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Food Chemistry 93 (2005) 227–235
Food Chemistry
www.elsevier.com/locate/foodchem

Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests

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Received 1 December 2003; received in revised form 22 September 2004; accepted 22 September 2004

Abstract

The effect of rosemary extract and tocopherols (-T) on the oxidative stability of rapeseed oil triacylglycerols (TAG) was evaluated by two instrumental tests: Rancimat® – measuring the generated carboxylic oxidation products and Oxidograph® – the measurement of oxygen absorption rate. Induction time and potential of the antioxidants to quench 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were used as major indicators of antioxidative potential of antioxidants used. The rosemary extract was a more effective antioxidant in the rapeseed oil TAG than α-, γ-, δ-tocopherols alone, and more effective than a homologous mixture composed of α-, γ-, δ-tocopherol, and butylated hydroxytoluene. Capacity to quench of DPPH radicals was measured before oxidation tests and when the end of stability was established. After the accelerated oxidation of systems consisting of TAG and antioxidants in Oxidograph and Rancimat, the ability to quench of the DPPH radical remained even after the end of induction time. The same systems oxidized in Rancimat possessed higher activity of quenching DPPH radicals than fresh prepared. This trend was observed in all samples with exception of the one to which α-T was added.

1. Introduction

Lipids undergo oxidative degradation during processing and storage, resulting in an alteration of major quality-control parameters such as colour, flavour, aroma, and nutritive value, affecting suitability for consumption. The majority of these changes are initiated by oxygen reactive species, which lead to the formation of various primary and secondary products (Andersson & Lingnert, 1999; Wagner & Elmadfa, 2001). Therefore, assessing the extent of oxidative degradation of fats, oils, and foods containing lipids is essential to the food industry (Addis, 1986).

Plant oils containing high levels of polyunsaturated fatty acids are susceptible to oxidation (Huang, Frankel, & German, 1995; Lampi & Kamal-Eldin, 1998; Lampi, Kataja, Kamal-Eldin, & Piironen, 1999; Lampi & Piironen, 1998). In addition, several compounds with antioxidant properties are either removed or changed during the refining process. Therefore, numerous studies have attempted to explain the mechanism of oxidation of lipids in oils as well as antioxidant activity of various natural and synthetic compounds (Frankel, 1993, 1996). Recently, the use of natural antioxidants in the food industry has increased rapidly and consequently many related studies have been reported (Beddows, Jagait, &

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doi:10.1016/j.foodchem.2004.09.021

Kelly, 2001; Korczak, Janitz, Hes, Nogala-Kalucka, & Gogolewski, 1999). Many herbs and spices are known to exhibit antioxidant activity in food lipids (Loeliger, 1991; Pokorny & Korczak, 2001). The majority of published work in the area of natural antioxidants has focused on tocopherols (Wagner & Elmadfa, 1998; Yanishlieva & Marinowa, 1998). As well, commercially available rosemary extracts exhibit potent antioxidant activity (Cuvelier, Richard, & Berset, 1996), and are widely used in the food industry. Many compounds have been isolated from rosemary, including flavones, diterpenes, steroids, and triterpenes. Of these, the antioxidant activity of rosemary extracts has been primarily related to two phenolic diterpenes: carnosic acid and carnosol (Chen, Shi, & Ho, 1992; Frankel, Huang, Aeschbach, & Prior, 1996; Schwarz & Ternes, 1992a, 1992b; Schwarz, Ternes, & Schmauderer, 1992).

In Poland, refined or cold pressed rapeseed oil is being frequently used in food production, cooking, and direct consumption (Krygier, 1997). Rapeseed oil consists mainly (95%) of triacylglycerols (TAG). Non-triacylglycerols, also known as minor components or unsaponifiable matter, make up the remaining 5%. They are primarily composed of phospholipids, tocopherols, tocotrienols, flavonoids, other phenolic compounds, pigments (carotenoids, chlorophylls), sterols, free fatty acids, and mono- and diacylglycerols (Shahidi & Shukla, 1996). Several classes of these components might be present in various oils and contribute to its oxidative stability (Hamilton, 1994; Shahidi, Amarowicz, Abou-Gharbia, & Shehata, 1997). Therefore, they must be removed when the oil is used for testing the effectiveness of natural and synthetic antioxidants. Oils and fats stripped of those components are named triacylglycerols (TAG). For example, TAG of edible oils have been used in to investigate the effects of tocopherols on autoxidation (Jung & Min, 1990).

