

ANTIOXIDANT PROPERTIES OF A MIXTURE OF *LAMIACEAE* PLANTS INTENDED TO USE AS A FOOD ADDITIVE

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Culinary herbs and medicinal plants could be used as natural food ingredients to replace synthetic antioxidants. Two natural antioxidant preparations, namely Rosmol (liquid) and Rosmol-P (powder) were produced by extraction from a mixture of medicinal plants belonging to the *Lamiaceae* family, such as rosemary (*Rosmarinus officinalis* L.), self heal (*Prunella vulgaris* L.), hyssop (*Hyssopus officinalis* L.) and lemon balm (*Melissa officinalis* L.). The main active compound of the extract is supposed to be a phenolic (caffeic) acid derivative. The total polyphenol content of the preparations is very high, 8.72 g l⁻¹ for Rosmol and 93.7 g kg⁻¹ for Rosmol-P. The products acted as primary and secondary antioxidants, chelating transitional metal ions and inhibiting the autoxidation of linoleic acid. Rosmol and Rosmol-P scavenged free radicals formed during Fenton type reaction measured by chemiluminometry, and also exhibited strong antioxidant property in Randox TAS measurement. The antioxidant activity of the products was unchanged after six months of storage. According to the in vitro studies, the products are suitable to use as antioxidant ingredients instead of synthetic ones in lipid containing food products.

Keywords: rosemary, self heal, hyssop, lemon balm, in vitro antioxidant activity

Oxidation of lipids in foodstuffs results in the development of off-flavour, rendering the product unacceptable for human consumption. The structure of foods is changed during processing and, as a result, lipids might become more exposed to oxygen. Antioxidants are substances that, when present in foods or in the body at low concentrations compared with that of an oxidizable substrate, markedly delay or prevent the oxidation of that substrate (PRYOR, 1994). Food manufacturers have used food-grade antioxidants to prevent quality deterioration of products and to maintain their nutritional value. Antioxidants have also been of interest to biochemists and health professionals, because they may help the body to protect itself against damage caused by reactive oxygen species and degenerative diseases. The role of antioxidants in today's food systems is constantly expanding, given the level of processing and handling that many foods encounter on their way to the consumer. However, the consumer is becoming increasingly concerned about the use of synthetic antioxidants, such as butylated

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hydroxytoluene and butylated hydroxyanisol, based on concerns that these compounds may be deleterious to human health (NAMIKI, 1990). These observations have led to a demand for antioxidants derived from naturally occurring sources.

Since ancient times, spices have been added to different types of food to improve flavour. Traditional culinary herbs and medicinal plants could be used as natural food antioxidants to replace synthetic ones. Since the early work of CHIPAULT and co-workers (1952), who investigated the antioxidant activity of spices such as rosemary, sage and clove in different substrates, interest in this field has increased and led to an increase in information about the compounds and mechanisms involved.

Early investigations have demonstrated that rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) are two of the most potent spices as natural antioxidants (CHIPAULT et al., 1956; HERRMANN, 1961). Both carnosol, an odourless and tasteless phenolic diterpene lactone, and carnosic acid have been found in rosemary and sage (WU et al., 1982). Rosmanol, epirosmanol, isorosmanol, rosmarinic acid and two diterpenes rosmaridiphenol and rosmariquinone have all been isolated from rosemary (NAKATANI & INATANI, 1981; INATANI et al., 1982). The antioxidant activity of whole spices and different extracts of rosemary and other spices belonging to *Lamiaceae* family was investigated in several model systems reviewed by LINDBERG-MADSEN and BERTELSEN (1995). Authors pointed out that the antioxidative activity of spices was significantly dependent on the substrate and radical generation system used in the evaluation. Especially in the case of food ingredients, the use of model systems is necessary, because storage experiments under realistic conditions are very time-consuming.

This study was focused on the antioxidant components and in vitro antioxidant properties of two plant preparations to be utilized as food ingredients to replace synthetic antioxidants. The antioxidant and free radical scavenging properties were studied in different chemical systems. The preparations were obtained from a special mixture of medicinal plants belonging to the *Lamiaceae* family.

1. Materials and methods

1.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, luminol, linoleic acid and tetramethylmurexide (TMM) were obtained from Sigma Chemical Co (MO, USA). Hexamethylene tetramine (hexamine) was purchased from Merck (Germany). Trolox was produced by Arcon Ltd (Belgium), while rosmarinic acid was purchased from ICN (Aurora, USA). Randox kit was obtained from Randox Laboratories Ltd. (Ardmore, UK). All other chemicals and reagents were of analytical grade from Chemolab (Hungary). Air-dried spices and medicinal plants such as hyssop, self heal, lemon balm and rosemary were of pharmacological grade according to PH. HG. (1986) and were purchased from local producers.

