Inhibition of lipid peroxidation
	DPPH	OH	NO	
IC_{50} [μg·ml^{-1}]	3.13 ± 0.21	ND	24.72 ± 1.98	879.25 ± 79.87
Concentration range [μg·ml^{-1}]	1–10	10–100	10–150	100–1 500
Regression equation	$12.95x + 9.49$	$10.11 \ln(x) - 9.37$	$9.36 \ln(x) + 19.96$	$18.63 \ln(x) - 76.26$
Coefficient of determination R^2	0.9952	0.9979	0.9961	0.9982

Values represent mean ± standard deviation of triplicate analysis.
IC_{50} – concentration of extract that caused 50% neutralization, DPPH – 2,2-diphenyl-1-picrylhydrazyl radical, OH – hydroxyl radical, NO – nitroso radical, ND – not detected.

were similar to the results obtained in the study of adding musk willow (*Salix aegyptiaca* L., Salicaceae) to sunflower oil and monitoring influence on the SO oxidative stability [35]. On the other hand, in samples of OO with added *S. kitaibelii* stored at 25 °C (OO25H), a faster decrease in *PV* followed by a faster increase was observed, compared to sunflower oil samples (Fig. 2A). This can be explained by the initial formation of hydroperoxides and their successive decomposition [36]. The highest fluctuations of *PV* were observed in samples of OO stored at 4 °C (OO4) but, at all sampling points, lower *PV* values were recorded in the samples with added *S. kitaibelii* compared to the control samples treated in the same manner (Fig. 2B). The peroxide value of SO and OO samples increased with heating time (180 °C, 225 min) and was found to be lower in SO or OO with added *S. kitaibelii* at each heating period (0, 45, 90, 135, 180 and 225 min), compared to the control samples, with the exception of OO sample stored at room temperature (Fig. 2C and Fig. 2D).

The results of previous studies showed that the phenolic compounds are promising agents in terms of the ability to prevent oxidative degradation of edible oils [10, 13, 29–31]. Namely, the addition of green tea extract (*Camellia sinensis* L., Theaceae) significantly improved the oxidative stability of rapeseed oil, which was the consequence of epicatechin, epicatechin gallate, epigallocatechin presence, as the authors concluded [31]. Sahin et al. [32, 33] showed that olive leaf extract rich in oleuropein improved the quality and enhanced the stability of the virgin olive oil. Sahin et al. [34] also verified that the addition of olive leaf extract was beneficial for sunflower oil during thermal oxidation. Taking into account the overall high content of total phenolics in the examined savory extract, it can be hypothesized that this class of secondary metabolites mostly contributes to oxidative stability of sunflower and olive oils.

The *PV* data on SO before heating (stored at 25 °C and 4 °C) and OO samples stored at 4 °C obtained in this study, with and without added *S. kitaibelii,* were approximately equal. It was observed that increase in temperature was followed by an increase in *PV*, which was in accordance with the results of previous studies [37]. Generally, *PV* tends to increase during the early phases of lipid oxidation, when the hydroperoxide formation rate is higher than the rate of their decomposition [37]. Additionally, SO samples stored at 4 °C (SO4H)