Several chemical, instrumental, and sensory techniques are commonly used to monitor oxidation of foods and to predict their shelf life stability and to evaluate effectiveness of antioxidants in different lipid systems (Gordon, 2001). While sensory methods are considered a gold standard in predicting the stability of lipids, they are cumbersome and not practical for routine analyses (Malcolmson, Vaisey-Jenser, Przybylski, & Eskin, 1994). Recently, a number of accelerated oxidation tests have been applied to examine the oxidative stability of edible oils and predict their shelf life. In these tests, lipid samples were placed in a forced-air heating to accelerate their oxidation (Frankel, 1993; Lampi, Piironen, Hopia, & Koivistoinen, 1997; Wan, 1995). For example, it has been shown that 1-h storage of rapeseed oil, at 100 °C in the Rancimat, was equivalent to 2-day storage at 20 °C (Gordon & Mursi, 1994). Maszewska (Maszewska, 2002) found that 1-h of storage of oils in Rancimat at 120 °C was equal to 5-months storage at 12 °C. For screening of natural substances for their antioxidant activity tests based on the examination of their antiradical potency are often used. The evaluation of the potency to quench of DPPH radical is an example (Auroma, 2003).

The goal of this study was to assess the antioxidant effectiveness of the rosemary extract, the tocopherol homologues and their mixture added to triacylglycerols (TAG) isolated from the rapeseed oil using Rancimat and Oxidograph instruments. Our secondary goal was to examine the antioxidative potential of tested antioxidants immediately after preparation and after determining their autooxidative stability.

2. Materials and methods

2.1. Materials

Low-erucic acid rapeseed oil was purchased from local from Plant Oil Refinery (Szamotuly, Poland). Rosemary extract was prepared from finely ground leaves of rosemary (*Rosmarinus officinalis* L.). Standards of the homologous α-tocopherol, γ-tocopherol, δ-tocopherol (99.8%) were purchased from Merck (Germany), and their mixture was purchased from Eisai (Japan). Homologous tocopherols mixture (mix-T) consisted of α-tocopherol (9.78 mg/100 mg), γ-tocopherol (20.7 mg/100 mg), and δ-tocopherol (24.3 mg/100 mg). Butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl, activated charcoal, and aluminium oxide (Al_2O_3) were also obtained from Merck (Germany). All other chemicals were ACS-grade or better.

2.2. Methods

2.2.1. Preparation of triacylglycerols

Triacylglycerols (TAG) of rapeseed oil were prepared using a column chromatographic method (Marinowa & Yanishlieva, 1992). A chromatographic column (2.5 cm i.d. × 20 cm) was connected to a vacuum water pump and packed sequentially with two adsorbent layers. The bottom layer consisted of 1 cm of anhydrous Na_2S0_4 and 15 cm Al_2O_3 activated for 3 h at 300 °C. The top layer was 3 cm of activated charcoal. All adsorbents were suspended in *n*-hexane. A 25% *n*-hexane solution of rapeseed oil was passed through the chromatographic column. The solvent in the eluent (TAG) was evaporated under vacuum at 40 °C.

2.2.2. Preparation of samples for accelerated oxidation tests

The rapeseed oil TAG samples (5 g) were prepared directly before the study of their oxidative stability.

2.2.2.1. Preparation of rosemary extract. The rosemary extract was obtained from finely ground rosemary leaves

by triple maceration with ethanol (96%) for 24 h (Pokorny, Nguyen, & Korczak, 1997). After extraction, the solvent was evaporated in the vacuum evaporator and the extract dried, ground, and stored (at 4 °C). Rosemary extract was added to the lipid sample at 500 ppm.

