ANTIOXIDANT ACTIVITY OF *LAMIACEAE* HERBS GROWN UNDER ORGANIC AND CONVENTIONAL FARMING*

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Lamiaceae herbs produce varieties of secondary metabolites which can be effectively used as food antioxidants. Antioxidant potential of selected widely used culinary herbs (two oregano species – *Origanum vulgare* L. and *Origanum heracleoticum* L. and spearmint *Mentha spicata* L.) from organic and conventional farming was therefore tested. Activity of aqueous extract was measured by using diphenylpicrylhydrazyl (DPPH) radical scavenging method, total phenolics content was determined by using the Folin-Ciocalteu reagent method. Antioxidant activity of both fresh and dried herbs was tested by the Schaal test based on the monitoring of the course of fat oxidation gravimetrically with free oxygen access in the dark at 60°C. Essential oils components which play an important role in antioxidant activity of herbs were determined by GC/MS analysis. Herbs from organic farming did not exhibit statistically significant higher antioxidant activity than those grown conventionally. Higher antioxidant activity of dried herbs aqueous extract than of extracts from fresh herbs was detected. The activity of herb extracts was different in hydrophilic and lipophilic matrices. Spearmint was the best antioxidant in an aqueous medium, while the effect of Greek oregano was ideal if fat containing medium was used. Its considerable antioxidant effect was because of high content of carvacrol and thymol in its essential oil.

Greek oregano; oregano; spearmint; Schaal test; DPPH method; phenolics

INTRODUCTION

Lipids, especially unsaturated fatty acids, belong to very reactive compounds present in food. Unsaturated fatty acids are usually slowly oxidized to hydro peroxides at lower temperatures (storage of food). Aldehydes, aldehydic fatty acids, and other products are consequently formed (Bilek, 2000; Frankel, 2005; Chrpová et al., 2010). Plant materials containing phenolic constituents are increasingly of interest because they inhibit oxidative degradation of lipids and therefore improve quality and nutritional value of food. Phenolics are vital substances that possess the ability to protect the body from damage caused by free radical-induced oxidative stress (Khalil et al., 2007). Medicinal plants are traditionally used in folk medicine as natural healing remedies with therapeutic effect. Pharmacological industry utilizes medicinal plants for their content of active chemical substances. Due to their preservative effects caused by the presence of antioxidant and antimicrobial constituents, they are valuable additives used also in food and cosmetic industry. Plants from several families, especially *Lamiaceae* (rosemary, sage, oregano, marjoram, basil, thyme, mints, balm), *Apiaceae* (cumin, fennel, caraway), and *Zingiberaceae* (turmeric, ginger) belong to commonly used medicinal plants with antioxidant activity known worldwide (Zeuthen, Bogh-Sorensen, 2003; Bouayed et al., 2007; Viuda-Martos et al., 2010; Škovránková et al., 2012). Culinary herbs are considered to be rich sources of antioxidants in direct consumption and in processed stage as well. Processing can cause degradation of many groups of biologically active chemical substances, which leads to their content reduction (Vábková, Neugebauerová, 2011). Plant antioxidants consist of a wide range of different substances such as ascorbic acid, tocopherols, polyphenolic compounds, or terpenoids. They perform several important funcions in plants and humans (Grassmann, 2005).

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Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as additives in foods to prevent oxidation of lipids. Their concentrations in food are restricted by legislative rules. There is a growing request for and interest in natural and safer antioxidants in food applications, and a growing trend as concerns consumers' preferences for natural antioxidants (Pokorný et al., 2001; Elmasta et al., 2005; Gülçin et al., 2006a, b). According to Faller, Fialho (2010), organic food is considered as food with a higher antioxidant capacity, because organic farming may induce the synthesis of secondary substances such as polyphenols. Pokorný et al. (2001) reported that organic products usually contain more phenolic and similar stress factors and less fat than conventional, normal products because of less favourable growing conditions.

MATERIAL AND METHODS

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

Standards. Ascorbic acid p.a. (Penta, Prague, Czech Republic), gallic acid (Sigma-Aldrich).

