

Antioxidant activity of rapeseed oil with octyl sinapate, an amphiphilic antioxidant, stored in various types of packages under various conditions

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Summary

This work focused on the estimation of effects of amphiphilic antioxidant octyl sinapate (OSA) and a well-known synthetic antioxidant butylated hydroxyanisole (BHA), type of packing and storage conditions on the antioxidant activity of refined rapeseed oil. The antioxidant activity of oils with added antioxidants was determined by four modified spectrophotometric methods: 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and Folin-Ciocalteu (FC) reducing capacity. Changes in the antioxidant activity of oils with OSA and BHA packed in transparent or amber glass bottles and stored under various conditions (various temperatures, various exposure to light) were estimated during four weeks. Oils with 200 mg·kg⁻¹ of OSA stored under various conditions had slightly lower values of antioxidant activity as determined by ABTS (11.45–19.38 mmol·kg⁻¹) and DPPH (3.24–5.62 mmol·kg⁻¹) assays than oils with the same amount of BHA (23.10–30.68 mmol·kg⁻¹, 4.44–7.50 mmol·kg⁻¹). The antioxidant activity of oils strongly depended on storage conditions, oil supplementation and antioxidants' contents. The lowest changes in antioxidant activity were observed for oils stored in the dark in a cupboard and at 4 °C in a refrigerator commonly used in households.

Keywords

octyl sinapate; rapeseed oil; antioxidant activity; storage; package

Rapeseed is the second most important oilseed crop in the world, behind soya. However, rapeseed oil produced from „double-low” rapeseed varieties, i. e. not containing erucic acid and with a reduced content of glucosinolates, is recognized as the most popular and “healthiest” vegetable oil in human nutrition. Its nutritional value is determined by the presence of bioactive components such as sterols, tocopherols, polyphenols, in particular sinapic acid and its derivatives, with health beneficial properties as well as the most favourable ratio of ω-6 to ω-3 fatty acids [1]. Unfortunately, antioxidants naturally present in rapeseed are lost at each technological stage of its processing, therefore their large amounts remain in by-products. Only a part of bioactive components is transferred to crude oils during pressing and refining processes cause a further decrease of their content thus reducing the biological quality of the oil [2]. Moreover, the remaining antioxidants can be

degraded by destructive reactions during storage [1, 2].

Liposoluble antioxidants play an important role in protecting oils against oxidative damage and extend their shelf life. However, commercially available synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) commonly used to delay fats' oxidation are associated with the incidence of liver damage and carcinogenesis in humans [3]. Supplementation of oils with synthetic antioxidants is strictly regulated by law, the contents of BHT and BHA in oils being limited to 200 mg·kg⁻¹ separately or together [4]. Therefore, interest increases in natural antioxidants such as phenolic acids, flavonoids, vitamins, anthocyanins, carotenoids and tocopherols extracted from various plants and vegetables such as potato peel, celery, rosemary, thyme, sweet grass or rice bran [5–8]. Sinapic acid and its choline es-

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ter sinapine are the main naturally present phenolic compounds in rapeseed and its products. Unfortunately, most of them are hydrophilic in nature, which limits their application in oils and emulsions. However, lipophilization involving esterification of carboxyl group of phenolic acids with alcohols allows to enhance the hydrophobic character of phenolic acids. Moreover, length of the alkyl chain of the alcohol molecule plays an important role in antioxidant properties of the synthesized esters, which is the “cut-off effect” [9]. Health beneficial properties of phenolic acid esters strongly depend on the number and distribution of methoxyl and hydroxyl groups on the aromatic ring. Substitution of the second hydroxyl group at *ortho* or *para* position or addition of the second methoxyl group cause an increase in antioxidant activity of bioactive compounds [10, 11]. For this reason, the antioxidative efficiency of dimethoxylated sinapic acid is higher than monomethoxylated ferulic acid [12].