1.2. Antioxidant materials

The antioxidant materials were prepared as follows: Rosmol – 100 g mixture of air-dry hyssop (*Hyssopus officinalis* L.), self heal (*Prunella vulgaris* L.), lemon balm (*Melissa officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) was percolated with ethanol (30 w/v% in water) to give 500 ml of brown percolate. The solution was mixed with 2 g of activated carbon for cleaning the percolate, then refrigerated and filtered. The filtrate was evaporated under reduced pressure. The obtained solid was dissolved in propylene-glycol–water (33 v/v%) solution to yield 300 ml antioxidant material. Rosmol-P was prepared similarly to the Rosmol, but the hydro-ethanolic solution was spray dried to get 30 g antioxidant powder by an APV NORDIC ANHYDRO (Denmark) type equipment (temp_{in}: 90 °C, temp_{out}: 160 °C). Maltodextrin was used as carrier. The concentration of the maltodextrin in the solution was 15%. Rosmol is a light brown liquid, while Rosmol-P is a light yellowish-brownish powder. Neither has significant spicy odour, practically they are odourless. Rosmol and Rosmol-P were prepared by FitoChem Ltd., Monor, Hungary. For all determinations Rosmol was diluted with distilled water, while Rosmol-P was dissolved in distilled water and filtered where appropriate.

1.3. Chemical analyses

The total polyphenol content present in Rosmol and Rosmol-P was determined spectrophotometrically using Folin-Denis reagent (A.O.A.C., 1990). The absorbance was determined at 760 nm and gallic acid was used as standard.

Rosmarinic acid content in the preparations was measured by an HPLC measurement. Twenty µl of diluted samples were injected to RP-HPLC (Perkin Elmer LC Oven 101, Diode Array Detector 235C, Binary Pump 250). Separation was carried out on a Eurosphere 100-C₈ column (0.4/25, Phenomenex, USA) using HCOOH/NH₄OH buffer (82.5 mM, pH 2.40) and acetonitrile (40:60), as a mobile phase and UV (254 nm) detection (LOPEZ-ARNALDOS et al., 1996).

1.4. In vitro studies

Hydrogen-donating ability of the samples was determined in the presence of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical as described by BLOIS (1958). The absorbance was measured spectrophotometrically at 517 nm. Hydrogen-donating ability is expressed as I₅₀, the amount of the sample that is needed for 50% inhibition of the colour development of DPPH. The lower value shows higher activity.

The reducing power of the samples was determined according to the method of OYAIKU (1986), based on the reduction reaction of Fe³⁺→Fe²⁺. The absorbance was read at 700 nm. Increased absorbance of the reaction mixture indicates elevated reducing power. Reducing power was expressed as ascorbic acid equivalent (ASE g⁻¹ or ASE ml⁻¹). ASE g⁻¹ or ASE ml⁻¹ means that 1 g or 1 ml sample exhibits the same reducing power as the given amount of ascorbic acid expressed in µmol.

The chelating activity of the samples on copper(II)-ions was measured according to SHIMADA and co-workers (1992), with a minor modification. Diluted samples of Rosmol and Rosmol-P in different volumes were added to hexamine buffer (10 mM, pH 5.0), containing KCl (10 mM) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (3 mM) and were shaken well. After 2 min, tetramethylmurexide (1 mM) was added. Absorbances at 485 and 530 nm were recorded and the chelating activity was determined as the absorbance ratio at 485 vs. 530 nm. Absorbance ratio of the reaction mixture containing 1 ml of distilled water was 3.55 ± 0.05 . Samples having lower absorbance ratios than 3.55 exhibit chelating activity.

The determination of total antioxidant status (TAS) of Rosmol and Rosmol-P was carried out using the Randox diagnostic kit with a COBAS MIRA automatic laboratory analyser. The measurement is based on the procedure of MILLER and co-workers (1993).

The scavenging capacity of Rosmol and Rosmol-P was studied by a chemiluminescence assay adapted to a Berthold Lumat LB 9501 manual instrument in the presence of H_2O_2 /OH-luminol-microperoxidase system (BLÁZOVICS et al., 1999). The H_2O_2 /OH-luminol-microperoxidase system without the sample gives the “standard light”, which is expressed by the instrumental method as relative light unit (RLU). In the presence of scavenger molecules, the intensity of chemiluminescence light can be lower than that of the standard one. The scavenging property of Rosmol and Rosmol-P was determined as the chemiluminescence light intensity measured in the presence of sample and expressed in the percent of standard light (RLU %). RLU % of the sample lower than 100 indicates a significant radical scavenging property.

The method for inhibition of the linoleic acid autoxidation was written by MITSUDA and co-workers (1966) and was slightly modified here. The lipid oxidation products formed during an incubation period at 40 °C in darkness from linoleic acid were measured by the thiocyanate method. The absorbance of the red colour was red at 500 nm. The colour reaction was carried out every day during a seven-day period. Antioxidant activity was judged from inhibition of the absorbance at the day when the absorbance of the control vial having no antioxidant material reached a maximum value (practically at 4th day).

1.5. Storage experiment

A portion of Rosmol and Rosmol-P samples was stored in dark during a 6 months period at ambient temperature. Liquid Rosmol was stored in capped, totally filled, brown glasses in 250 ml volume. The air-space above the liquid surface was minimised. The powder Rosmol-P was placed in well-closed, totally filled plastic bags covered by brown paper with minimum amount of oxygen. Total polyphenol content, hydrogen donating ability and reducing power were measured in stored samples for checking the stability of antioxidant properties.

1.6. Statistics

Two independent portions of both preparations, Rosmol and Rosmol-P were investigated in three replicate measurements. Statistical evaluation was carried out by SPSS for Windows 9.0, and parameters were expressed as mean and standard deviation.

