

Antioxidant Activity of Selected Phenols and Herbs Used in Diets for Medical Conditions

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Abstract

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The antioxidant capacity of some herbs used in dietology practice was determined by the DPPH free radical method, which was calibrated with ascorbic acid. Partially hydrophilic phenolic compounds are the most active compounds in plants, and therefore water was used as the extraction agent. Besides antioxidant capacity, the content of total phenolic compounds was also measured and a strong correlation between these two variables was found. The extracts of lemon balm (*Melissa officinalis* L.), peppermint (*Mentha x piperita* L.), oregano (*Origanum vulgare* L.), Greek oregano (*Origanum heracleoticum* L.), sage (*Salvia officinalis* L.) and winter savory (*Satureja montana* L.) showed very significant activity. It was comparable with the activity of green tea in the case of oregano and peppermint. Lower activity was observed in the case of rosemary (*Rosmarinus officinalis* L.), marjoram (*Majorana hortensis*), hyssop (*Hyssopus officinalis* L.), sweet basil (*Ocimum basilicum*), and lovage (*Levisticum officinale* Koch.). The inhibitory activity of the herb extracts was monitored also during the autooxidation of lard. Very high antioxidant capacity was observed mainly in sage samples, but also in marjoram and Greek oregano. The extracts of peppermint, oregano, rosemary, winter savory, lemon balm and hyssop showed middle activity comparable to that of α -tocopherol. The antioxidant capacity of sweet basil and lovage was insignificant.

Keywords: herbs; antioxidant activity; DPPH; fats

Food prepared by technology for diets aimed at patients suffering from medical conditions is applied in the cases of indigestion and some diseases

of the digestive system. It is usually quite tasteless with only weak aroma. The motivation for patients to modify their eating habits and accept these di-

ets is therefore very low. For this reason, modern dietology recommends the use of several aromatic herbs (fresh or dry) to improve and enhance the flavour and aroma of the prepared meals. These plants, moreover, often contain compounds with antioxidant activity, mainly phenolic compounds and ascorbic acid.

Most foods of plant origin contain components which are active as inhibitors of undesirable oxidative processes not only in food but very often in the human body as well. These are mainly compounds of natural origin, which are formed by regulated biosynthesis in plants. Phenolics belong to the very important group of plant antioxidants. They are always substituted phenolic compounds with one to three hydroxyl groups on the aromatic ring in different positions. A carboxylic acid group may also be present as the main substituent or another ring may be linked to the aromatic ring. The antioxidant effect depends mainly on the number and position of hydroxyl groups and the identity of the main substituents, but also on many other factors.

Total antioxidant capacity of plant material depends not only on the content and composition of phenolics, but also on the contents of other antioxidants, for example ascorbic acid. Besides that there could be synergism or antagonism between the active compounds. Therefore, the estimation of antioxidant activity in real materials is very difficult and the total antioxidant effect must be determined experimentally (POKORNÝ 1991; PÁNEK 2001; SCHMIDT & POKORNÝ 2005; DIMITRIOS 2006; YANISHLIEVA *et al.* 2006; HORVATHOVA *et al.* 2007; VELÍŠEK 2009).

The aim of this work was to evaluate the antioxidant capacity of selected aromatic herbs suitable for inclusion in foods for patients with medical conditions. The diphenylpicrylhydrazyl (DPPH) radical-scavenging method was applied together with the Schaal test using pork fat.

MATERIAL AND METHODS

Chemicals. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Sigma-Aldrich, St. Louis, USA); Folin-Ciocalteu phenol reagent (Merck, Darmstadt, Germany)

Standards: ascorbic acid, gallic acid, thymol puriss, caffeic acid, rosmarinic acid (97%), carvacrol (98%), Trolox, catechin, α -tocopherol, all chemicals from Sigma-Aldrich (St. Louis, USA).

