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Effects of blackcurrant seeds and rosemary extracts on oxidative stability of bulk and emulsified lipid substrates

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Abstract

The effects of blackcurrant seeds extract and commercially available rosemary extracts on rapeseed oil and rapeseed oil triacylglycerol oxidative stability were evaluated. The antioxidant activity of plant extracts was investigated, both in bulk and emulsified lipid substrates, and compared with those of α-tocopherol and BHT. The investigation showed that blackcurrant seeds and rosemary extracts are the source of active antioxidants and are appropriate for food lipid stabilization. The extracts performed better in stripped substrates as they may interfere with native tocopherols present in rapeseed oil and show different activity according to the presence of water. α-Tocopherol, a 200 ppm, was inactive in bulk and emulsified rapeseed oil but was an effective antioxidant in triacylglycerols. BHT showed the best performance of all used additives in emulsified substrates.

Keywords: Natural antioxidants; Antioxidant activity; Rosemary; Blackcurrant; Oxidative stability; Hexanal

1. Introduction

Autoxidation of unsaturated fatty acids affects quality of lipid-containing food by decreasing its sensory and nutritional values. Oxidative changes implicate, not only fatty acids, but also minor compounds, such as vitamins or sterols (Hwang, 1991; Kubow, 1992). As oxidation processes have been associated with higher incidence and mortality rates of numerous human illnesses, including cancer, atherosclerosis and heart disease, an attempt has been made to explore effective food antioxidants for disease prevention. Recently, the attention of researchers has been focussed on natural antioxidants. Plant extracts provide phenolic antioxidants that might exhibit strong activity. Subjects of investigation have been various spice and herb extracts, as well as fruits and vegetables (e.g. rosemary, pepper, evening primrose, tea, coffee, grape skin, grains, tomato seeds and amaranth peel) (Klimczak, Małecka, & Pachołek, 2002; Małecka, 2002; Schmidt, Niklová, Pokorný, Farkaš, & Sekretár, 2003; Schwarz et al., 2001).

The effect of natural extracts on inhibition of oxidation in lipid systems is determined by multiple factors. Antioxidants are active over certain optimal ranges of concentration. Exceeding them results in decrease of activity and even the occurence of a prooxidative effect. When used in excessive amounts, phenolic antioxidants become prooxidants by regenerating peroxyl radicals (Frankel, 1998).

In multicomponent systems, direct interactions occur between antioxidants. The cooperative effect is known as synergism and often occurs when free radical-scavengers are present together or combined with chelators. A strong synergistic inhibition of lipid oxidation is observed between tocopherols in the presence of ascorbic acid and is explained by the regeneration of the tocopheroxyl radical back to α-tocopherol (Decker, 2002).

The presence of water in a system results in partition of antioxidants in the medium between polar and apolar phases and this should be considered when explaining antioxidant activity. According to the polar paradox (Porter, Black, & Drolet, 1989), hydrophilic antioxidants are more

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effective in non-polar media, while lipophilic compounds are better antioxidants in polar media. This thesis was confirmed by Frankel, Huang, Kanner, and German (1994), when lipophilic α-tocopherol and ascorbyl palmitate were shown to be more effective in oil-in-water emulsion than in bulk oil, while the inverse dependence was found for trolox and ascorbic acid. On the other hand, some authors have reported that some compounds do not follow the polar paradox rule or the partitioning of antioxidants between the oil and water phases depend on different factors. This suggests that antioxidant activity is affected by complex phenomena and that polarity is not the only parameter to be taken into account (Cuvelier, Bondet, & Berset, 2000; Gordon, Paiva-Martins, & Almeida, 2001). Additionally, plant materials contain many compounds that may interfere with each other or minor lipid components and also contribute to the autoxidation process (Yanishlieva & Marinova, 1996). To ensure effective protection of food lipids by antioxidants, the efficient compounds or their mixtures should be used most favourably in a particular system.

The aim of this research was to investigate the effects of blackcurrant seed extract and commercially available rosemary extracts on rapeseed oil and rapeseed oil triacylglycerol oxidative stability. The antioxidant activity of plant extracts was investigated, both in bulk and emulsified lipid substrates and compared with those of α-tocopherol and BHT.