Herbs (fresh and dried leaves). Oregano *(Origanum vulgare* L.*),* Greek oregano (*Origanum heracleoticum* L.), and spearmint (*Mentha spicata* L.); all these pe rennial plants were planted under conventional and organic farming conditions in the experimental station of the Czech University of Life Sciences Prague, in Prague-Troja. The organic and conventional experimental fields were located at the same place. They were separated by a mechanical barrier in accordance with organic farming legislation. Oregano and Greek oregano were one-year-old plants, spearmint was 4 years old. Mineral fertilizer YaraMila^{TM} COMPLEX (Yara International ASA, Oslo, Norway) in the amount of 8.3 g/m^2 was applied to the conventional field while the same amount of the organic fertilizer Organica N (McMon B.V., Arnhem, the Netherlands) was applied to the ecological field. Fresh and dried leaves from two harvests were used for analysis. Leaves were collected before flowering at the end of June and at the beginning of September. They were dried in the open air at room temperature.

Pork lard. The lard used for the experiments was produced by Masokombinát Plzeň (Plzeň, Czech Republic) and its expiry dates were as follows: 8/2011 (for fresh herbs from the 1st harvest), 11/2011 (for fresh leaves from the 2nd harvest and dried leaves from the 1st harvest), 1/2012 (for dried leaves from the 2nd harvest).

Software programs. Data were processed using the programs MS Excel 2010 and STATISTICA, Version 10, 2011.

Herbs extraction. Fresh leaves (8 g) or the equivalent amount of dried leaves (1.6 g) were taken for the extraction. Average dry matter content of fresh leaves was 16%, of dried leaves 80%. The leaves were extracted twice in 50 ml of hot (70°C) demineralized water. Each extraction with occasional mixing lasted for 10 min. Samples were filtered after extraction.

Determination of antioxidant activity by the DPPH method. Antioxidant activity of the water extract was measured in terms of radical scavenging ability using the stable radical DPPH. The method is based on the measuring intensity of the violet DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical solution at 522 nm. The radical is decolourized by compounds with antioxidant activity. The method was calibrated with ascorbic acid and the results were expressed as equivalents of ascorbic acid per unit mass of sample and recalculated per dry mass. This method was taken from Chrpová et al. (2010), Buřičová et al. (2011), and Koksal et al. (2011).

Determination of total phenolic compounds (TPC). The content of total phenolics was determined spectrophotometrically at 760 nm wavelength by using the Folin-Ciocalteu reagent. The results were expressed as the content of gallic acid per unit mass of the sample and recalculated per dry mass. The method described by Dorman et al. (2003) and Stratil et al. (2008) was slightly modified (60 min incubation in the dark).

Determination of antioxidant activity by the Schaal test. The principle of this method is based on gravimetric monitoring of fat oxidation with free oxygen access in the dark at 60°C. Weight changes were monitored in two paralel determinations in lard without additive and in lard with the addition of fresh (80 g/kg lard) or dried (16 g/kg lard) herbs. The ratio of fresh and dried herbs was calculated on their dry matter content values. Active compounds from leaves were transferred into lard by diffusion. Induction period (IP) value as a criterion of stability of lard during storage was determined by mathematical linear regression from relative weight changes (RWC):

RWC = ((sample weight on a given day) – (sample weight at the beginning of the experiment))/(sample weight at the beginning of the experiment)

Protection factor (PF) was calculated as follows:

PF = IP with additive/IP without additive

where:

PF = criterion of antioxidants activity (in this case the mixture of antioxidants from the relevant herb)

The method was taken over from Frankel (2005) and Chrpová et al. (2010).

Essential oils. Essential oils were obtained by hydrodistillation of herbs using Clevenger apparatus. The method was taken from The Czech Pharmacopoeia (Pharmacopoea Bohemica, 2009) and modified (distillation time only 180 min). The qualitative and semi-quantitative determination of essential oils volatile substances was carried out using the Agilent