Herbs, used parts – dry leaves. Peppermint (*Mentha × piperita* L.), rosemary (*Rosmarinus officinalis* L.), oregano (*Origanum vulgare* L.), winter savory (*Satureja montana* L.), sage (*Salvia officinalis* L.), hyssop (*Hyssopus officinalis* L.); lovage (*Levisticum officinale* Koch.); lemon balm (*Melissa officinalis* L.), sweet basil type “Compatto” (*Ocimum basilicum* L., var. Compatto). All these hardy annual plants (9 years old) and therophyte sweet basil were planted in the locality of the Czech town region Kolin in loamy-sandy and less permeable humus soil. Leaves were collected at the beginning of July before flowering. The leaves were dried in the shade in the open air room at ambient temperature.

Marjoram (*Origanum majorana* L.) was grown in the Czech University of Life Sciences in Prague (CULS) greenhouse and fertilised with mineral fertiliser. Leaves were dried at 40°C in a dryer with air circulation.

Citrus type of peppermint *Mentha × piperita* var. Citrata (*Mentha citrata*, *Mentha odorata*) grew wildly in the university campus in Prague. Leaves were collected in August before flowering and dried in the same way as marjoram

Herbs from the Greek market. Rigani Charma – Greek oregano “rigani” (*Origanum heracleoticum* L.). Dried leaves were purchased in a retail shop in Greece. The country of origin is Greece, region Ioannina, growing and drying conditions unknown, and expiry date 12/2010.

Green tea. All green tea samples were purchased in their original packagings in the tea-shop Teatao in Prague. Green tea Sencha Classic, growing region Japan; GABA green tea (organic) – China; Xi Hu Long Jing “Dragon’s well” from province Zhe Jiang, region Xi Hu in China.

Pork lard. Edible animal fat, expiry date 5/2009; manufacturer Meat Factory Pilsen, Czech Republic.

Peroxide value of lard before use was determined as 1.27 milliequivalents of active oxygen/kg

Sample extraction. Dry leaves (1.5–2 g) were extracted twice with 50 ml of hot demineralised water. Each extraction lasted 10 min at 70°C with occasional mixing.

Determination of antioxidant activity by DPPH method. Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. This method was taken from DORMAN *et al.* (2003), KULISIC *et al.* (2004), SU *et al.* (2007), BUŘIČOVÁ and RÉBLOVÁ (2008),

LO SCALZO (2008) and modified. The method was calibrated with ascorbic acid and the results were expressed as equivalents of ascorbic acid per unit mass of the sample (AAE). The principle of this spectrophotometric method is based on the intensity of the violet DPPH radical solution measurement at 522 nm. The radical is decolourised by phenolic compounds with antioxidant activity. The reaction equilibrium is usually reached after two hours for most compounds. The extract (0.2–1.0 ml) was dissolved in demineralised water (50 ml). Radical solution (2 ml; 0.005 g per 100 ml methanol) was added to a cuvette, and 1 ml of the dissolved extract was added prior to the measurement.

Determination of total phenolic compounds.

The content of total phenolics was determined spectrophotometrically at 760 nm by using Folin-Ciocalteu reagent. The results were expressed as the content of gallic acid per unit mass of the sample (DORMAN *et al.* 2003; STRATIL *et al.* 2008).

Antioxidant activity by the Schaal test. The principle of the method is based on the monitoring of the course of fat oxidation gravimetrically with free oxygen access in the dark at 60°C. The

weight changes were monitored in two parallel determinations in lard without any additive and in lard with the addition of herb extract (extract from 40 g of dried herbs) per 1 kg of lard, or with the addition of pure antioxidant (500 mg) per 1 kg of lard. The dried extracts and pure compounds were dissolved in diethyl ether, and the solution was added to lard. The induction period (IP) values were determined by the linear regression method using MS Excel software. The protection factor (PF) was calculated by the formula: PF = IP with additive/IP without additive.