2. Materials and methods

2.1. Materials

The blackcurrant seeds, a by-product in the production of juices, were obtained from Kotlin Sp. z o.o. (Poland). Seeds were extracted, thrice for 1 h with the use of 80% ethanol. The extracts were combined, evaporated using a Bűchi R-114 rotary evaporator and the dry residue was dissolved in 96% ethanol (POCh, Poland). The rosemary extracts, Stabiloton oil-soluble (OS) and Stabiloton water-soluble (WS), were from Raps (Germany). As stated by the manufacturer, OS extract contained 30% (±3%) of acitve substances (phenolic diterpenes), WS −9% (±1%). Moreover, the rosemary extracts contained additives: edible oil and silicon dioxide in both extracts and sodium chloride, mono- and diacetyl tartaric acid esters of mono-and diglycerides of fatty acids in WS extract. (+)-α-Tocopherol (Fluka, Germany) and butylated hydroxytoluene (BHT, Sigma–Aldrich, Germany) were used for comparison. The rosemary extracts, α-tocopherol and BHT were dissolved in absolute ethanol (SDS, France).

Commercially available rapeseed oil (RO) (Z.T. Kruszwica S.A., Poland) was used. RO was purified to produce triacylglycerols (RO TAG) according to Popov, Yanishlieva, and Slavceva (1968). RO contained 669 ppm of tocopherols as measured by high-performance liquid chromatography (HPLC). No residues of tocopherols in RO TAG were found.

All reagents and solvents were either of HPLC or analytical grade.

2.2. Determination of total phenolics content and antiradical efficiency of extracts towards DPPH·

Total phenolics content in rosemary and blackcurrant seeds extracts was determined using Folin–Ciocalteu's reagent (Sigma–Aldrich, USA) (Singleton & Rossi, 1965). Caffeic acid (Sigma–Aldrich, Germany) was used as a standard.

The antiradical efficiency of extracts was measured using the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH·, Sigma–Aldrich, Germany) (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998).

All absorbance measurements were carried out on a Genesys 2 UV–Vis (Milton Roy) spectrophotometer.

2.3. Preparation of lipid substrates

The 5% (w/v) oil-in-water emulsions were prepared with RO or RO TAG and Tween 20 (Fluka, Germany) in phosphate buffer (pH 6.5), that was made according to the AOAC Official Method 941.17 (1995) with the use of water purified with a Milli-Q purification system (Millipore). Emulsification was carried out on a magnetic stirrer for 5 min.

Antioxidants dissolved in ethanol were added to bulk and emulsified RO and RO TAG (blackcurrant seeds extract 3000 ppm, rosemary extract OS 200 ppm, rosemary extract WS 700 ppm in bulk oils and 500 ppm in emulsions, α-tocopherol 200 ppm and BHT 200 ppm, lipid weight basis). Ethanol was removed by an extended flushing with argon and agitation of the sample. The choice of concentrations of used antioxidants was based on the content of active compounds, suggestions of the rosemary extract's manufacturer and preliminary experiments.

Four millilitre portions of each sample were placed in glass vials (22 ml), closed and oxidized at 60 °C (bulk oils) or 40 °C (emulsions) under dark conditions. The vials were opened and shaken for 5 s every two days.

2.4. Oxidative stability determination

Oxidative stability was evaluated by analyzing samples periodically for primary and secondary oxidation product formation. Hydroperoxides were measured as peroxide value (PV) (in bulk oils) according to the AOAC procedure (AOAC Official Method 957.13, 1995) or by using the ferric thiocyanate method (in emulsions) as described by Inatani, Nakatani, and Fuwa (1983).

Hexanal formation was monitored by static headspace analysis. The analyses were carried out on a Varian 3800 gas chromatograph equipped with a FID detector and autosampler, Tekmar 7000. A CP Sil 8CB (30 m × 0.53 mm × 1.5 μm) column was used. Samples were mixed for 40 min at 50 °C to reach equilibrium and then analyses

were performed. The initial column temperature was 40 °C (2 min), then it was raised to 100 °C (8 °C/min) and to 200 °C (20 °C/min), then held for 5.5 min. The headspace conditions were as follows: vial pressurisation 5 psi, pressurize time 0.3 min, sample equilibration 0.05 min, loop fill 0.6 min, loop equilibration 0.05 min, injection 0.5 min, vial needle flow 65 ml/min. Hexanal was determined by comparison of retention time with that of a known hexanal standard (Sigma–Aldrich, Germany) and quantified from a standard curve. The standard was added to the matrix prepared with the use of RO bulk or emulsified.