It is known that antioxidants protect the unsaturated fatty acids against oxidation processes depending on availability of oxygen, presence of light and temperature. Polyunsaturated fatty acids of edible oils can be easily oxidized following exposure to light in the presence of oxygen and a photosensitizer, due to activation of diatomic oxygen to singlet oxygen [13]. Moreover, the type of containers in which oils are stored directly influence its quality by protecting them from light and oxygen. The most common containers are made of plastics, mainly polyethylene terephthalate (PET), or glass bottles of different shapes, sizes and colours are used. Glass containers, in comparison with plastic ones, provide greater protection against photooxidation and are impermeable to gases. Also, concentration and type of oxygen significantly affect oxidation of oils, which is an important indicator of oil quality and shelf life. Therefore, oil stored with a bigger headspace in the bottle, e. g. after opening, is oxidized faster. Recent studies demonstrated that incorporation of a UV-blocker, pigment or oxygen scavenger could successfully protect the oil from photooxidative degradation [13–15]. The antioxidant and pro-oxidant effects of natural compounds such as chlorophyll, β -carotene, phenolic compounds or tocopherols depend on their concentration, the presence of oxygen and trace metals, exposure to light, the storage time and temperature as well as composition of oil play a role [16–18].

To the best of our knowledge, no study has been carried out yet on changes of antioxidant activity of rapeseed oil spiked with different amounts of amphiphilic antioxidant octyl sinapate (OSA),

packed in transparent and amber glass bottles and stored in a refrigerator, in a dark place or exposed to light. For this reason, this work focused on the estimation of effects of OSA and a synthetic antioxidant BHA, type of packaging and storage conditions on antioxidant activity of refined rapeseed oil. Antioxidant potential of the studied oil samples was determined by four modified spectrophotometric methods, namely, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and Folin-Ciocalteu (FC) reducing capacity. The obtained results on antioxidant activity were compared, discussed and used as descriptors for hierarchical cluster analysis (HCA) in order to differentiate rapeseed oils without and with antioxidants stored in various conditions.

MATERIALS AND METHODS

Reagents

Butylated hydroxyanisole (BHA, 99%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, 95%), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, $\geq 98\%$), Folin-Ciocalteu reagent (FC reagent, 2 mol·l⁻¹), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ, 99%), potassium persulfate, ethanol (96%), methanol ($\geq 99.9\%$), acetic acid (80%), iron(III) chloride hexahydrate, sodium acetate and sodium carbonate were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Re-distilled water was used for preparation of solutions.

Oil samples enriched with antioxidants

(Octyl(E)-3-(4-hydroxy-3,5-dimethoxyphenyl) propenoate (OSA) was synthesized for the first time in our research group, purified and identified according to procedures and analytical methods described in our previous report [19]. The refined rapeseed oil in the original packing (polypropylene bottles, volume 1 l) was kindly provided by a local vegetable oil factory in Kruszwica, Poland. Two antioxidants, namely, OSA and BHA were added to the refined rapeseed oil and placed in an ultrasonic bath SW 6H (Sonoswiss, Ramsen, Switzerland) for 5 min, to enhance solubility and to obtain products with final contents of 200 mg·kg⁻¹ OSA, 5000 mg·kg⁻¹ OSA and 200 mg·kg⁻¹ BHA.

Storage of oil samples

Oil samples (50 ml) supplemented with 200 mg·kg⁻¹ OSA, 5000 mg·kg⁻¹ OSA and

Tab. 1. Analytical parameters of the modified spectrophotometric methods used for antioxidant activity determination.