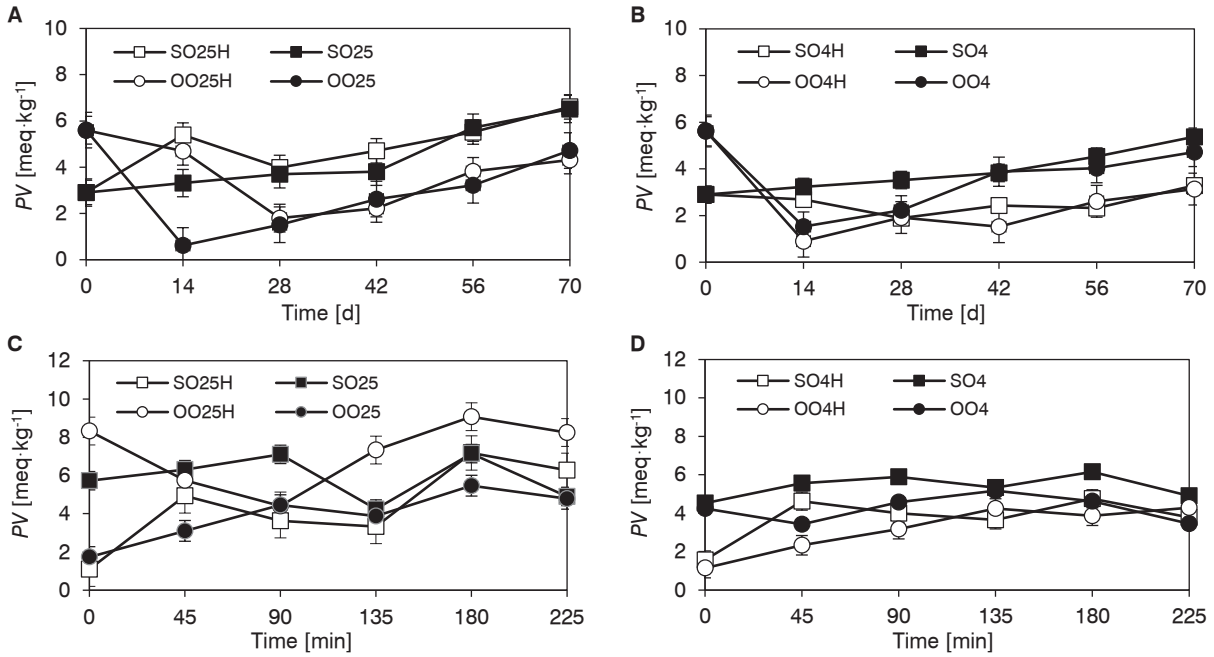

Fig. 2. Changes in peroxide value during the storage time and subsequent heating process.

A – storage at 25 °C, prior to heating; B – storage at 4 °C, prior to heating; C – storage at 25 °C, after heating; D – storage at 4 °C, after heating.

Data are expressed as mean ± standard deviation (n = 3). *PV* – peroxide value expressed as milliequivalents of peroxide per kilogram of oil. SO25H, SO4H – sunflower oil with added herb; SO25, SO4 – sunflower oil without added herb; OO25H, OO4H – olive oil with added herb; OO25, OO4 – olive oil without added herb.