2.5. Statistical analysis

All analytical values represent means of duplicate analyses done on at least two different experiments. Significant differences between the samples were calculated using a Tukey's HSD test. Differences with $p < 0.05$ were considered significant. The Statistica 6.0 software (StatSoft, Poland) was used for analysis.

3. Results and discussion

3.1. Phenolic compounds and antiradical efficiency of natural extracts

Phenolic compound contents in rosemary and blackcurrant seed extracts and their antiradical properties are shown in Table 1. Rosemary extract OS contained the highest amount of total phenolics, twice more than WS and three times more than blackcurrant seeds extract.

The assay with DPPH$^{\cdot}$ radical showed that tested extracts are the source of active antioxidant compounds. The reaction between phenols and DPPH$^{\cdot}$ in ethanol assay is mainly based on a fast electron transfer process whilst hydrogen atom abstraction becomes a marginal reaction path (Foti, Daquino, & Geraci, 2004). The measure of antiradical efficiency (AE) involved the potency (EC_{50}) and the reaction time ($T\,EC_{50}$). Good antioxidants should act rapidly and at low concentration. The lower concentration of antioxidant and the more the rapid reaction with radical, the higher is the antiradical efficiency (Sánchez-Moreno et al., 1998). Phenolic compounds of rosemary extract OS exhibited the highest antiradical efficiency. This extract showed comparable potency (EC_{50}) to blackcurrant seeds extract but its reaction with the radical was more rapid.

The phenolics of rosemary and blackcurrant seed extracts are a complex mixture of active compounds. They showed lower antiradical efficiency than e.g. ascorbic acid but they were more effective than BHA and resveratrol due to their relatively faster action (Sánchez-Moreno et al., 1998).

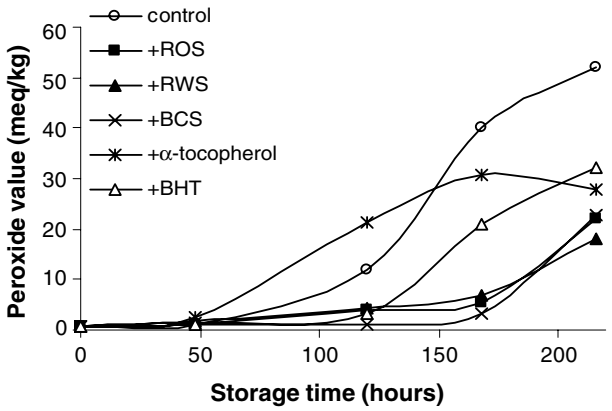

Fig. 1. Effect of blackcurrant seed extract (BCS, 3000 ppm), rosemary OS (ROS, 200 ppm) and rosemary WS (RWS, 700 ppm) extracts, α-tocopherol (200 ppm) and butylated hydroxytoluene (BHT, 200 ppm) on the formation of hydroperoxides in rapeseed oil at 60 °C.

3.2. Stability of bulk oil and triacylglycerols

The effect of antioxidants on oxidation of RO samples during storage at 60 °C is presented in Fig. 1. Plant extracts and BHT inhibited oxidative changes in RO but the natural compounds provided longer protection. The samples with the addition of α-tocopherol showed higher hydroperoxide contents than did the controls and, after 168 h of incubation, the rate of decomposition exceeded the rate of primary products formation.