2.2.2.2. Preparation of tocopherols and BHT samples. Homologous tocopherols (alpha-T, α-T or gamma-T, γ-T and delta-T, δ-T), their mixture (mix-T) and BHT (butylated hydroxytoluene) were added at 100 ppm to the rapeseed oil TAG. Tocopherols and BHT were dissolved in small amount methanol and added to a lipid model system. After mixing, methanol was evaporated under vacuum at 30 °C.

2.2.3. Accelerated oxidation tests

2.2.3.1. Auto-oxidation stability in Oxidograph. Stability of the samples was measured using an Oxidograph® instrument (Mikrolab, Denmark) (Larsen, 1989). Briefly, 5 g lipid samples were placed in reaction vessels, flushed with the oxygen (30 s), tightly closed, heated to 110 °C on a heating block and stirred with a magnetic stirrer. Pressure inside the reaction vessels was measured continuously. The results are expressed as induction time (h) after which the pressure rapidly dropped due to absorption of the oxygen by the lipids undergoing oxidation.

2.2.3.2. Auto-oxidation resistance in Rancimat. The resistance to auto-oxidation was measured using the Rancimat 679® (Metrohm AG, Herisau, Switzerland) instrument at 110 °C with the airflow rate of 20 L/h (Läubli & Bruttel, 1986; Platek, 1995). The oxidative stability was expressed as induction time (h). The antioxidant activity of added antioxidants in both tests was expressed by calculating a protection factor (PF), the ratio of induction time of the sample with and without antioxidant added.

2.2.4. Scavenging effect on (1,1-diphenyl-2-picrylhydrazyl) DPPH radical

The capacity of antioxidants to quench DPPH radical in rapeseed oil TAG was determined before and after accelerated oxidation tests (Bondet, Brand-Williams, & Berset, 1997; Espin, Soler-Rivas, & Wichers, 2000; Yoshida et al., 1989). Ethyl acetate was used as a better solvent of hydrophobic compounds. To 0.2 ml of the sample ethyl acetate was added to obtain 4 ml of the mixture and 1 ml of DPPH (6.09×10^{-5} mol/L) solution in ethyl acetate were added (total volume, 5 ml). After 10 min. from addition of reagents the colour was stable, and the absorbance was measured at wavelength $\lambda = 520$ nm. The reference sample used was:1 ml of DPPH solution and 4 ml ethyl acetate. The effect of antioxidants on DPPH radical-quenching was expressed as the absorbance. A lower value of absorbance reflects stronger ability of an antioxidant to quench radicals.

2.3. Statistical analysis

Results are presented as means ± standard deviation from three replicates of each experiment. A P-value < 0.05 was used to denote significance differences among mean values determined by analysis of variance (ANOVA).

3. Results and discussion

3.1. General

The most important finding of this study is that rosemary extract inhibits oxidation of rapeseed oil TGA more than tocopherols and BHT. Moreover, the mechanism by which rosemary extract inhibits lipid oxidation might be different from tocopherols.

3.2. Effect of rosemary extract and tocopherols on rapeseed oil TAG stability.

Induction times of rapeseed oil TAG using accelerated oxidation tests are presented in Table 1. Endogenous antioxidants were removed from the rapeseed oil during isolation of TAG. The stability of TAG did not exceed 1 h in either test whereas induction time of the

Table 1
Stability of rapeseed oil triacylglycerols systems with antioxidants estimated by Oxidograph and Rancimat tests (induction time in h)

Antioxidant	Concentration (ppm)	Oxidograph (h)	Rancimat (h)
TAG (control)[A]	0	0.43 ± 0.03 (a)[B]	0.84 ± 0.04 (a)
TAG + α-T	100	2.60 ± 0.05 (c,d)	4.50 ± 0.11 (c,d)
TAG + γ-T	100	2.87 ± 0.13 (d,e)	6.44 ± 0.07 (e)
TAG + δ-T	100	1.78 ± 0.26 (b)	4.29 ± 0.12 (c)
TAG + mix-T	100	1.92 ± 0.08 (b,c)	3.48 ± 0.06 (b)
TAG + rosemary	500	3.90 ± 0.40 (e)	7.61 ± 0.45 (f)
TAG + BHT	100	3.80 ± 0.15 (e)	4.83 ± 0.05 (d)

[A] TAG, rapeseed oil triacylglycerols; α-T, alpha-tocopherol; γ-T, gamma-tocopherol; δ-T, delta-tocopherol; mix-T, tocopherol mixture; BHT, butylated hydroxytoluene.