2. Results and discussion

Rosmol and Rosmol-P contained significant amount of polyphenolic compounds, as can be seen in Table 1. From the literature it was clear that rosmarinic acid is the main antioxidant compound in the extracts. In liquid Rosmol the concentrations of the phenolic substances and rosmarinic acid are $8.72 \pm 0.75 \text{ g l}^{-1}$ and $2.69 \pm 0.32 \text{ g l}^{-1}$, respectively. In solid Rosmol-P the total polyphenol content is $93.7 \pm 5.4 \text{ g kg}^{-1}$ and the rosmarinic acid is $38.5 \pm 1.1 \text{ g kg}^{-1}$. In Rosmol-P the bioactive compounds are more concentrated than in Rosmol. During a six months storage period, the total polyphenol content of the samples did not change significantly. Concentration of polyphenols in Rosmol and Rosmol-P after storage was $9.24 \pm 0.65 \text{ g l}^{-1}$ and $96.5 \pm 4.3 \text{ g kg}^{-1}$, respectively. Rosmarinic acid is an ester of caffeic and 3,4-dihydroxyphenyllactic acid. It is commonly found in species of the *Boraginaceae* and the subfamily *Nepetoideae* of the *Lamiaceae*. Rosmarinic acid has a number of interesting biological activities, e.g. antiviral, antibacterial, antiinflammatory and antioxidant (FRANKEL et al., 1996). In plants, rosmarinic acid is supposed to act as a preformed constitutively accumulated defence compound.

Table 1. Concentration of biologically active compounds and antioxidant properties (mean $\pm$ SD) of Rosmol and Rosmol-P

Property	Rosmol	Rosmol-P
Total polyphenol content	$8.72 \pm 0.75 \text{ g l}^{-1}$	$93.7 \pm 5.4 \text{ g kg}^{-1}$
Rosmarinic acid content	$2.69 \pm 0.32 \text{ g l}^{-1}$	$38.5 \pm 1.1 \text{ g kg}^{-1}$
Hydrogen-donating ability (I_{50}) ^a	$1.98 \pm 0.21 \text{ }\mu\text{l}$	$0.180 \pm 0.010 \text{ mg}$
Reducing power ^b	$65.4 \pm 3.4 \text{ ASE ml}^{-1}$	$857 \pm 25 \text{ ASE g}^{-1}$
Total antioxidant status ^c	$45.5 \pm 2.4 \text{ mmol l}^{-1}$	$620 \pm 37 \text{ mmol kg}^{-1}$
Chelating ability ^d	2.05 ± 0.10	1.90 ± 0.10
Antioxidant capacity (I_{50}) ^e	$0.13 \pm 0.02 \text{ }\mu\text{l}$	$0.020 \pm 0.005 \text{ mg}$

^a I_{50} is the amount of the sample needed for 50% inhibition of the colour development of DPPH. The lower value shows higher activity. ^bASE ml⁻¹ or ASE g⁻¹ means that 1 ml or 1 g sample exhibits the same reducing power as the given amount (in μmol) of ascorbic acid. ^cExpressed in Trolox equivalent measured by Randox TAS kit. ^dAbsorbance ratio at 485 vs. 530 given by the reaction mixture containing 100 μl of Rosmol or 10 mg of Rosmol-P. The control mixture did not contain chelating material and gave an absorbance ratio of 3.55. Samples with ratio lower than 3.55 are chelating agents. ^e I_{50} is the amount of the sample needed for 50% inhibition of linoleic acid autooxidation during a 4-day incubation period.

Rosmol and Rosmol-P exhibited strong hydrogen-donating ability in a concentration dependent manner (Fig. 1). Hydrogen-donating abilities of solutions prepared from Rosmol (2 ml l^{-1}) and Rosmol-P (200 mg l^{-1}) were compared to those of ascorbic acid (20 mg l^{-1}) and Trolox (20 mg l^{-1}). Hydrogen-donating ability of 0.1–1.5 ml of Rosmol and Rosmol-P was in the same range as that of ascorbic acid and Trolox in the concentration mentioned above. For the easy comparison of H-donating effect of different materials, the I_{50} value, the amount of the sample needed for the 50% discolouration of DPPH radical, was calculated. I_{50} -s of Rosmol and Rosmol-P are $1.98 \pm 0.21 \text{ } \mu\text{l}$ and $0.180 \pm 0.010 \text{ mg}$, respectively (Table 1). In case of ascorbic acid and Trolox, I_{50} was $0.013 \pm 0.001 \text{ mg}$ and $0.018 \pm 0.001 \text{ mg}$, respectively. It seems that ascorbic acid and Trolox are ten times stronger antioxidants than Rosmol and Rosmol-P in this system.