Analytical method	Calibration equation	R^2	Linear range [mmol·l ⁻¹]	LOD [mmol·l ⁻¹]	LOQ [mmol·l ⁻¹]
ABTS method	$y = (314.44 \pm 7.65)x + (10.11 \pm 0.62)$	0.9970	0.01–0.15	8.53×10^{-3}	2.84×10^{-2}
DPPH method	$y = (790.86 \pm 11.53)x - (0.31 \pm 0.78)$	0.9990	0.02–0.10	3.24×10^{-3}	1.08×10^{-2}
FRAP method	$y = (49.53 \pm 1.26)x + (0.037 \pm 0.013)$	0.9980	0.002–0.018	1.13×10^{-3}	3.77×10^{-3}
FC method	$y = (6.22 \pm 0.16)x + (0.044 \pm 0.011)$	0.9960	0.007–0.114	7.53×10^{-3}	2.51×10^{-2}

The equations of the calibration curves were obtained by least-squares linear regression method. The slope and intercept of the calibration curves represent mean \pm standard deviation ($n = 5$).

ABTS – 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, DPPH – 2,2-diphenyl-1-picrylhydrazyl, FRAP – ferric reducing antioxidant power, FC – Folin-Ciocalteu. R^2 – determination coefficient, LOD – limit of detection, LOQ – limit of quantification.

200 mg·kg⁻¹ BHA were transferred to Duran transparent glass bottles and to Duran amber glass bottles with blue polypropylene screw caps and drip-free sealing rings. The first series of oils in transparent glass bottles was transferred placed in a refrigerator at 4 °C, the second series in transparent glass containers was placed in a dark place and the last two series in transparent or amber glass bottles were kept on the lab shelf at ambient temperature with sunlight exposure to simulate supermarket storage conditions and household use over 28 days long period.

Determination of oil antioxidant activity

Methanolic extracts of rapeseed oils without and with synthetic antioxidants were obtained according to the procedure described previously [1, 20]. Briefly, the test tubes with oils (2.00 g) and methanol (5 ml) were shaken for 30 min using a shaker SHKA 2508-1CE (Labo Plus, Warsaw, Poland) at room temperature in the dark. The extracts were then separated from oils in a freezer (–20 °C, 30 min) and transferred quantitatively into glass bottles.

Four spectrophotometric methods using ABTS, DPPH, FRAP and FC developed by RE et al. [21], BRAND-WILLIAMS et al. [22], BENZIE and STRAIN [23] and SINGLETON and ROSSI [24], respectively, were applied with some modifications to determine antioxidant activity of rapeseed oils before and after supplementation with OSA or BHA and stored at various conditions.

The procedures of antioxidant activity determination by modified ABTS, DPPH, FRAP and FC methods were described in our previous article [20]. Antioxidant activity of oil samples analysed by using the ABTS method (AA_{ABTS}), DPPH method (AA_{DPPH}), FRAP method (AA_{FRAP}) and FC method (AA_{FC}) was expressed as millimoles of Trolox equivalents per kilogram of

sample. The UV–Vis spectra were recorded using a Hitachi U-2900 spectrophotometer (Hitachi, Tokyo, Japan) in a 1 cm quartz cell. Calibration curves for each method were constructed on the same day. The linear concentration ranges, calibration equations, regression coefficients, limit of detection (LOD) and limit of quantification (LOQ) for the standard antioxidant (Trolox solutions) using the modified analytical methods are summarized in Tab. 1.

Statistical analysis

Antioxidant activity of rapeseed oils without and with antioxidants stored in various conditions was determined, five portions of each oil extract being analysed within 1 day, by the ABTS, DPPH, FRAP and FC methods, respectively. The obtained results were presented as mean \pm standard deviation. One-way analysis of variance (ANOVA), followed by Duncan's test, was performed to analyse the significance of differences between data ($p < 0.05$).

Hierarchical cluster analysis (HCA) was used for detection of differences and grouping of the investigated rapeseed oils stored in various types of packages and subjected to variable storage conditions during four weeks. Statistical analyses of data were carried out using the Statistica 8.0 software (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Antioxidant activity of supplemented rapeseed oil samples before and after storage

The effects of addition of OSA at two levels (200 mg·kg⁻¹ and 5000 mg·kg⁻¹) and BHA (200 mg·kg⁻¹) on antioxidant activity of rapeseed oils stored in various types of packages and subjected to various storage conditions in order to

simulate household use and storage on supermarket shelves were analysed by the ABTS, DPPH, FRAP and FC methods and the results are presented in Tab. 2.