[B] Values are means of three determinations ± SD. Values within each column with different letters are different ($P < 0.05$).

refined rapeseed oil before elimination of the anioxidants was considerably longer (4.4 h in Oxidograph and 10.4 in Rancimat (Hes, Korczak, Nogala-Kalucka, Jedrusek-Golinska, & Gramza, 2001)).The activity of tocopherols was evaluated at the concentration of 100 ppm whereas the rosemary extract was added at the level 500 ppm (Korczak et al., 1999). The most effective antioxidant in stabilizing rapeseed oil TAG in the Oxidograph test was rosemary extract (3.9 ± 0.4 h) followed by BHT (3.8 ± 0.15 h). Both, rosemary extract and BHT were significantly (all $P < 0.05$) more effective than tocopherols tested. Among tocopherols, γ- and α-tocopherol increased induction time significantly more than δ-tocopherol (2.87 and 2.60 h vs. 1.78 h, respectively) or the tocopherol homologues mixture (1.92 h).

The effect of antioxidants on the resistance of rapeseed oil TAG to oxidation measured in the Rancimat showed a similar pattern. Rosemary extract was significantly more effective (7.61 h) than BHT (4.83 h) or tocopherols ($P < 0.05$), and γ-tocopherol (6.44) was more effective than BHT, α-tocopherol (4.50 h), δ-tocopherol (4.29 h), and tocopherol homologue mixture (3.48 h).

Relative antioxidative efficiencies calculated as a protection factor (PF) of added antioxidants are presented in Table 2. In the tests, the antioxidant activity of natural antioxidants in TAG isolated from the rapeseed oil, expressed as PF, was much higher than in the refined oil (Korczak et al., 1999).

There were no significant differences (all $P > 0.05$) between PFs calculated from the Oxidograph and Rancimat, except for BHT (8.83 vs. 5.75). This difference could be explained by higher volatility of BHT at high temperature (Frankel, 1993) combined with a constant air flow in Rancimat than BHT volatility in a hermetically closed Oxidograph. As expected from the induction and resistance times (Tables 1 and 2), the rosemary extract has a significantly higher PF value than tocopherols and BHT.

The ethanol rosemary extract contains approximately 10–20% active antioxidants (Nakatani & Inatani, 1984).

Table 2
Antioxidant activity of tocopherols and rosemary extract in rapeseed oil triacylglycerols systems estimated by Oxidograph and Rancimat tests (protection factor – PF)

Antioxidant	Protection factor	
	By Oxidograph	By Rancimat
α-T[A]	6.05 ± 0.12 (b,c)[B]	5.35 ± 0.13 (b,c)
γ-T	6.67 ± 1.12 (c,d)	7.67 ± 0.08 (d)
δ-T	4.13 ± 0.60 (a)	5.10 ± 0.14 (b)
Mix-T	4.46 ± 0.18 (a,b)	4.15 ± 0.07 (a)
Rosemary	9.07 ± 0.90 (d)	9.06 ± 0.53 (e)
BHT	8.83 ± 0.35 (d)	5.75 ± 0.06 (c)

[A] Abbreviations see Table 1.

[B] Values are means of three determinations ± SD. Values within each column with different letters are different ($P < 0.05$).