RESULTS AND DISCUSSION

Determination of antioxidant capacity of extracts

The antioxidant capacity of the selected herbs and green tea samples determined by the DPPH method are expressed in units of mg equivalent of ascorbic acid (AAE) per gram of dried leaves (Table 1); the total phenolic content values are expressed as mg equivalent of gallic acid (GAE)

Table 1. Antioxidant capacity and total phenolic contents of plant family Lamiaceae, lovage and green tea samples (*Camelia sinensis* L.)

Herbs	Latin name	Family	DPPH (mg AAE/g)	Phenols (mg GAE/g)
Oregano	<i>Origanum vulgare</i> L.	Lamiaceae	209.1	91.4
Peppermint – citrus type	<i>Mentha × piperita</i>	Lamiaceae	203.8	89.6
Lemon balm	<i>Melissa officinalis</i> L.	Lamiaceae	171.5	85.0
Peppermint	<i>Mentha × piperita</i> L.	Lamiaceae	147.5	63.0
Winter savory	<i>Satureja montana</i> L.	Lamiaceae	69.5	27.1
Sage	<i>Salvia officinalis</i> L.	Lamiaceae	60.6	24.3
Greek oregano (“Rigani”)	<i>Origanum heracleoticum</i> L.	Lamiaceae	56.2	27.0
Basil Compatto	<i>Ocimum basilicum</i> L.	Lamiaceae	55.5	20.3
Rosemary	<i>Rosmarinus officinalis</i> L.	Lamiaceae	44.7	–
Hyssop	<i>Hyssopus officinalis</i> L.	Lamiaceae	43.5	20.1
Marjoram	<i>Origanum majorana</i> L.	Lamiaceae	42.1	27.7
Lovage	<i>Levisticum officinale</i> Koch.	Apiaceae	34.1	19.7
Green tea “Xi Hu Long Jing”	<i>Camelia sinensis</i> L.	Theaceae	221.7	91.0
Green tea “GABA”	<i>Camelia sinensis</i> L.	Theaceae	216.2	112.8
Green tea “Sencha”	<i>Camelia sinensis</i> L.	Theaceae	138.1	63.7

AAE = ascorbic acid equivalent; GAE = gallic acid equivalent

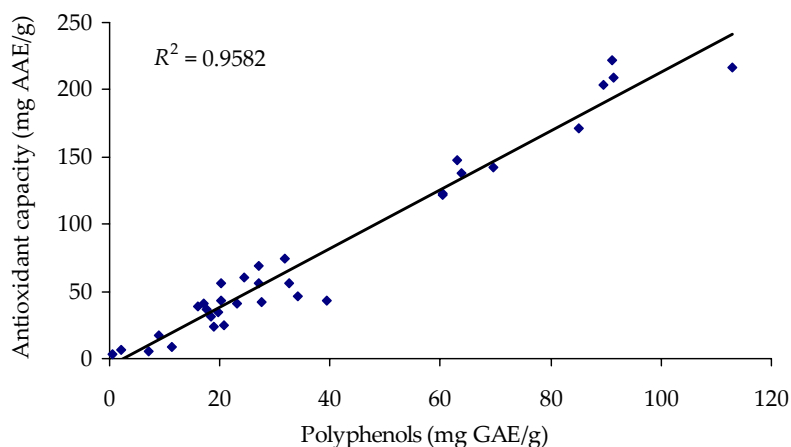


Figure 1. Correlation between total phenolic content and antioxidant capacity of herbs

per gram of dried leaves. Besides that, the repeatability of the method was determined by analysing the antioxidant capacity of nine oregano samples. Relative standard deviation (RSD) was 2.79% which shows very good repeatability.

Figure 1 displays a very interesting correlation in the samples between the antioxidant capacity determined by the DPPH method and the total phenolic content. Antioxidant effects will probably depend mainly on the content of total phenolic compounds in the herb. Extremely high antioxidant capacity, comparable with Chinese green tea samples, could be observed in oregano and peppermint extracts. High antioxidant capacity was also found for lemon balm, winter savory, and sage samples. Wild citrus type of peppermint also had higher values than cultured peppermint. This could confirm the literature statements that widely grown plants without fertilisation produce more secondary metabolites for their protection. Also other tested plants from the Lamiaceae family, like marjoram, rosemary, and hyssop, showed quite good antioxidant effects.