It can be noted that OSA addition helped to improve antioxidant activity, which increased with increasing its content in the refined rapeseed oil. The values of antioxidant activity determined by ABTS method ($AA_{ABTS} = 72.51\text{--}128.55\text{ mmol}\cdot\text{kg}^{-1}$), DPPH method ($AA_{DPPH} = 16.50\text{--}24.82\text{ mmol}\cdot\text{kg}^{-1}$), FRAP method ($AA_{FRAP} = 6.21\text{--}11.00\text{ mmol}\cdot\text{kg}^{-1}$) and FC method ($AA_{FC} = 15.34\text{--}21.12\text{ mmol}\cdot\text{kg}^{-1}$) were the highest for rapeseed oils with OSA at a level of 5 000 mg·kg⁻¹.

The lowest values ($AA_{ABTS} = 8.20\text{ mmol}\cdot\text{kg}^{-1}$, $AA_{DPPH} = 2.75\text{ mmol}\cdot\text{kg}^{-1}$, $AA_{FRAP} = 0.23\text{ mmol}\cdot\text{kg}^{-1}$ and $AA_{FC} = 0.98\text{ mmol}\cdot\text{kg}^{-1}$) were determined for the control oil without antioxidants in transparent glass containers exposed to light and stored at room temperature during 28 days. Our data suggest that supplementation of oil with OSA created an effective defence system against free-radical attack. The enhancement of antioxidant activity along with an increase in contents of OSA or BHA added to rapeseed oil can be explained by the synergism of naturally present antioxidants and other components of oil with the synthetic antioxidants.

Tab. 2. Antioxidant activity of refined rapeseed oils without and with antioxidants before and after 28 days of storage.