Simultaneously, there was a significant increase in hexanal content in RO samples with added α-tocopherol

Table 1
Total phenolics of rosemary and blackcurrant seed extracts and comparison of their antiradical activities with activities of some standards

Extracts/standards	Total phenolics (mg of caffeic acid/g of extracts)	EC_{50} (g of antioxidant/kg DPPH$^{\cdot}$)[a]	$T\,EC_{50}$ (min)[b]	AE × 10^{-3} [c]
Rosemary OS[d]	140 ± 1.3	106 ± 2.5	21 ± 1.5	0.45
Rosemary WS[d]	67 ± 0.7	146 ± 5.2	28 ± 2.2	0.25
Blackcurrant seeds[d]	46 ± 0.1	103 ± 3.5	28 ± 2.4	0.35
Ascorbic acid[e]		76 ± 7	1.15 ± 0.08	11.4
BHA[e]		93 ± 6	104 ± 7.21	0.10
Resveratrol[e]		337 ± 12	60.5 ± 4.25	0.05

[a] Amount of antioxidant that causes a decrease in the initial concentration of DPPH$^{\cdot}$ by 50%.
[b] The time needed to reach the steady state with EC_{50} concentration.
[c] Antiradical efficiency, calculated as follows: $AE = 1/EC_{50}\ T\,EC_{50}$.
[d] Own investigation.
[e] Adapted from: Sánchez-Moreno et al. (1998).

Table 2
Effects of blackcurrant seed extract (BCS, 3000 ppm), rosemary OS (ROS, 200 ppm) and rosemary WS (RWS, 700 ppm in bulk oils, 500 ppm in emulsions) extracts, α-tocopherol (200 ppm) and butylated hydroxytoluene (BHT, 200 ppm) on hexanal formation in lipid substrates (μg/ml)

Lipid substrates	Storage time (h)	Control	+ ROS	+ RWS	+ BCS	+ α-Tocopherol	+ BHT
RO in bulk	0	N.d.[a]	N.d.	N.d.	N.d.	N.d.	N.d.
	120	19.4 ± 0.75	8.08 ± 0.31	11.6 ± 0.24	13.7 ± 0.14	18.70 ± 0.50	14.73 ± 0.75
	216	38.9 ± 1.80	24.8 ± 1.04	14.6 ± 0.45	21.5 ± 0.80	47.0 ± 2.55	16.82 ± 0.83
RO TAG in bulk	0	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.
	288	62.8 ± 0.52	21.4 ± 0.90	16.0 ± 0.59	54.3 ± 1.46	54.6 ± 1.23	1.05 ± 0.12
	576	65.4 ± 1.23	51.5 ± 1.83	50.9 ± 2.00	108 ± 2.07	115 ± 1.64	24.0 ± 0.51
RO in emulsion	0	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.
	480	N.d.	N.d.	N.d.	0.03 ± 0.01	0.06 ± 0.02	N.d.
	672	0.04 ± 0.00	0.03 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.01	0.03 ± 0.00
RO TAG in emulsion	0	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
	360	41.3 ± 1.83	32.2 ± 1.62	35.6 ± 1.91	46.60 ± 1.20	71.6 ± 1.23	0.38 ± 0.07
	672	52.7 ± 1.75	6.29 ± 0.26	13.9 ± 0.64	14.32 ± 0.31	58.6 ± 2.55	34.6 ± 1.07

[a] Not detected.

(Table 2). The blackcurrant seed extract and rosemary extract greatly inhibited hydroperoxide breakdown and hexanal formation. Hexanal is one of the characteristic products of linoleate deterioration and thus is an appropriate indicator of rancidity development in food lipids rich in $n-3$ and $n-6$ acids (Gordon, 2001; Shahidi, 1998).

The use of RO TAG allowed elimination of the effect of minor compounds on fatty acid oxidation. The rosemary extracts markedly retarded the course of oxidation in RO TAG (Fig. 2). Nevertheless, BHT inhibited oxidation the most. The addition of α-tocopherol and blackcurrant seed extract improved RO TAG stability only during the early period of incubation; then the hydroperoxides formation was faster than in the controls.

The rosemary extracts and BHT retarded hexanal formation in RO TAG samples during the entire period of incubation. α-Tocopherol and blackcurrant seed extract showed protective effects in inhibiting hexanal after 288 h of heating but showed prooxidant activity after 576 h (Table 2).