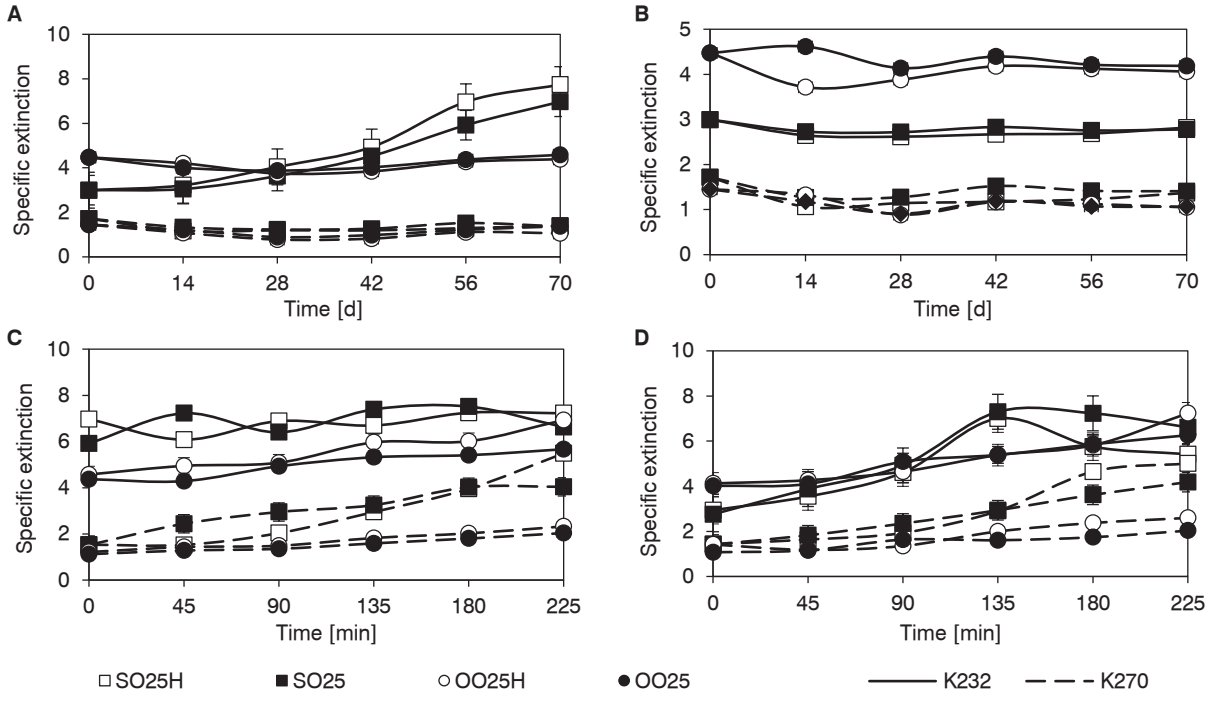

Fig. 3. Changes in specific extinction during the storage time and subsequent heating process.

A – storage at 25 °C, prior to heating; B – storage at 4 °C, prior to heating; C – storage at 25 °C, after heating; D – storage at 4 °C, after heating.
Data are expressed as mean ± standard deviation (n = 3). K232 – specific extinction at 232 nm, K270 – specific extinction at 270 nm. SO25H, SO4H – sunflower oil with added herb; SO25, SO4 – sunflower oil without added herb; OO25H, OO4H – olive oil with added herb; OO25, OO4 – olive oil without added herb.