The comparison of antioxidant activity results published in many papers is difficult, because the data are significantly influenced by the method of extraction and the analytical method used for their determination. Compounds with different antioxidant capacity are extracted depending on the solvent polarity. Most authors in the studies of this type use concentrations of herbs similar to the portions used in common culinary practice.

The results obtained are in a good agreement with the literature data (DORMAN *et al.* 2004), where the authors determined the antioxidant capacity and total phenolic content of some less well known plants of *Thymbra*, *Satureja* and *Origanum* genera. Only the reported phenolic content of *Satureja*

cuneifolia Ten. was significantly higher than we observed. DORMAN *et al.* (2003) monitored the antioxidant capacity of aqueous herb extracts by different methods (Fe^{3+} reduction, DPPH, ABTS, hydroxyl radical, LDL oxidation). The results are in some cases different according to the analytical method. But generally, according to this study, the highest activity was reported for sage and rosemary. Oregano samples revealed lower values, contrary to our results. DRAGLAND *et al.* (2003) monitored the total antioxidant capacity by the FRAP method in planted herbs and in herbs from the market which are important for culinary use. Their results of antioxidant capacity of samples of the Lamiaceae family of herbs expressed in AAE are very comparable with the data and the order of activity for particular herbs in our study. SHAN *et al.* (2005) determined total antioxidant capacity using the ABTS radical scavenging method and the Folin-Ciocalteu method for 26 methanolic extracts of plants from 12 families. In agreement with our results, oregano had extremely high antioxidant capacity, whereas peppermint, rosemary, and sage had medium activity, and basil had weak activity. A very good correlation ($R = 0.96$) between total phenolic content and antioxidant activity was found by the above mentioned method.

For better results, the antioxidant activities of three phenolic acids and synthetic 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were determined for comparison. The results in AAE are given in Table 2. The highest antioxidant activity of the selected standards was found for gallic acid, which is naturally present linked to other molecules in some kinds of vegetable, green tea, and other food. The results obtained show a very strong antioxidant effect, approximately 3.5 times higher than the antioxidant effect

Table 2. Antioxidant activity of selected standards

	mg AAE/g of standard
Gallic acid	3541.3
Rosmarinic acid	1530.8
Caffeic acid	1484.4
Trolox	791.3

of ascorbic acid. This ratio is about 1.5 in the case of rosmarinic and caffeic acids.

Determination of antioxidant activity of the extracts using the Schaal test Lard was chosen as the fat material for the evaluation of antioxidant activity. Its disadvantage is high stability, but it does not contain natural antioxidants which would affect the results. The weight increase by oxygen absorption and hydroperoxide formation was monitored during the Schaal test. The temperature of 60°C was selected because of practical reasons, i.e. to obtain the results in a reasonable time period. Many published papers use various modifications of the test and different plant oils (for example BEDDOWS *et al.* 2001; HOULOULA *et al.* 2004). A rapid Rancimat test is also often used (BEDDOWS *et al.* 2000; TRIANTAPHYLLOU *et al.* 2001; EXARCHOU *et al.* 2002). Many studies are focused on the use of herb extracts for meat and fish fat stabilisation (RACCANICI *et al.* 2004; BHALE *et al.* 2007; FASSEAS *et al.* 2007; SIMITZIS *et al.* 2008).

Only a small concentration of hydroperoxides is formed during the first oxidation step, where the activity of the antioxidant is important. The dura-

tion of this period is characterised as the induction period. The activity of a particular antioxidant is expressed as the protection factor value, which is the ratio of the inhibited and non-inhibited induction periods of oxidation. Hydroperoxide formation is very fast during the second oxidation step, which reflects the rapid sample weight increase. The used fat loses its quality rapidly during this oxidation stage (POKORNÝ 1991; PÁNEK 2001).