3.3. Stability of emulsions

The course of oxidation in RO emulsion is presented in Fig. 3. Only the synthetic compound BHT exhibited a marked effect on the inhibition of hydroperoxide formation over a long period. Blackcurrant seed and rosemary WS extracts exerted slight protection at early stages of incubation. The rosemary OS extract did not improve lipid substrate stability, whereas the level of hydroperoxides in samples with α-tocopherol added was higher than that in controls during the whole storage period. The prooxidative effect of α-tocopherol was confirmed on the basis of hexanal measurements (Table 2).

The hydroperoxides formation in RO TAG emulsion was markedly inhibited by all additives (Fig. 4). The high-

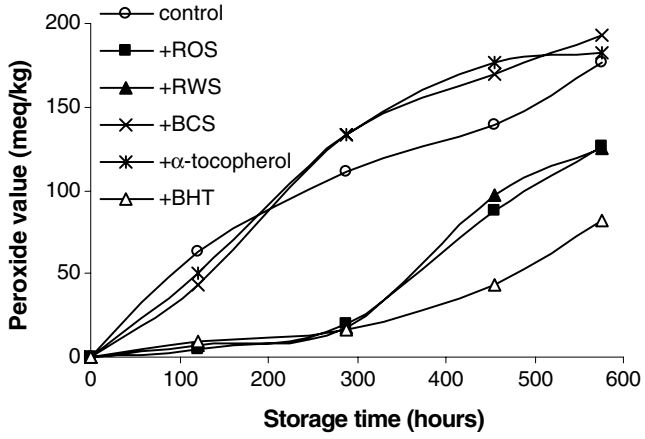

Fig. 2. Effect of blackcurrant seed extract (BCS, 3000 ppm), rosemary OS (ROS, 200 ppm) and rosemary WS (RWS, 700 ppm) extracts, α-tocopherol (200 ppm) and butylated hydroxytoluene (BHT, 200 ppm) on the formation of hydroperoxides in rapeseed oil triacylglycerols at 60 °C.

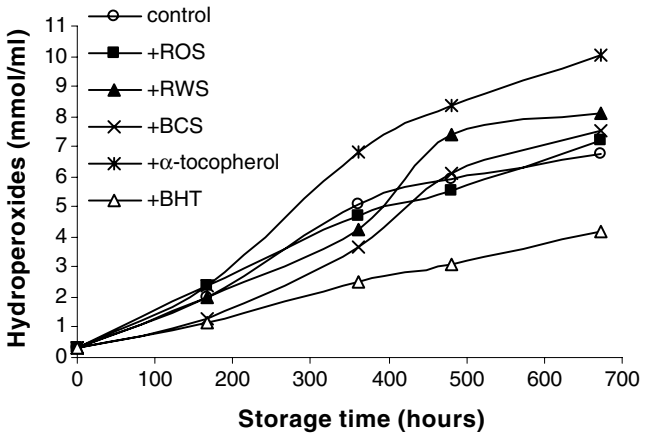

Fig. 3. Effect of blackcurrant seed extract (BCS, 3000 ppm), rosemary OS (ROS, 200 ppm) and rosemary WS (RWS, 500 ppm) extracts, α-tocopherol (200 ppm) and butylated hydroxytoluene (BHT, 200 ppm) on the formation of hydroperoxides in emulsified rapeseed oil at 40 °C.

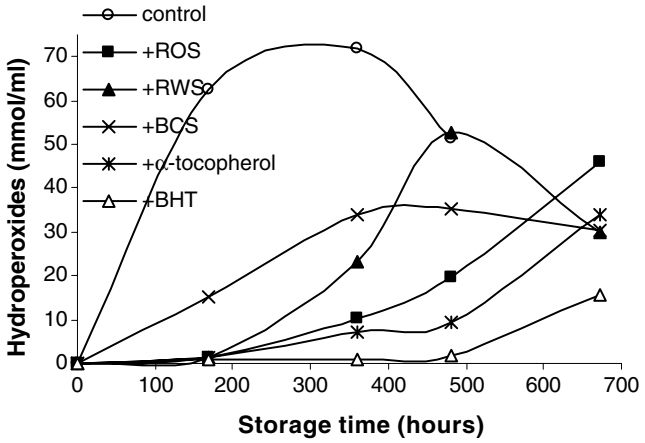

Fig. 4. Effect of blackcurrant seed extract (BCS, 3000 ppm), rosemary OS (ROS, 200 ppm) and rosemary WS (RWS, 500 ppm) extracts, α-tocopherol (200 ppm) and butylated hydroxytoluene (BHT, 200 ppm) on the formation of hydroperoxides in emulsified rapeseed oil triacylglycerols at 40 °C.

est stability of lipid substrate was achieved with BHT. This synthetic additive was followed by α-tocopherol, rosemary extract OS and rosemary extract WS. These additives were still efficient, even when the rate of hydroperoxide breakdown in control samples was higher than the rate of their formation, and performed better than did blackcurrant seed extract.