In an early study of 16 compounds isolated from rosemary, including flavones, diterpenes, steroids, and triterpenes, concluded that the antioxidant activity of rosemary extracts is primarily related to two phenolic diterpenes: carnosic acid and carnosol. This was confirmed by others (Chen et al., 1992; Frankel et al., 1996; Schwarz & Ternes, 1992a). It was also found that antioxidant activity of rosemary extracts is related to the presence of other phenolic diterpenes such as methyl carnosate, rosmanol, isorosmanol, epirosmanol and 7-methylepirosmanol (Cuvelier et al., 1996; Schwarz & Ternes, 1992b), rosmaridiphenol (Houlihan, Ho, & Chang, 1984), rosmariquinone (Houlihan, Ho, & Chang, 1985), and phenolic acids such as rosmarinic acid (Frankel et al., 1996). In fresh rosemary leaves carnosic acid is the major phenolic diterpene. During the extraction of rosemary extracts carnosic acid is partially converted either into carnosol or into other diterpenes, which can degrade further to produce other phenolic diterpenes with γ- and δ-lactone structure (Geoffroy, Lambelet, & Richert, 1994; Schwarz & Ternes, 1992a) or compounds of unknown structure (Hopia, Huang, Schwarz, German, & Frankel, 1996). These compounds are more lipophilic than carnosic acid and act as antioxidants (Chen et al., 1992; Hopia et al., 1996; Nakatani & Inatani, 1984), but their antioxidant activities are lower than those of carnosic acid and methyl carnosate (Cuvelier, Berset, & Richard, 1994). Antioxidative activity of these degradation products is relatively high in temperatures similar to frying temperature (Loeliger, Lambelet, Aeschbach, & Prior, 1996; Pokorny & Korczak, 2001). When compared to other antioxidants, rosemary diterpenes were reported to have higher antioxidant activity than many commonly used phenolic antioxidants. For example, under the conditions of the AOM test, rosmanol and carnosol demonstrated markedly greater activity than α-tocopherol (Nakatani & Inatani, 1984).

PF values were significantly higher for γ- and α-tocopherol than for δ-tocopherol and the tocopherol mixture, the latter two did not differ from one another. This would suggest that γ- and α-tocopherol were more efficient than δ-tocopherol and tocopherol mixture in preventing rapeseed oil TAG oxidation.

Tocopherols are considered to be primary or chain-breaking antioxidants in free radical chain reactions, converting lipid radicals to more stable products, thus extending the shelf life of edible oils (Gordon, 1990). The antioxidant activity of tocopherols is dependent on concentration, temperature, light, type of substrate and solvent, as well as the presence of synergists and other chemical species that may act as pro-oxidants (Kamal-Eldin & Appelqvist, 1996). The inhibition of peroxides' production and the efficiency of homologous tocopherols depend on their concentration and the lipid system being tested (Nogala-Kalucka, Wagner, & Elmadfa, 1998; Wagner & Elmadfa, 2001). Gordon and

Table 3
The effect of quenching the DPPH radicals by model rapeseed oil triacylglycerols systems with antioxidants before and after oxidation in Oxidograph and Rancimat tests (extinction value at 520 nm)

System	Oxidograph		Rancimat	
	Before oxidation [A]	After oxidation [A]	Before oxidation [A]	After oxidation [A]
TAG[A]	0.565 ± 0.014 (e)[B]	0.594 ± 0.004 (a)	0.553 ± 0.021 (e)	0.489 ± 0.021 (c)
TAG + α-T	0.407 ± 0.026 (a)	0.549 ± 0.030 (a)	0.368 ± 0.020 (a)	0.393 ± 0.021 (a)
TAG + γ-T	0.446 ± 0.005 (a,b)	0.544 ± 0.022 (a)	0.414 ± 0.016 (b)	0.367 ± 0.016 (a)
TAG + δ-T	0.525 ± 0.010 (d,e)	0.572 ± 0.029 (a)	0.504 ± 0.009 (d)	0.408 ± 0.009 (a,b)
TAG + mix-T	0.473 ± 0.003 (b,c)	0.564 ± 0.023 (a)	0.466 ± 0.003 (c,d)	0.443 ± 0.003 (b)
TAG + rosemary	0.485 ± 0.020 (b,c,d)	0.535 ± 0.033 (a)	0.460 ± 0.015 (c)	0.366 ± 0.015 (a)
TAG + BHT	0.506 ± 0.019 (c,d)	0.549 ± 0.024 (a)	0.482 ± 0.016 (c,d)	0.377 ± 0.017 (a)

[A] Abbreviations see Table 1.
[B] Values are means of three determinations ± SD. Values within each column with different letters are different ($P < 0.05$).