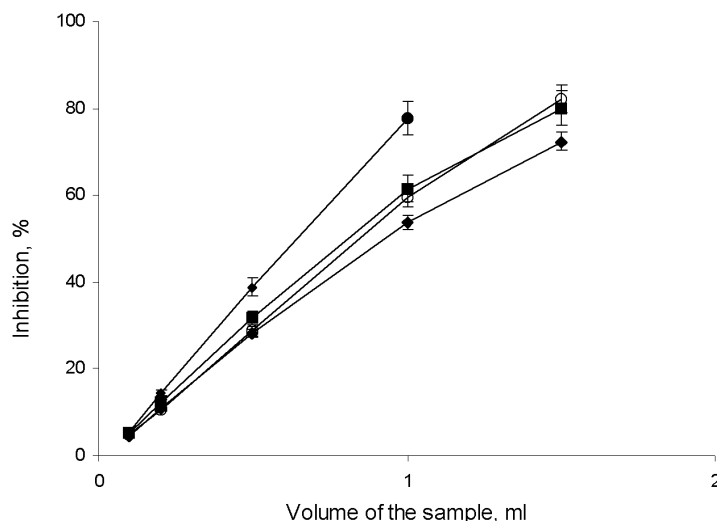


Fig. 1. Hydrogen-donating ability of Rosmol, Rosmol-P, ascorbic acid and Trolox. ◆: Rosmol, 2 ml l^{-1} ; ■: Rosmol-P, 0.2 g l^{-1} ; ●: ascorbic acid, 20 mg l^{-1} ; ○: Trolox, 20 mg l^{-1}

Hydrogen-donating ability of Rosmol and Rosmol-P did not change during the six months storage period at ambient temperature. Concentration-dependent H-donor properties of antioxidant materials can be seen in Fig. 2. Linearity of the curves is proven, and the correlation between the amount of sample and hydrogen-donating ability was statistically not different before and after the storage. I_{50} values of Rosmol and Rosmol-P were the same before and after storage: $1.98 \pm 0.21 \text{ } \mu\text{l}$ vs. $1.91 \pm 0.20 \text{ } \mu\text{l}$ for Rosmol and $0.180 \pm 0.010 \text{ mg}$ vs. $0.175 \pm 0.012 \text{ mg}$ for Rosmol-P.

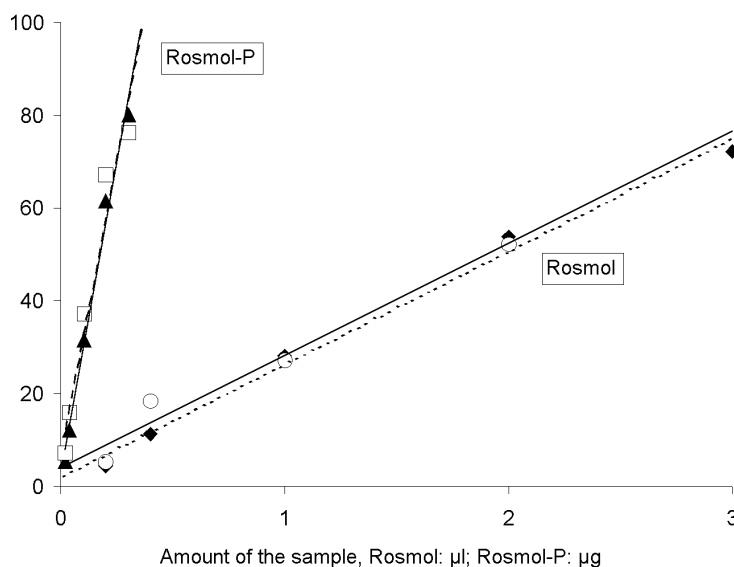


Fig. 2. Hydrogen-donating ability of Rosmol and Rosmol-P before and after storage. $\cdots\blacklozenge\cdots$: Rosmol before storage: $y=24.402x+1.7299$, $R^2=0.9919$; $-\circ-$: Rosmol after storage: $y=24.153x+3.9622$, $R^2=0.9696$; $-\blacktriangle-$: Rosmol-P before storage: $y=271.47x+2.2666$, $R^2=0.9880$; $-\square-$: Rosmol-P after storage: $y=253.98x+7.2347$, $R^2=0.9503$

Hydrogen-donating ability is an index of the primary antioxidant activity. Primary or chain-breaking antioxidants give hydrogen to free radicals, particularly the lipid hydroperoxide radicals that are the major propagators of the chain autoxidation of fats. This conversion leads to non-radical species, therefore inhibiting the propagation phase of lipid peroxidation. According to the results, Rosmol and Rosmol-P can act as primary antioxidants in delaying the induction period of lipid oxidation.

Rosmol and Rosmol-P are able to reduce ferrous to ferric ions (Fig. 3). Reducing power of the antioxidants was concentration dependent in the range of 0.1–1.0 mg for Rosmol-P and 1.0–10 μl for Rosmol. As can be seen in Fig. 3, reducing power of working solutions of Rosmol (10 ml l^{-1}), Rosmol-P (1 g l^{-1}), ascorbic acid (0.1 g l^{-1}) and Trolox (0.1 g l^{-1}) was in the same range. For the characterization of reducing power, an index as ascorbic acid equivalent (ASE) was set. The reducing power of 1 ml Rosmol and 1 g Rosmol-P is $65.4\pm3.4 \text{ ASE ml}^{-1}$ and $857\pm25 \text{ ASE g}^{-1}$, respectively. Reducing power can be interpreted as an index of secondary antioxidant activity. Secondary or preventive antioxidants are the most important in the initial phase of lipid oxidation, as they chelate multivalent metal catalysers, bind singlet oxygen and act in many other ways. Rosmol and Rosmol-P can act as secondary antioxidants in the inhibition of lipid peroxidation.