The oxidation changes of lard containing herb extracts are shown in Figure 2, and the samples containing α -tocopherol (as positive control) and pure phenols are shown in Figure 3. The induction periods and protection factor values are summarised in Table 3. The antioxidant capacity of the herb extracts and pure standards is compared with that of α -tocopherol (PF 2.5), which has a significant antioxidant activity in more saturated fats, and with the synthetic hydrophilic analogue 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox).

The protection factors of most herbs (oregano, rosemary, peppermint, hyssop, and savory) are comparable with the tocopherol values. Only the activity of basil was significantly lower and lovage was practically ineffective. On the other hand, marjoram and Greek oregano showed higher activity. Their protection factors were above 3.5 which can be considered as very significant. Sage extracts showed extremely high activity with PF 8.9.

The values of the antioxidant activity of the samples in lard do not correlate with the results using the DPPH method or total phenolics de-

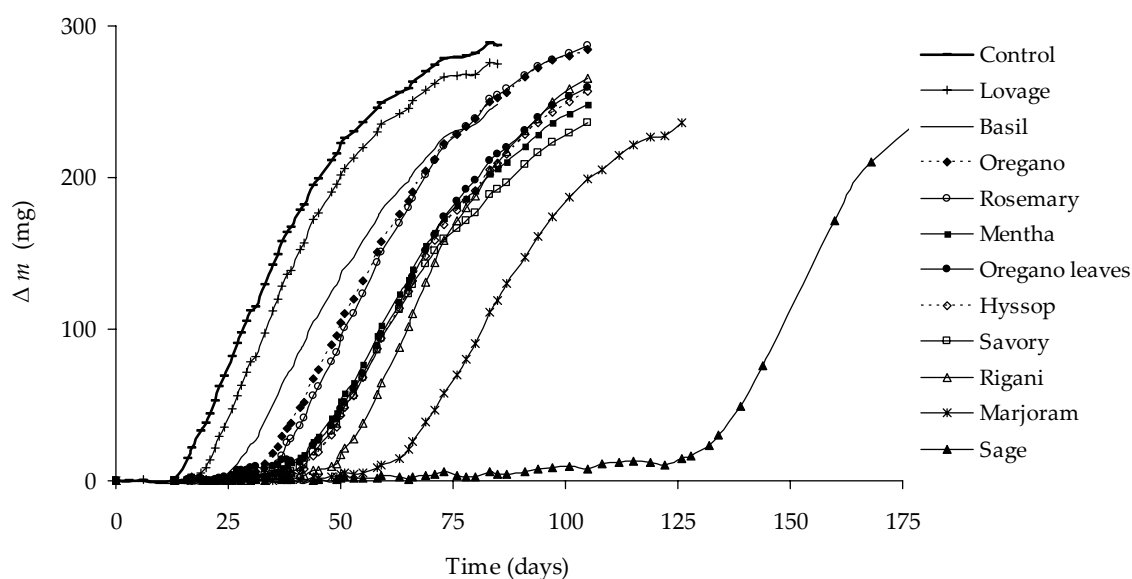


Figure 2. Oxidation of lard inhibited by herbs extracts

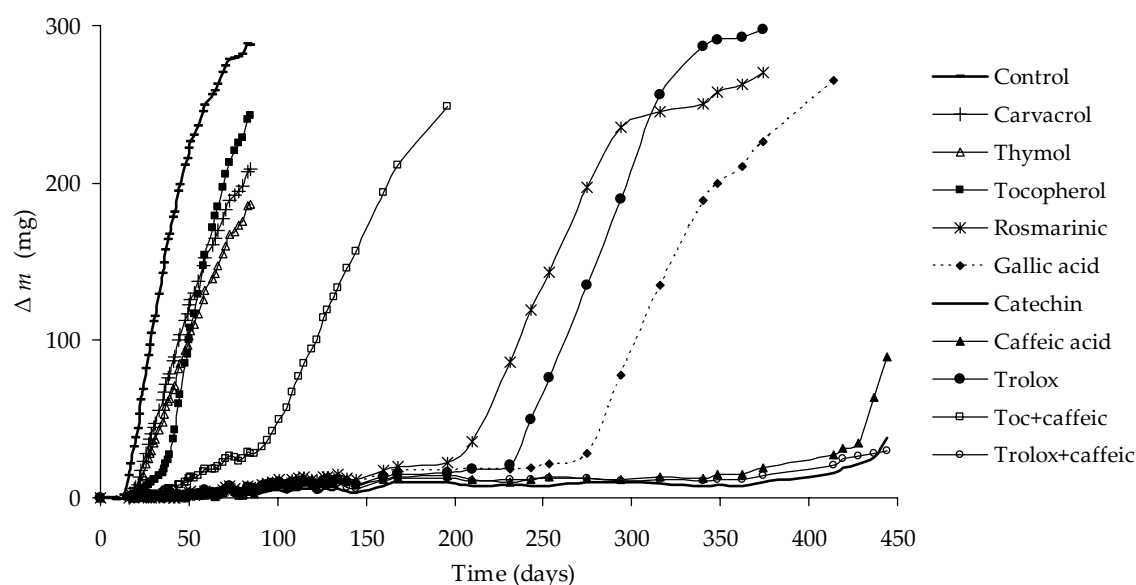


Figure 3. Oxidation of lard inhibited by α -tocopherol and phenolic compounds

termination. Oregano or peppermint showed the highest capacity by the DPPH method, but their values by the Schaal test are close to the average. On the other hand, marjoram and sage, with medium capacity using the DPPH method, were very effective in lard.