No	Oil samples	Antioxidant activity [mmol·kg ⁻¹]			
		ABTS method	DPPH method	FRAP method	FC method
Rapeseed oil					
RO1	Control	10.75 ± 0.44 ^b	4.18 ± 0.21 ^c	1.18 ± 0.05 ^d	1.48 ± 0.08 ^b
RO2	TGR	9.87 ± 0.36 ^a	3.24 ± 0.08 ^{ab}	0.88 ± 0.03 ^{bc}	1.39 ± 0.06 ^{ab}
RO3	TGD	10.63 ± 0.43 ^{ab}	3.17 ± 0.09 ^{ab}	0.94 ± 0.03 ^c	1.18 ± 0.07 ^a
RO4	TGL	8.20 ± 0.31 ^a	2.75 ± 0.10 ^a	0.23 ± 0.01 ^a	0.98 ± 0.04 ^a
RO5	AGL	9.03 ± 0.72 ^a	2.88 ± 0.13 ^a	0.70 ± 0.01 ^b	1.17 ± 0.07 ^a
Rapeseed oil + BHA (200 mg·kg ⁻¹)					
RO6	Control	30.68 ± 0.72 ^g	7.50 ± 0.06 ^f	3.92 ± 0.25 ^h	3.16 ± 0.18 ^e
RO7	TGR	24.41 ± 0.81 ^f	5.44 ± 0.17 ^e	2.16 ± 0.08 ^g	2.64 ± 0.11 ^d
RO8	TGD	25.31 ± 0.91 ^f	4.51 ± 0.23 ^c	2.44 ± 0.04 ^g	2.66 ± 0.09 ^d
RO9	TGL	23.10 ± 0.80 ^f	4.44 ± 0.17 ^c	1.39 ± 0.02 ^e	2.27 ± 0.12 ^d
RO10	AGL	24.60 ± 0.70 ^f	4.99 ± 0.24 ^d	1.82 ± 0.02 ^f	2.60 ± 0.09 ^d
Rapeseed oil + OSA (200 mg·kg ⁻¹)					
RO11	Control	19.38 ± 0.13 ^e	5.62 ± 0.13 ^e	3.17 ± 0.08 ^h	3.04 ± 0.10 ^e
RO12	TGR	15.71 ± 0.51 ^d	4.32 ± 0.11 ^c	1.53 ± 0.05 ^f	2.21 ± 0.09 ^d
RO13	TGD	11.82 ± 0.31 ^c	4.15 ± 0.11 ^c	1.66 ± 0.02 ^f	1.83 ± 0.08 ^c
RO14	TGL	11.45 ± 0.28 ^c	3.24 ± 0.09 ^{ab}	0.98 ± 0.01 ^c	1.63 ± 0.03 ^b
RO15	AGL	12.68 ± 0.35 ^c	3.63 ± 0.10 ^b	1.31 ± 0.01 ^e	2.31 ± 0.11 ^d
Rapeseed oil + OSA (5000 mg·kg ⁻¹)					
RO16	Control	128.55 ± 6.54 ^l	24.82 ± 0.15 ^k	11.00 ± 0.49 ^k	21.12 ± 0.95 ⁱ
RO17	TGR	114.91 ± 6.00 ^k	20.27 ± 0.59 ⁱ	9.36 ± 0.31 ^j	17.89 ± 0.88 ^g
RO18	TGD	92.54 ± 5.08 ⁱ	22.50 ± 0.13 ^j	9.08 ± 0.41 ^j	18.92 ± 0.67 ^h
RO19	TGL	72.51 ± 3.71 ^h	16.50 ± 0.84 ^g	6.21 ± 0.25 ⁱ	15.34 ± 0.25 ^f
RO20	AGL	100.42 ± 3.33 ^j	18.45 ± 0.32 ^h	8.86 ± 0.32 ^j	17.63 ± 0.24 ^g

Antioxidant activity is expressed in millimoles of Trolox equivalents per kilogram of sample, values represent mean \pm standard deviation ($n = 5$). Different letters within the same column indicate significant differences (one-way ANOVA and Duncan's test, $p < 0.05$) between antioxidant activity of the studied oils packaged in various containers and stored under various conditions. ABTS – 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, DPPH – 2,2-diphenyl-1-picrylhydrazyl, FRAP – ferric reducing antioxidant power, FC – Folin-Ciocalteu, RO – rapeseed oil, BHA – butylated hydroxyanisole, OSA – octyl sinapate, TGR – transparent glass bottles at refrigeration, TGD – transparent glass bottles in the dark at room temperature, TGL – transparent glass bottles exposed to light, AGL – amber glass bottles exposed to light.

The antioxidant activity results indicated that OSA at a higher content ($5000 \text{ mg}\cdot\text{kg}^{-1}$) induced approximately a 6-, 4-, 4-, 7-fold increase in AA_{ABTS} , AA_{DPPH} , AA_{FRAP} and AA_{FC} compared to oil containing $200 \text{ mg}\cdot\text{kg}^{-1}$ of OSA before storage (Tab. 2). Significant differences in antioxidant activity of rapeseed oils supplemented with OSA and BHA at $200 \text{ mg}\cdot\text{kg}^{-1}$ were observed.