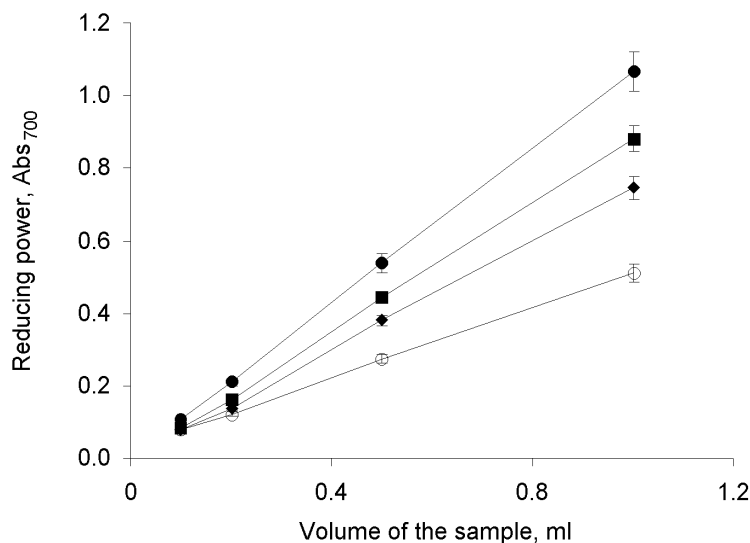


Fig. 3. Reducing power of Rosmol, Rosmol-P, ascorbic acid and Trolox. ◆: Rosmol, 10 ml l⁻¹; ■: Rosmol-P, 1 g l⁻¹; ●: ascorbic acid, 0.1 g l⁻¹; ○: Trolox, 0.1 g l⁻¹

Reducing power of Rosmol and Rosmol-P did not change during the six-month storage period. Reducing power of Rosmol and Rosmol-P was 69.7 ± 3.2 ASE ml⁻¹ and 907 ± 37 ASE g⁻¹, respectively. The total antioxidant status of Rosmol and Rosmol-P measured by Randox diagnostic kit was 45.5 ± 2.4 mmol l⁻¹ and 620 ± 37 mmol kg⁻¹, respectively (Table 1).

Chelating ability of a natural product exists when the absorbance ratio at 485 vs. 530 nm of the reaction mixture is lower than that of the control mixture having no antioxidants. The absorbance ratio at 485 vs. 530 nm of the control mixture (containing distilled water) is 3.55 ± 0.02 . For better comparison, this ratio is 2.70 ± 0.05 , 1.34 ± 0.02 and 0.62 ± 0.01 when the reaction mixture contains 2.25, 2.50 and 2.75 mM EDTA, a well-known chelating substance, respectively (LUGASI, unpublished data). Both preparations Rosmol and Rosmol-P can definitely chelate copper ions in a concentration dependent manner as can be seen in Fig. 4. Copper-chelating ability of Rosmol (100 µl) and Rosmol-P (10 mg) was 2.05 ± 0.10 and 1.90 ± 0.10 , respectively.

Although chelating agents such as EDTA are not antioxidants, they play an important role in the prevention of lipid oxidation. During the propagation phase, decomposition of the lipid hydroperoxides catalysed by transitional metal ions (Fe²⁺, Cu²⁺) results in an explosive increase of free radicals and consecutive chain reactions.

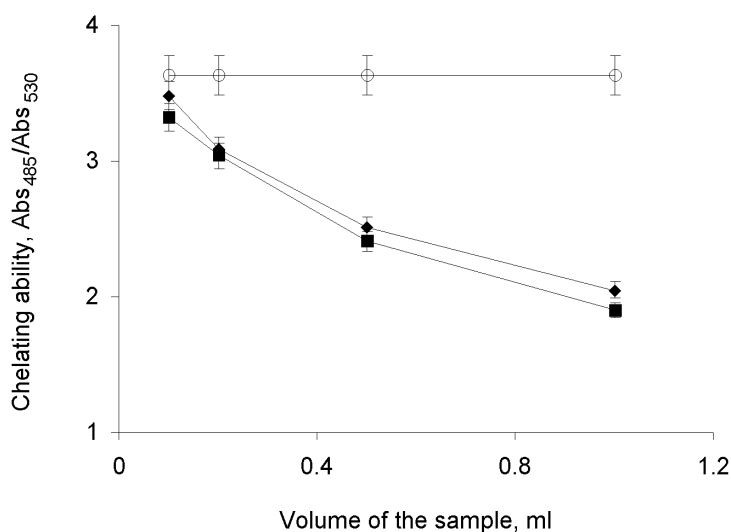


Fig. 4. Chelating ability of Rosmol and Rosmol-P. ○: control; ◆: Rosmol, 100 ml l⁻¹; ■: Rosmol-P, 10 g l⁻¹

They also reduce the activation energy at the initiation step, therefore they may generate free radicals directly from unsaturated fatty acids via single-electron transfer and proton abstraction. It has been considered that flavonoids and phenolic acids as primary antioxidants that act as free radical acceptors and chain breakers. Besides these activities, phenolics are known to chelate metal ions. More than twenty-seven different phenolic compounds including flavonoids such as hesperetin, apigenin, luteolin-glucoside, genkwanin, cirsimaritin and scutellarin could be isolated from extracts of rosemary and other plants of *Lamiaceae* family (OKAMURA et al., 1994; CUVELIER et al., 1996; LU & FOO, 2000; PATORA & KLIMEK, 2002). Although detailed composition of Rosmol and Rosmol-P is not known, it is supposed that besides rosmarinic acid, other phenolic compounds including flavonoids are present in both preparations. These compounds could be responsible for the significant chelating ability of the extracts.