Most study results (for example BEDDOWS *et al.* 2000, 2001; EXARCHOU *et al.* 2002; FASSEAS *et al.* 2007; CELIKEL & KAVAS 2008; CALIKOGLU *et al.* 2009) indicate high activity in sage extracts. But none of these papers reported such high values in sage extracts and so different from the values obtained with other plants as we obtained from our results. The main sage antioxidants are rosmarinic acid, carnosic acid,

and α -tocopherol. While the α -tocopherol content is more or less similar in different herb species and varieties, the carnosic acid content can differ by a factor of up to twenty (ABREU *et al.* 2008). This can be the explanation of the differences between our values and the published data.

The activity of marjoram in lard is also surprisingly high, because its effect is usually considered as medium. Only TRIANTAPHYLLOU *et al.* (2001) found results similar to our data. On the other hand, a high antioxidant activity is commonly reported for rosemary extracts (BEDDOWS *et al.* 2000; RACCANICI *et al.* 2004; BHALE *et al.* 2007) as well as oregano (BEDDOWS *et al.* 2001; GOVARIS

Table 3. Values of induction period (IP) and protection factors (PF) of lard with herbs and pure phenolics

	IP (days)	PF		IP (days)	PF
Lard	14.9	1.0	Sage	132.9	8.9
Lovage	18.9	1.3	Carvacrol	20.0	1.3
Basil	25.2	1.7	Thymol	20.0	1.3
Oregano	32.1	2.2	Tocopherol	37.8	2.5
Rosemary	33.8	2.3	Tocopherol + caffeic acid	94.6	6.3
Peppermint	37.1	2.5	Rosmarinic acid	203.6	13.7
Hyssop	42.1	2.8	Trolox	232.4	15.6
Savory	42.2	2.8	Gallic acid	272.1	18.3
Oregano leaves	43.6	2.9	Catechin	> 374	> 25.1
Marjoram	54.3	3.6	Caffeic acid	> 374	> 25.1
Greek oregano	54.5	3.7	Trolox + caffeic acid	> 374	> 25.1

et al. 2004; HOUHOULA *et al.* 2004, BHALE *et al.* 2007; FASSEAS *et al.* 2007; SIMITZIS *et al.* 2008; AMAROWICZ *et al.* 2009). However, we have found the antioxidant activity in lard to be only moderate. The moderate or weak activity of other plants agrees with literature conclusions.