Kourimska (1995) have examined the effects of different antioxidants on the oxidative stability of rapeseed oil during heating and the order of antioxidant activity was *t*-butylhydroquinone > lecithin > ascorbyl palmitate > rosemary extract > BHT, BHA and δ-tocopherol.

In the present study, we also found that the PF values for the mixture of homologous tocopherols did not differ between Oxidograph and Rancimat, despite significant differences between induction periods in both methods (Table 2). In the case of Oxidograph, induction

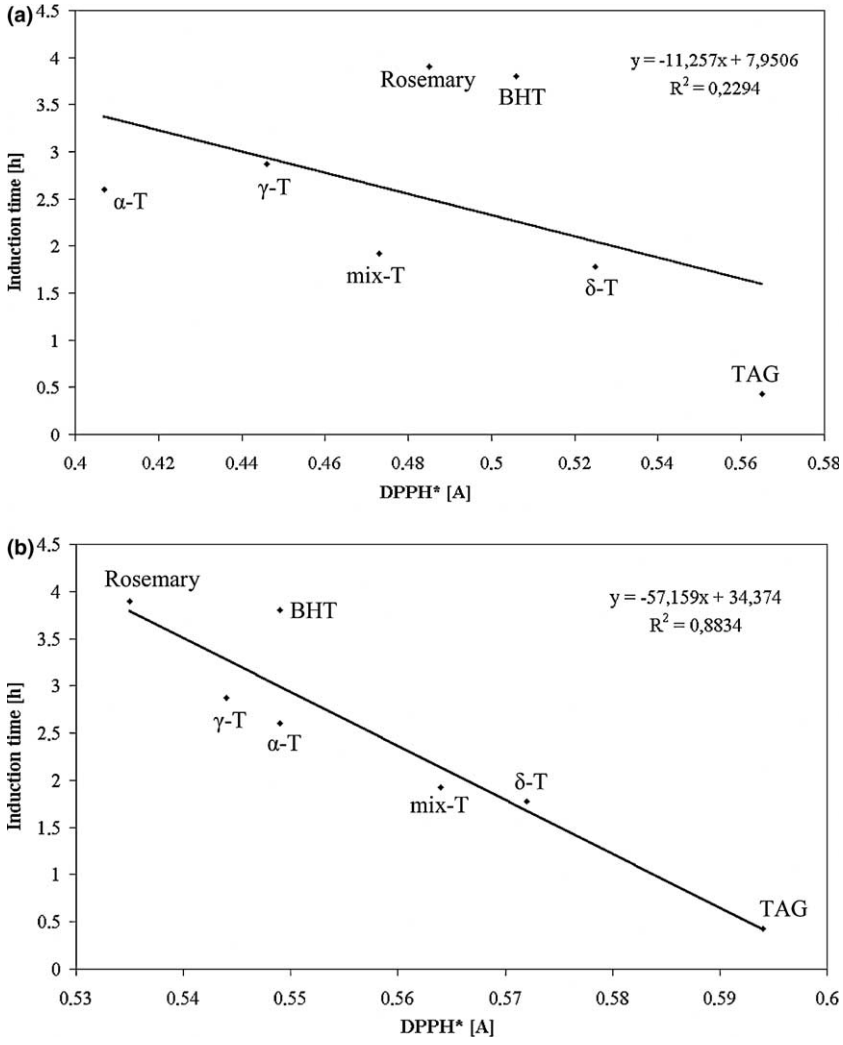

Fig. 1. Correlation between stability of rapeseed oil TAG with antioxidants estimated by Oxidograph test and their effectiveness of quenching the DPPH radicals in model systems before oxidation (a) and after oxidation stability test (b). *Abbreviations see Table 1.