Oils with OSA stored under various conditions had a slightly weaker antioxidant activity ($AA_{ABTS} = 11.45\text{--}19.38 \text{ mmol}\cdot\text{kg}^{-1}$, $AA_{DPPH} = 3.24\text{--}5.62 \text{ mmol}\cdot\text{kg}^{-1}$) than oils containing BHA ($AA_{ABTS} = 23.10\text{--}30.68 \text{ mmol}\cdot\text{kg}^{-1}$, $AA_{DPPH} = 4.44\text{--}7.50 \text{ mmol}\cdot\text{kg}^{-1}$). These differences in antioxidant activity values may be due to the addition of synthetic antioxidants to refined rapeseed oil at various contents, differences in types of packaging, storage conditions and the mechanism of the applied analytical methods. It is established that the ABTS assay allows determination of hydrophilic and hydrophobic antioxidants, whereas only hydrophobic antioxidants can be analysed by DPPH method. Lower values of AA_{FRAP} and AA_{FC} in comparison with AA_{ABTS} and AA_{DPPH} can be explained by the fact that FRAP and FC assays are taken as inapplicable to lipophilic antioxidants [25].

Antioxidant activity of refined rapeseed oil

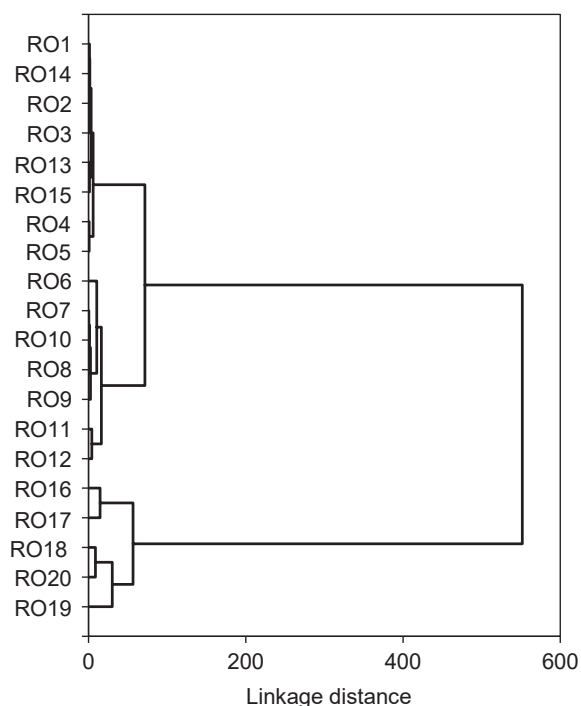


Fig. 1. Hierarchical cluster analysis of studied rapeseed oils.

Designation of samples (RO01–RO20) is explained in Tab. 2.

without and with antioxidants changed significantly during storage under various conditions during 28 days (Tab. 2). As can be seen, the highest decrease in antioxidant activity was observed for oil samples in transparent bottles exposed to light. However, oil samples packaged in amber glass bottles preserved the antioxidant activity for 28 days better than those packaged in transparent glass bottles. Furthermore, rapeseed oils before and after supplementation stored in transparent bottles in the complete absence of light (dark place and refrigerator) had higher antioxidant activity than those that were exposed to light during 28 days (Tab. 2). This suggests that the natural pigment (chlorophyll) in rapeseed oil kept in dark place acted synergistically with naturally present antioxidants and added synthetic antioxidants to inhibit autooxidation. An increase in temperature (dark place) had no significant effect on antioxidant activity of the studied oils. No significant differences were determined between antioxidant activity values of oils stored in dark place and refrigerator (except AA_{ABTS} and AA_{FC} for rapeseed oil with OSA and AA_{DPPH} for rapeseed oil with BHA; Tab. 2).

Loss of antioxidants during storage of oils is probably related to their decomposition and oxidation, which causes decrease of quality of oils. Therefore OSA, in particular at a higher content, effectively protected the refined rapeseed oil from unfavorable reactions and enhanced its antioxidant activity due to presence of hydrogen-donating or electron-donating substituents in the aromatic ring, which are able to reduce free radicals. Antioxidant effect of phenolic acids esters strongly depends on the chemical structure, mainly on the number and arrangement of hydroxyl and methoxyl groups [26].