Both preparations can prevent the autoxidation of linoleic acid at 40 °C during a 7-day incubation period in a concentration-dependent manner (Fig. 5). The induction time of the oxidation measured by the thiocyanate method was longer when the samples were present in the reaction mixture. For the 50% inhibition of the autoxidation of linoleic acid, 0.13±0.02 µl Rosmol and 20±0.5 µg Rosmol-P were needed. It has been proposed that flavonoids react with lipid peroxyl radicals leading to termination of radical chain reactions. The autoxidation of linoleic acid and methyl linoleate was inhibited by flavonoids such as catechin, quercetin, rutin, luteolin, kaempferol and morin (KANDASWAMI & MIDDLETON, 1997) and different plant extracts such as rosemary and sage (CUVELIER et al., 1996). From the inhibition of the formation of trans-trans hydroperoxide isomers of linoleic acid by flavonoids, inhibition of autoxidation of fatty acids by radical chain reaction termination was discerned.

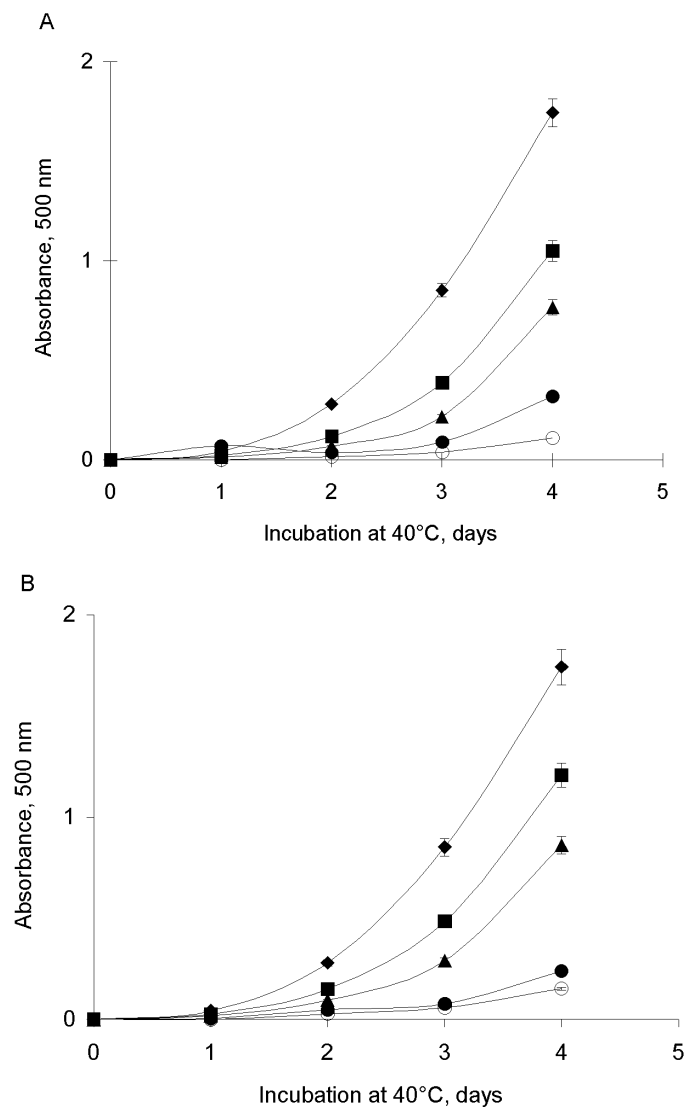


Fig. 5. Effect of Rosmol and Rosmol-P on the autoxidation of linoleic acid. A: Rosmol, 1 ml l⁻¹; ◆: control; ■: 0.1 ml; ▲: 0.2 ml; ●: 0.5 ml; ○: 1.0 ml; B: Rosmol-P, 0.1 g l⁻¹; ◆: control; ■: 0.1 ml; ▲: 0.2 ml; ●: 0.5 ml; ○: 1.0 ml

Rosmol and Rosmol-P showed significant free radical scavenging activity in H₂O₂/·OH-luminol system (Fig. 6). The highest chemiluminescence light intensity could be detected in the measuring system where only H₂O₂, luminol and

microperoxidase were present. Rosmol and Rosmol-P at different concentrations decreased the chemiluminescence light intensity, thus it is supposed that these preparations are good scavengers of free radicals.