The antioxidant activity of pure synthetic antioxidant compounds was monitored at the same time, using the same methods and lard, as for the herb samples. Phenolic compounds with reported antioxidant activity were chosen with respect to their natural presence in plants. Simple phenols like thymol and carvacrol are the main phenolic components of oregano, savory, and other herbs oils (KULISIC *et al.* 2005; HRÁDKOVÁ *et al.* 2009; OKE *et al.* 2009 etc.). Caffeic acid and gallic acid are naturally found in the majority of herbs and are present in the free form or commonly bound as esters, depsides, and/or glycosides. Rosmarinic acid is a typical active compound of sage, marjoram, oregano and other herbs; (+)-catechin is free or as an ester mainly with gallic acid (ZIAKOVÁ & BRANDŠTETEROVÁ 2003; YANISHLIEVA *et al.* 2006; KIVILOMPOLO & HYÖTYLÄINEN 2007; KULISIC *et al.* 2007; VELÍŠEK 2009 etc.).

The induction period of hydrophilic Trolox is significantly longer than the IP of α -tocopherol. This phenomenon is described in many papers as the so-called polar paradox. The antioxidant activity of thymol and carvacrol (PF 1.3) is very low. However, both these compounds significantly slowed down the second fast oxidation period. The activity of caffeic acid, gallic acid, rosmarinic acid, and catechin was extremely high at the applied temperature and concentrations. One year after the beginning of the experiment, the samples of catechin and caffeic acid in lard were still in the first stage of the induction period. Their PFs were higher than 25. The use of a combination of α -tocopherol and caffeic acid showed a significant reduction of antioxidant activity, compared to the effect of caffeic acid alone. This reflects the possible prooxidant effect of tocopherol. This effect was not observed in the case of a caffeic acid and Trolox combination.

CONCLUSIONS

The activity of plant extracts from the family Lamiaceae and other selected herbs against undesirable oxidation processes in food was evaluated

according to their ability to scavenge the DPPH radical and their ability to inhibit fat autooxidation in lard. The determination by the DPPH radical scavenging method was investigated together with spectrophotometric determination of the total phenolic content of herb extracts.

Hot water as the extraction agent was used because of the hydrophilic character of the compounds with expected antioxidant activity, because of similar procedures in the literature, and also because water is the main medium used to prepare foods for diets for patients suffering from medical conditions.

The following main conclusions were obtained by using the DPPH radical method:

Very good repeatability of the determination; RSD ($n = 9$) was below 3%.

There was a significant correlation ($R > 0.95$; $n = 34$) between the antioxidant capacity and total phenolic content of extracts.

The extracts of oregano, peppermint, and lemon balm showed the highest antioxidant activity comparable to the activity of green tea extracts. Hyssop, sage, winter savory, rosemary and Greek oregano had moderate activity.

Antioxidant activity in lard monitored by the Schaal test was evaluated by the ability of the antioxidant to extend the induction period, and protection factors were calculated. The results are different from those found in the DPPH assay, because of the lipophilic medium and different types of reactions. Besides the ability to inhibit radical reactions, there is also the ability to reduce hydroperoxides as they are formed. Both types of reactions lead to the retardation of the autooxidation reaction.

The extracts of sage, marjoram, and Greek oregano showed very high antioxidant effects. Moderate activity, comparable to the effect of α -tocopherol, was found in the extracts of peppermint, oregano, rosemary, winter savory, and hyssop while the activity of basil and lovage was negligible.

To make a comparison, the activity of phenolic antioxidant standards was tested. Gallic, caffeic, and rosmarinic acids, catechin and the synthetic Trolox showed very high activity, while the activity of thymol and carvacrol extracts was very low.

The majority of herbs potentially applicable to flavour meals showed significant antioxidant effects. This fact, together with the increase in taste diversity, could contribute to the justification of the increased consumption of these important food commodities.

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