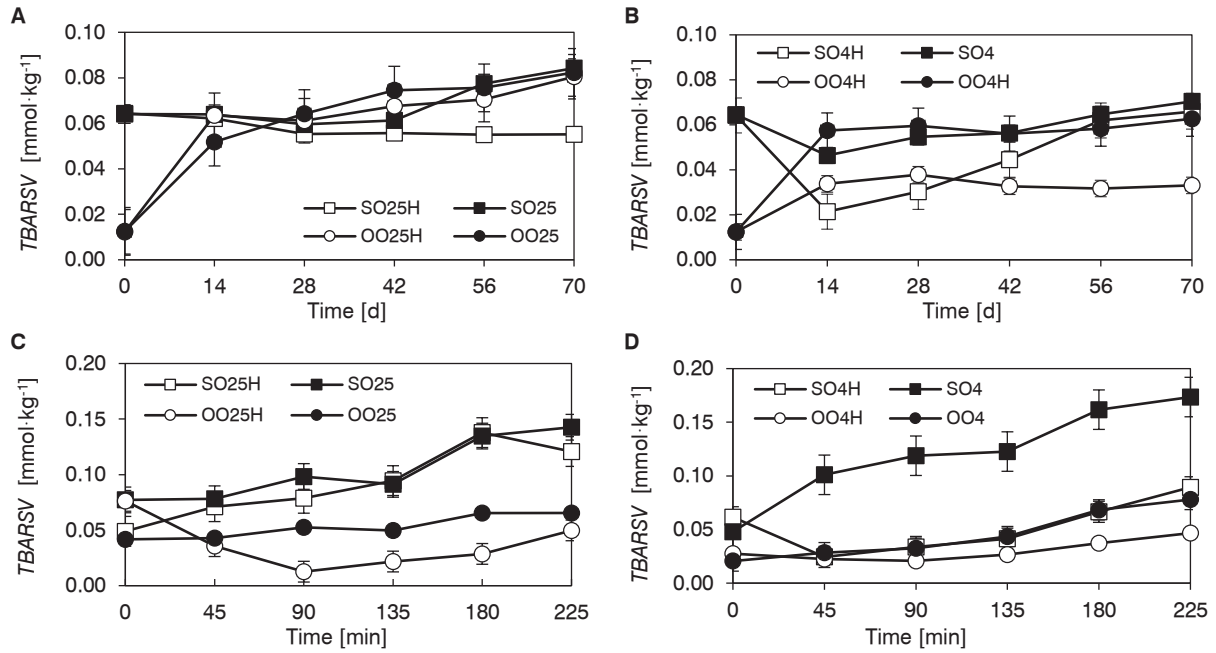

Fig. 4. Changes in TBARS value during the storage time and subsequent heating process.

A – storage at 25 °C, prior to heating; B – storage at 4 °C, prior to heating; C – storage at 25 °C, after heating; D – storage at 4 °C, after heating.
Data are expressed as mean ± standard deviation (n = 3). SO25H, SO4H – sunflower oil with added herb; SO25, SO4 – sunflower oil without added herb; OO25H, OO4H – olive oil with added herb; OO25, OO4 – olive oil without added herb.

and OO stored at 25 °C (OO25H) with added *S. kitaibelii* showed significantly different *PV* values compared to the control samples ($p < 0.05$).

The formation of hydroperoxides, as a result of PUFA oxidation, leads to a further transposition of unconjugated double bonds and the formation of CD, which show a characteristic maximum of absorption at 232 nm. When PUFA containing three or more double bonds undergo oxidation, the conjugation of CD parts can be extended to include another double bond resulting in the formation of CT [38]. The presence of CD and CT is a better parameter for determining the degree of oil oxidation than *PV* as the resulting products remain in oil even after frying. Our results of CD and CT determination showed a slight increase in the specific extinction of samples at 232 nm and 270 nm during storage (Fig. 3A and Fig. 3B). A significant difference ($p < 0.05$) in the K_{232} value was observed only in the case of OO samples stored at 4 °C (OO4H) compared to control samples. On the other hand, the significant decrease ($p < 0.05$) in K_{270} values among SO samples stored at 4 °C with added *S. kitaibelii* (SO4H) and control samples (SO4) could be observed. Several studies showed that adding garlic (*Allium sativum* L., Alliaceae), lemon (*Citrus limonum* L., Rutaceae), oregano, and rosemary to OO samples leads to a decrease in the formation of CD and CT [37]. However, it was concluded that antioxidant activity depends not only on the concentration of phenolic compounds but also on the specific chemical structure of each phenolic compound and its polarity [30].

During the heating process, an increase in the specific extinction values was observed in all examined oil samples at both wavelengths, being more pronounced for CD compared to CT (Fig. 3C and Fig. 3D). However, there was no statistically significant difference ($p < 0.05$) between the samples with added *S. kitaibelii* and the control samples. The obtained results were in accordance with previous studies [37, 39] where it was demonstrated that adding grape seed extract and conehead thyme (*Thymus capitatus* L., Lamiaceae) increased the oxidative stability of sunflower and maize oils during the heating process. Our results support the findings of Upadhyay et al. [12], who suggested that CD quantification is convenient for controlling the quality of the heating process. In order to gain a more comprehensive insight into the oxidative processes occurring in the examined oil samples during storage time and subsequent heating, the content of TBARS was determined as an indicator of the quantity of secondary oxidation products (Fig. 4). As determination of *PV* provides information only regarding the initial phases of lipid oxidation, it is necessary to examine the inhibitory effect of the plant material at later phases. However, this method was criticized as non-specific and insensitive to low levels of malondialdehyde, while, on the other hand, it may be useful for comparing samples of one type on various oxidation stages [40]. The obtained results were consistent with the previous study of the antioxidative ability of chrysanthemum (*Chrysanthemum morifolium* L., Asteraceae) in the experimental system with soybean oil, suggesting a decrease in TBARS as a result of adding the plant material [41]. In the present study, TBARS content showed increasing trends during the thermal treatment for both oil samples, but no regular pattern of increase was observed (Fig. 4C and Fig. 4D). In the TBARS assay, a statistically significant difference ($p < 0.05$) was