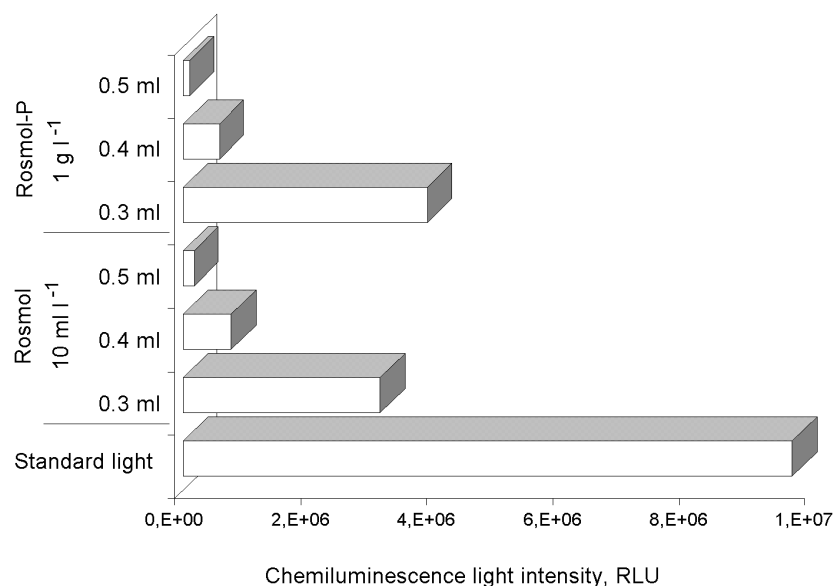


Fig. 6. Scavenging property of Rosmol and Rosmol-P in $H_2O_2/-OH$ -luminol system

3. Conclusion

A number of compounds such as flavonoids, phenolic diterpenes, carnosic acid, carnosol, rosmaridiphenol, rosmarinic acid and rosmariquinone have been identified as antioxidants in rosemary, sage, oregano, thyme, summer savoury, and other herbs belonging to the *Lamiaceae* family (FRANKEL et al., 1996; MARKOVA et al., 1997; TRIANTAPHYLLOU et al., 2001). Screening of antioxidant activity in various model systems is important prior to testing or application of antioxidants in foods.

In present study, two antioxidant preparations were tested in different model systems. The results proved that both products, Rosmol and Rosmol-P, could inhibit or delay the lipid peroxidation processes via different mechanisms. The extracts have hydrogen-donating property, thus they can act as primary (chain breaking) antioxidants. They could also prevent the oxidation as secondary antioxidants, and are strong chelating agents. The bioactive compounds in the extracts could scavenge free radicals and inhibit the oxidation process in lipid model systems.

Rosmol-P, the spray-dried powder form contains ten times higher concentration of polyphenolic compounds than liquid Rosmol. Rosmarinic acid constitutes 30.8 and 41.0% of total polyphenols in Rosmol and Rosmol-P, respectively. The concentration of rosmarinic acid of the preparations depends on its content of the plants used as basic materials and some structural changes of the molecule can be also taken into consideration during the procedure (OFFORD et al., 1997). Plants in the *Lamiaceae* family have other components with high antioxidant capacity such as carnosol, carnosic acid, rosmanol and epirosmanol. Although carnosol and carnosic acid are reported to account for over 90% of the antioxidant activity of rosemary, it must be borne in mind that carnosic acid is relatively unstable (CUVELIER et al., 1994). Antioxidant properties of these compounds are different and effected by the chemical structure (NAKATANI & INATANI, 1981; INATANI et al., 1982). It is supposed that beyond rosmarinic acid, other phenolics contribute to the antioxidant properties of Rosmol and Rosmol-P.

It seems that Rosmol and Rosmol-P are useful antioxidants in vitro, and in different food products, as well. Rosmol-P effectively inhibited the oxidation of lipids and cholesterol and suppressed the protein damages in experiments with horse mackerel fish (*Trachurus trachurus*) during freezing storage (AUBOURG et al., 2004). The preparation was also effective in an improvement of sensory characteristics and inhibition of lipid peroxidation in special Hungarian sausages (LUGASI & BLÁZOVICS, 2004). A four-step procedure may prove the usefulness of a new antioxidant in the food industry as a healthy, natural food ingredient; first is to determine the quality and quantity of the active compounds, second is to characterise the antioxidant and radical scavenging activities of the extract, third is to test it in several model systems, and fourth is to apply it in different food products having significant lipid content such as meat products, mayonnaise, and vegetarian creams.

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References

- A.O.A.C. (1990): *Official Methods of Analysis*. 15th ed, Arlington USA 952.03/A-C.
- AUBOURG, S., LUGASI, A., HÓVÁRI, J., PINEIRO, C., LÉBOVICS, V. & JAKÓCZI, I. (2004): Damage inhibition during frozen storage of horse mackerel (*Trachurus trachurus*) fillets by a previous plant extract treatment. *J. Fd Sci.*, 69, 136–141.
- BLÁZOVICS, A., KOVÁCS, Á., LUGASI, A., HAGYMÁSI, K., BÍRÓ, L. & FEHÉR, J. (1999): Antioxidant defence in erythrocytes and plasma of patients with active and quiescent Crohn's disease and ulcerative colitis: a chemiluminescence study. *Clin. Chem.*, 45, 895–896.
- BLOIS, M.S. (1958): Antioxidant determination by the use of a stable free radical. *Nature*, 4617, 1198–1200.
- CHIPAULT, J.R., MIZUNO, G.R., HAWKINS, J.M. & LUNDBERG, W.O. (1952): The antioxidant properties of natural spices. *Fd Res.*, 17, 46–55.