periods for all samples were shorter than for the same samples tested on the Rancimat. Only BHT displayed lower activity in the Rancimat than in the Oxidograph. We reported previously that in the rapeseed oil TAG with the addition of antioxidants used in the present study had greater stability than refined rapeseed oil with antioxidants added in the same amounts (Hes et al., 2001). A plausible explanation is that refined rapeseed oil contained the optimal amount of endogenous antioxidants, and introducing additional quantities, even differing in nature, will not result in increasing the stabilizing effect (Loeliger et al., 1996).

3.3. Evaluation of the DPPH radical scavenging activity

Antioxidant potential was assessed by the antioxidants' capacity to quench the free DPPH radicals (Table 3). The process of inhibiting the autoxidation of lipids by antioxidants is related to the antioxidants' ability to break the radical formation reaction. Therefore, antioxidant potential could also be estimated in systems in which radicals are generated chemically (Lambelet, Ducret, Saucy, Savoy, & Loeliger, 1987; Lambelet, Saucy, & Loeliger, 1998; Loeliger et al., 1996). In the present study, potential quenching of the DPPH radicals by antioxidants and rapeseed oil TAG was measured before oxidation and after completing the accelerated tests. In the Rancimat, this potential was determined at the moment of rapid increase of the carboxylic volatile products of oxidation changing conductivity when dissolved in water, and in the Oxidograph at the moment of rapid pressure drop. Before conducting accelerated tests, the ability to quench free DPPH radicals was in the following order: α-tocopherol > γ-tocopherol > rosemary extract = tocopherol mixture > δ-tocopherol = BHT. After the accelerated oxidation in the Oxidograph, the DPPH radical quenching potential, although still significant, decreased by 8% for system with BHT