For comparison, walnut oils with natural ($800 \text{ mg}\cdot\text{kg}^{-1}$ of rosemary extract) and synthetic ($100 \text{ mg}\cdot\text{kg}^{-1}$ and $200 \text{ mg}\cdot\text{kg}^{-1}$ of ascorbyl palmitate and TBHQ) antioxidants stored in darkness had higher AA_{DPPH} values than oils stored exposed to light [27]. Ascorbyl palmitate and the rosemary extract added to walnut oil at various amounts produced insignificant effects on antioxidant activity of oils analysed by DPPH method, while a strong impact of TBHQ content on antioxidant potential of fortified oils was observed by these authors.

Moreover, WRONIAK and REKAS [13] found the highest rates of tocopherols and sterols degradation in the cold-pressed rapeseed oils exposed to oxygen and stored at room temperature, whereas the lowest loss of bioactive compounds was determined in oils kept closed and stored at 4°C in a refrigerator.

Hierarchical cluster analysis

HCA was applied to group the studied rapeseed oils without and with antioxidants stored in various conditions based on similarities in their antioxidant activity determined by the modified ABTS, DPPH, FRAP and FC methods. The obtained results were presented as a dendrogram in Fig. 1. It is noteworthy that the twenty oil samples were classified into two main clusters (Fig. 1). The dendrogram depicted a clear separation of oils containing 5000 mg·kg⁻¹ of OSA, with the highest antioxidant activity, from all other oil samples (Tab. 2). Five rapeseed oils with 200 mg·kg⁻¹ of BHA and two oils with 200 mg·kg⁻¹ of OSA, immediately after the addition of antioxidant and after 28 days of storage at 4 °C in a refrigerator had approximately 4 times weaker antioxidant activity and arranged in one group. These two oils with OSA having a lower antioxidant activity values created an inter-cluster (Fig. 1). The second group included all refined rapeseed oils without antioxidants and with 200 mg·kg⁻¹ of OSA, exposed to light and kept in dark at room temperature, because these oils had low *AA*_{ABTS}, *AA*_{DPPH}, *AA*_{FRAP} and *AA*_{FC}. As can be seen, antioxidant properties of the studied rapeseed oils were different from each other. The dendrogram revealed that the twenty oil samples were grouped depending on the presence of synthetic antioxidants, their contents, type of packing and storage conditions, which could affect the total level of antioxidants in oils.

CONCLUSIONS

The effectiveness of OSA on antioxidant activity of refined rapeseed oil packed in various containers (transparent and amber glass bottles) and stored under various conditions (at 4 °C in a refrigerator, in the dark and exposed to light) commonly used in household or retail markets was evaluated. The used ABTS, DPPH, FRAP and FC methods are relatively simple, precise and convenient for the determination of antioxidant properties of the investigated rapeseed oils. The addition of the amphiphilic antioxidant improved storage stability of the refined rapeseed oil in terms of its antioxidant activity. Moreover, antioxidant activity of rapeseed oil increased with increasing the OSA content, as significantly higher *AA*_{ABTS}, *AA*_{DPPH}, *AA*_{FRAP} and *AA*_{FC} values were determined for oils supplemented with 5000 mg·kg⁻¹ of OSA than for those supplemented with this antioxidant at 200 mg·kg⁻¹. However, the common synthetic antioxidant BHA at 200 mg·kg⁻¹ stabilized the rape-

seed oil to a greater extent than OSA at the same content (200 mg·kg⁻¹). On the other hand, the refined rapeseed oil without and with antioxidants stored in darkness at room temperature presented a similar rate of antioxidant activity decrease to that stored at 4 °C in a refrigerator. Amber glass bottles were found to be more suitable containers than transparent glass bottles for packaging refined rapeseed oils.

Two main groups were identified by HCA, rapeseed oils with the higher OSA content exhibiting higher antioxidant activity values regardless of the storage conditions and the type of packaging. The synthesized amphiphilic antioxidant OSA can be used as an efficient supplement of oils to delay oxidation reactions, to enhance antioxidant activity of oils and to prolong their shelf life.

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