The synthetic antioxidant, BHT, was also the most efficient additive in retarding formation of hexanal in RO TAG emulsion (Table 2). By contrast, α-tocopherol did not inhibit hexanal formation. The protective effect of α-tocopherol toward conjugated hydroperoxides and simultaneous lack of protection against hexanal formation in emulsified sunflower oil TAG was described by Cuvelier, Lagunes-Galvez, and Berset (2003).

3.4. Activity of natural antioxidants in lipid substrates

To compare activities of antioxidants, the protective factor (PF) t_s/t_c was used, where t_s is time required for the stabilized sample to reach a PV value of 12 (in bulk oils) or hydroperoxides = 4 mmol/ml (in emulsions), t_c is respective time for the controls. PF is a measure of the lipid substrate stability and describes the ability of antioxidants to retard oxidation. The higher the PF value, the higher is the stability of a lipid substrate and the higher is the activity of tested antioxidant. PF < 1 indicates prooxidant properties of the additive.

On the basis of PF, blackcurrant seeds and rosemary extracts performed slightly better in bulk RO than in emulsified RO (Fig. 5). After stripping of tocopherols, the activity of rosemary extracts significantly increased, and was significantly higher than was blackcurrant seed extract. In bulk RO, activities of blackcurrant and rosemary extracts were higher than was BHT whereas, in emulsified substrates, the synthetic BHT showed the best performance of all used additives. α-Tocopherol exhibited antioxidant activity in triacylglycerols, which was higher in emulsified TAG than in bulk TAG.

Extract OS contained higher amounts of active compounds than did WS and for this reason it was used at lower concentration in lipid substrates. Despite the fact that both extracts contained various substances added for

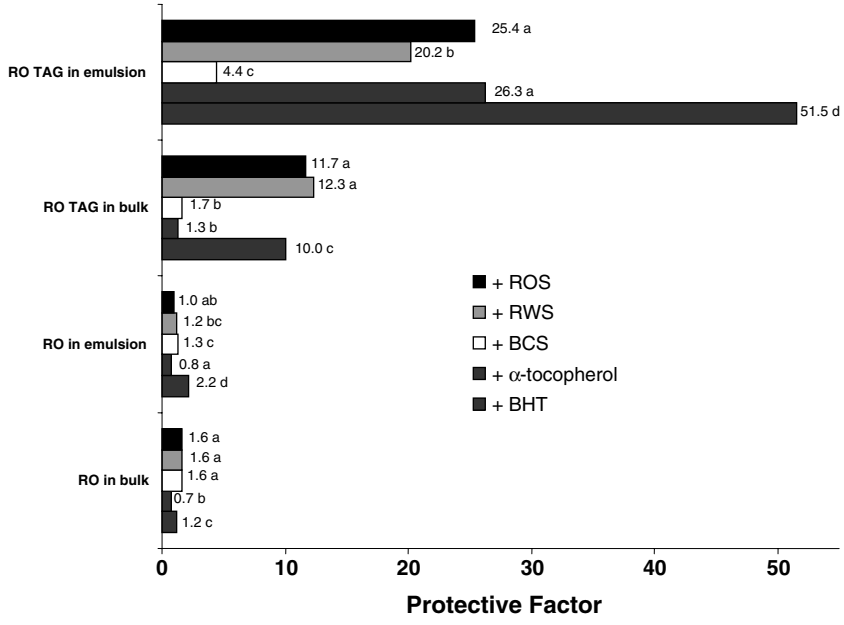

Fig. 5. Antioxidant activity of blackcurrant seed extract (BCS, 3000 ppm), rosemary OS (ROS, 200 ppm) and rosemary WS (RWS, 700 ppm in bulk oils, 500 ppm in emulsions) extracts, α-tocopherol (200 ppm) and butylated hydroxytoluene (BHT, 200 ppm) in lipid substrates. PF (Protective Factor) = t_s/t_c, where t_s is time required for the stabilized sample to reach a PV value = 12 (in bulk oils) or hydroperoxides = 4 mmol/ml (in emulsions) and t_c is respective time for the controls. Mean values followed by different letters are significantly different (within the same lipid substrate) ($p < 0.05$).