- CHIPAULT, J., MIZUMO, G. & LUNDBERG, W. (1956): The antioxidant properties of spices in foods. *Fd Techn.*, 10, 209–211.
- CUVELIER, M.E., BERSET, C. & RICHARD, H. (1994): Antioxidant constituents in sage (*Salvia officinalis*). *J. agric. Fd Chem.*, 42, 665–669.
- CUVELIER, M., RICHARD, H. & BERSET, C. (1996): Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *J. Am. Oil Chem. Soc.*, 73, 645–652.
- FRANKEL, E.N., HUANG, S., AESCHBACH, R. & PRIOR, E. (1996): Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. *J. agric. Fd Chem.*, 44, 131–135.
- HERRMANN, K. (1961): The antioxidative activity of *Labiatae* drugs and their constituent *Labiatae* acid. *Z. Lebensmittelunters.-Forsch.*, 116, 224–228.
- INATANI, R., NAKATANI, N., FUWA, H. & SETO, H. (1982): Structure of a new antioxidative phenolic diterpene isolated from rosemary. *Agric. biol. Chem.*, 46, 1661–1666.
- KANDASWAMI, C. & MIDDLETON, E. (1997): Flavonoids as antioxidants. -in: SHAHIDI, F. (Ed) *Natural antioxidants. Chemistry, health effects and application*. AOCS Press, Champaign, IL, pp. 182–203.
- LINDBERG-MADSEN, H. & BERTELSEN, G. (1995): Spices as antioxidants. *Trends Fd Sci. Technol.*, 6, 271–277.
- LOPEZ-ARNALDOS, T., ZAPATA, J.M., CALDERÓN, A.A. & ROS BARCELO, A. (1996): Antioxidant activity of lavandin (*Lavandula x intermedia*) cell cultures in relation to their rosmarinic acid content. -in: RISCH, S.J. & HO, CH-T. (Eds) *Spices. Flavour chemistry and antioxidant properties*. ACS Symposium Series 660. American Chemical Society, Washington DC, pp. 206–218.
- LU, Y. & FOO, L.Y. (2000): Flavonoid and phenolic glycosides from *Salvia officinalis*. *Phytochemistry*, 55, 263–267.
- LUGASI, A. & BLÁZOVICS, A. (2004): *Az egészséges táplálkozás tudományos alapjai*. Széchenyi füzetek 4. (Scientific basis of healthy nutrition. Széchenyi Booklets No 4.) Országos Élelmezés- és Táplálkozástudományi Intézet, Budapest, Hungary, pp. 74–86.
- MARKOVA, H., SOUSEK, J. & ULRICHOVA, J. (1997): *Prunella vulgaris* L. – a rediscovered medicinal plant. *Ceska Slov. Farm.*, 46, 58–63.
- MILLER, N.J., RICE-EVANS, C., DAVIES, M.J., GOPINATHAN, V. & MILNER, A. (1993): A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.*, 84, 407–412.
- MITSUMA, H., YASUMOTO, K. & IWAMI, K. (1966): Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyoto Shokuryo*, 19, 273–300.
- NAKATANI, N. & INATANI, R. (1981): Structure of rosmanol, a new antioxidant from rosemary. *Agric. biol. Chem.*, 45, 2385–2386.
- NAMIKI, M. (1990): Antioxidants/antimutagens in food. *Crit. Rev. Fd Sci. Nutr.*, 29, 273–300.
- OFFORD, E.A., GUILLOT, F., AESCHBACH, R., LÖLIGER, J. & PFEIFER, A.M.A. (1997): Antioxidant and biological properties of rosemary components: implications for food and health. -in: SHAHIDI, F. (Ed) *Natural antioxidants. Chemistry, health effects, and applications*. AOCS Press, Champaign, IL, pp. 88–96.
- OKAMURA, N., HARAGUCHI, H., HASHIMOTO, K. & YAGI, A. (1994): Flavonoids in *Rosmarinus officinalis* leaves. *Phytochemistry*, 37, 1463–1466.
- OYAZU, M. (1986): Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.*, 44, 307–315.
- PATORA, J. & KLIMEK, B. (2002): Flavonoids from lemon balm (*Melissa officinalis* L., *Lamiaceae*). *Acta Pol. Pharm.*, 59, 139–143.
- PH. HG. (1986): *Pharmacopoeia Hungarica*. Ed. VII, Medicina Könyvkiadó, Budapest, pp. 374–399.
- PRYOR, W.A. (1994): Free radicals and lipid peroxidation: What they are and how they got that way. -in: FREI, B. (Ed) *Natural antioxidants in human health and disease*. Academic Press, San Diego, CA, pp. 1–25.
- SHIMADA, K., FUJIKAWA, K., YAHARA, K. & NAKAMURA, T. (1992): Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. agric. Fd Chem.*, 40, 945–948.
- TRIANTAPHYLLOU, K., BLEKAS, G. & BOSKOU, D. (2001): Antioxidative properties of water extracts obtained from herbs of the species *Lamiaceae*. *Int. J. Fd Sci. Nutr.*, 52, 313–317.
- WU, J.W., LEE, M-H., HO, C-T. & CHANG, S.S. (1982): Elucidation of the chemical structure of natural antioxidants isolated from rosemary. *J. Am. Oil Chem. Soc.*, 59, 339–345.