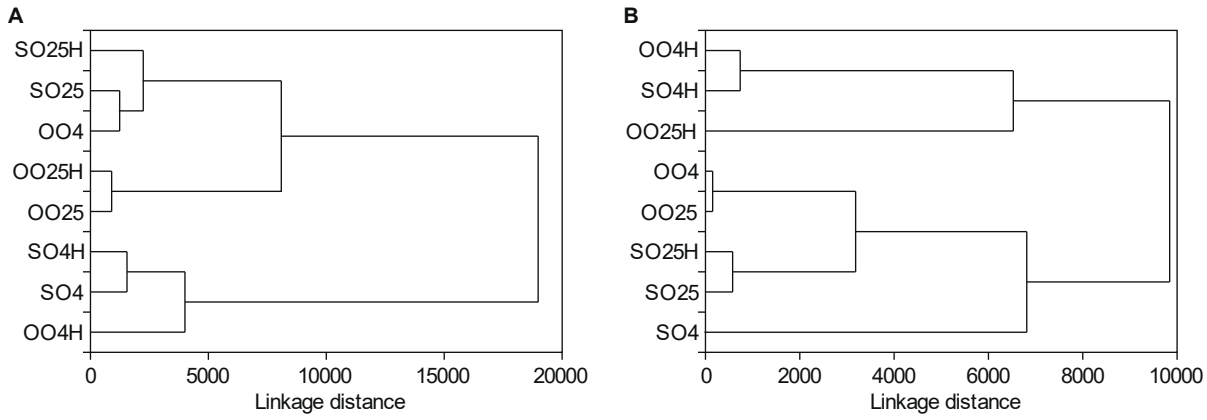

Fig. 5. Cluster analysis of the examined oil samples.

A – storage time, B – heating process.

observed only for SO samples during storage at 4 °C (SO4H) when compared to control samples (Fig. 4B).

Canonical discriminant analysis was applied on the dataset describing oxidative stability of the samples (*PV*, K_{232}, K_{270}, *TBARSV*). The obtained Mahalanobis distances were used as input data for consequent hierarchical cluster analysis. It was noticed (Fig. 5A) that during storage, no significant differences existed in oxidation processes occurring in oils stabilized with *S. kitaibelii*, when compared to the oils without the herb. The only exception were the olive oil samples stored at 4 °C (OO4H and OO4), which belonged to different clusters, suggesting the possible applicability of savory as a preservation agent in this case. On the other hand, it was noted that adding of *S. kitaibelii* highly affected the oxidative stability of the examined oils during heating processes. Namely, Fig. 5B shows that the oil samples with the added herb in most of cases belonged to clusters different than those of the samples without *S. kitaibelii*. This indicates the possible applicability of savory in food production involving various thermal treatments.

CONCLUSIONS

The results obtained by HPLC analysis and evaluation of antioxidant potential indicate that *S. kitaibelii* extract is a rich source of phenolic compounds, which is in correlation with the strong antioxidant potential. Stabilization with savory had a remarkable effect on *PV*, specific extinction values and TBARS value in sunflower and olive oil samples, in particular during the heating process. Namely, during storage at 4 °C for 70 days and subsequent heating at 180 °C, sunflower oil samples enriched with savory showed a significant decrease in *PV* (constituting 17–49 % and 16–65 %, respectively, depending of the measurement time point), compared to control samples. The obtained results highlight the potential of *S. kitaibelii* as a novel valuable source of natural antioxidants, suggesting its potential to be used as beneficial ingredient and make impact on the profitability of the functional food industry. However, further studies are recommended to reveal the mechanisms involved in the inhibition of lipid oxidation in edible oils and to assess the overall safety of this plant as a possible food improvement agent.

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