technological reasons, results were similar, with the exception of emulsified RO TAG, where OS showed a higher antioxidant effect than did WS.

Results obtained by Lu and Foo (2003) showed the presence of an array of polyphenols in blackcurrant seeds, such as anthocyanins, consisting of the rutinosides and glucosides of delphinidin, cyanidin, myricetin, quercetin, kaempferol-3-glucoside, dihydroquercetin and aureusidin, as well as the phenolic acids 1-cinnamoyl- and 1-*p*-coumaroyl-β-D-glucosides. Rosemary extracts contain a large number of components that exhibit antioxidant activity, such as carnosic acid, carnosol, rosmarinic acid, rosmanol and epirosmanol (Inatani et al., 1983; Sáenz-López, Fernández-Zurbano, & Tena, 2002). Earlier studies showed that rosemary extract, carnosic and rosmarinic acid were more active when added to bulk than emulsified corn oil. Moreover, rosmarinic acid acted as a prooxidant in emulsion. Rosemary extracts, carnosol and carnosic acid efficiently inhibited hydroperoxide formation in bulk soybean and peanut oils whereas, in emulsions, they showed prooxidant activity (Frankel, Huang, Prior, & Aeschbach, 1996).

The antioxidant potential of plant extracts is a result of presence, not only of the active phenolic compounds, but also concomitant components. Earlier reports showed a synergistic antioxidant effect of rosemary extracts and α-tocopherol (500 + 200 ppm) in a sardine oil model system and frozen-crushed fish meat (Wada & Fang, 1992). Peyrat-Maillard, Cuvelier, and Berset (2003) observed interaction between phenolic antioxidants and α-tocopherol in linoleic acid emulsion. An antagonistic effect has been found in mixtures of α-tocopherol/rosmarinic acid and α-tocopherol/caffeic acid. The increase of activity of blackcurrant seeds and commercial rosemary extracts examined in our study in stripped oil (RO TAG, bulk and emulsified) suggests antagonistic interaction of their components with tocopherols. More research is required to verify the hipothesis about influence of native tocopherols present in edible oils on the antioxidant activity of plant extracts added.

The activity of α-tocopherol significantly differs from the activity of tested extracts. α-Tocopherol did not show antioxidant activity in bulk and emulsified RO. Lack of activity might be explained by the beyond-optimal tocopherol concentration in oil after addition of 200 ppm of α-tocopherol, as RO contained 669 ppm of native tocopherols.

Some reports have indicated that addition of tocopherols to polyunsaturated oils was inefficient because their natural concentration was close to the optimum (Yanishlieva & Marinova, 2001). Frankel, Cooney, Moser, Cowan, and Evans (1959) observed that the presence of native tocopherols in soybean oil did not have a possitive effect on its stability. After removing some of the tocopherols, the oxidative and flavour stability increased markedly. In our study, α-tocopherol showed very high antioxidant activity in RO TAG emulsion (Fig. 5). It was due to absence of native tocopherols but also because of the presence of a polar medium. Because of the lipofilic character of the molecule, the ability of α-tocopherol to stabilize the emulsion confirmed the polar paradox, explained by the interfacial phenomena (Frankel et al., 1994; Porter et al., 1989).

4. Conclusion

Blackcurrant seed extract and rosemary extracts are sources of active antioxidants and might be used in food lipids stabilization. The investigation showed higher activity of natural components in stripped oils, as they may interfere with minor constituents of plant oils. Manufacturers have to pay special attention when stabilizing edible oils with tocopherols as there is a risk of exceeding their optimal concentration. The polarity of antioxidants and lipid substrates should be considered during application of natural extracts, as natural compounds can show different activities according to the amounts of water in food.

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