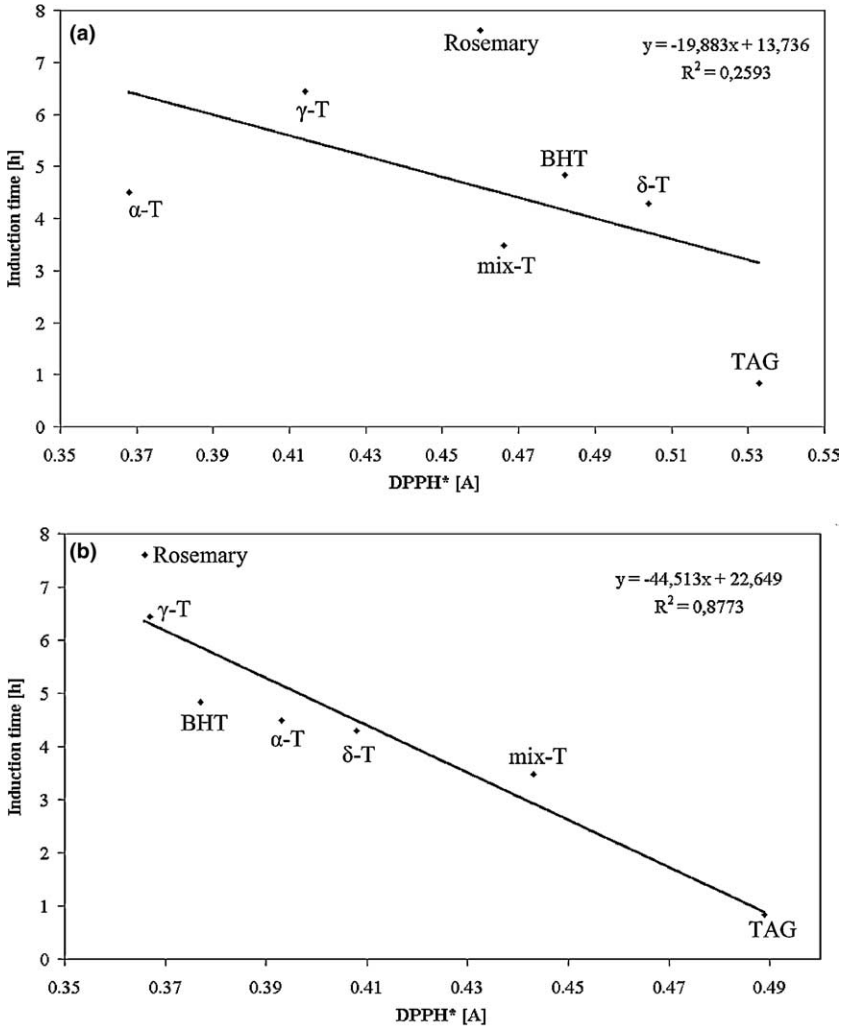

Fig. 2. Correlation between stability of rapeseed oil TAG with antioxidants estimated by Rancimat test and their effectiveness of quenching the DPPH radicals in model systems before oxidation (a) and after oxidation stability test (b). *Abbreviations see Table 1.

to 35% for α-tocopherol. The TAG samples with addition of antioxidants had higher antioxidant potential than those without antioxidants. This suggests that either active compounds were not depleted, or some new products with antioxidant activity were formed. Examples of such products are tocopherol dimers formed from tocopherols (Nogala-Kalucka, Gogolewski, & Luczyński, 1982) or ketophenoxy radical formed from carnosic acid and carnosol (Geoffroy et al., 1994).

Oxidation in Rancimat led to lower measured absorption values during experiments, suggesting greater effectiveness in quenching the $DPPH^+$ radicals after completion of oxidation processes, than for fresh prepared systems. This finding maybe explained by the removal of volatile compounds formed in the Rancimat during oxidation in the air stream (Wan, 1995). Lack of these compounds could lead to increase in the antioxidant potential of the system despite the advanced oxidation changes.

The status of antioxidant potency of systems oxidized in Oxidograph and Rancimat could differ because of different principles of estimation of the end of induction time in both instruments. Alternatively, higher partial pressure of oxygen in Oxidograph and faster oxidation of antioxidants may also explain differences seen.

In this study, α-tocopherol had the strongest activity in quenching DPPH radicals in system before oxidation, whereas its antioxidant activity evaluated by both tests was lower than the activity of γ-tocopherol, rosemary extract, and BHT. In the same systems δ-tocopherol had the lowest antioxidant activity and a very low capacity to quench radicals before and after oxidation in both Rancimat and Oxidograph tests. The primary antioxidant BHT possessed high antioxidant effect on TAG in the Oxidograph, but was not as active in quenching free radicals. Rosemary extract, the most active antioxidant, had lower capacity to destroy DPPH radicals than α- and γ-tocopherol in unoxidized systems, but it had the highest capacity in systems after estimation of the stability of TAG in both instruments.

Correlation between stability and the antioxidant potential of TAG without and with addition of antioxidants, before and after Oxidograph and Rancimat accelerated oxidation tests are shown in Figs. 1 and 2.

The ability of systems consisting of antioxidants and TAG to quench DPPH radicals was related to their stability. Correlation coefficients between the stability of TAG and their ability to quench DPPH radicals in the oxidized samples were higher than in the fresh samples in both Rancimat ($R^2 = 0.88$ vs. 0.23) and Oxidograph ($R^2 = 0.88$ vs. 0.26), respectively. These results suggest that in oxidized samples the ability of antioxidants to quench DPPH radicals did not differ from their effect on induction time. In contrast, in unoxidized rapeseed TAG the ability to quench DPPH radical and induction times at least for rosemary and BHT were different. This may suggest different antioxidant mechanism of single compound (BHT or tocopherols) vs. that in the mixture of antioxidants (rosemary extract).

In summary, we showed that rosemary extract was a more effective antioxidant than tocopherols in rapeseed oil TAG. Our results also suggest different mechanisms of antioxidant action of tocopherols and rosemary extract during the accelerated tests in the rapeseed oil TAG.

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