Table 1. Antioxidant activity of selected herbs measured by DPPH method

Herb	Harvest	Herbs condition	DPPH (mg AA/100 g)			
			organic		conventional	
				to dry mass		to dry mass
Oregano	1.	fresh	1190±60	4310±217	810±47	2860±166
		dried	5140±240	6500±304	3690±175	4580±217
	2.	fresh	1980±95	8330±400	1160±63	4830±262
		dried	3760±198	4320±227	15560±731	17840±838
Greek oregano	1.	fresh	510±30	1970±116	540±33	2190±134
		dried	13160±530	16660±671	10420±520	13240±661
	2.	fresh	1240±73	5560±327	1550±80	7740±399
		dried	11560±428	13470±499	4920±235	5720±273
Spearmint	1.	fresh	290±27	1510±141	650±41	4010±253
		dried	17570±645	21820±801	9230±401	11880±516
	2.	fresh	970±51	5590±294	1170±63	7530±405
		dried	14800±740	17410±871	6400±289	7640±345

AA = ascorbic acid

6890N GC with the Agilent 5973 quadrupole mass detector system (Agilent Technologies, Palo Alto, USA); column HP Innowax (Agilent Technologies), length 30 m, internal diameter 0.25 mm, and film thickness 0.25 µm; carrier gas He; inlet 220°C; column temperature was programmed from 40 to 240°C with a linear gradient (40°C per 3 min, then 5°C/min to 150°C, 10°C/min to 240°C, and hold at this temperature for 30 min).

RESULTS AND DISCUSSION

Antioxidant activity determined by the DPPH, TPC and Schaal test

Spectrophotometric determination of antioxidant activities of oregano, Greek oregano, and spearmint by the DPPH method was monitored in two parallel determinations. The results were calculated in mg of ascorbic acid per 100 g and recalculated per dry mass of the samples (Table 1). Spectrophotometric monitoring of the TPC in herbs was realized in two parallel determinations. The results of TPC (Table 2) are expressed in mg of gallic acid per 100 g and also recalculated per dry mass. From the results (Table 1, 2) it can be seen that oregano, Greek oregano, and spearmint show great scavenging ability and also contain large amounts of phenolic compounds. However, Kahkonen et al. (1999) stated that antioxidants activity does not necessarily correlate with high amounts of phenolics. Herbs from *Lamiaceae* family show a significant antioxidant activity, which is attributed to the content of phenolic compounds (Dorman et al., 2003; Kiselova et al., 2006; Coultate, 2009; Chrpová et al., 2010; Gramza-Michalowska et al., 2011). The content of these compounds in plants is affected by endogenous (genetic) and exogenous factors such as location, climatic conditions, altitude, sunlight, etc. Plants that are exposed to the stress factors of the environment (e.g. lack of nutrients, pests, etc.) tend to create more secondary metabolites including phenolic compounds, flavonoids, and essential oils, which have antioxidant activity. Wild herbs usually contain maximum of secondary products and exhibit a higher antioxidant activity than herbs from agricultural planting. Organic agriculture generally produces food with a similar or slightly higher content of polyphenols and higher antioxidant capacity than the conventional farming system (Faller, Fialho, 2010; Škovránková et al., 2012). In our experiment, statistically significant differences were observed only between types of processing. Dried herbs had generally higher content of phenols and higher scavenging activity. This fact can be caused by the damage of the plant tissues during the drying process. Antioxidants are probably more extractable from dried tissues. Our results are not in concordance with some literature sources e.g. Capecka et al. (2005).

Concerning antioxidant activities and phenolic content, no statistically significant differences between organic and conventional farming were observed. These results are inconsistent with the findings of Faller, Fialho (2010) and other authors. In the design of our experiment, nutrition requirements of plants were ensured at both farming conditions. Plants were not in the state of metabolic stress and therefore they

Table 2. Total phenolic compounds (TPC) of selected herbs

Herb	Harvest	Herbs condition	TPC (mg GA/ 100 g)			
			organic		conventional	
				to dry mass		to dry mass
Oregano	1.	fresh	530±55	1930±200	580±61	2050±216
		dried	3310±301	4190±381	3390±284	4210±353
	2.	fresh	920±89	3870±374	740±73	3080±304
		dried	3530±289	4050±332	2790±241	3200±276
Greek oregano	1.	fresh	460±51	1790±198	380±43	1520±172
		dried	4590±485	5810±513	2900±246	3680±312
	2.	fresh	700±70	3510±351	590±61	2960±306
		dried	3400±302	3970±353	3480±281	4050±327
Spearmint	1.	fresh	670±71	3540±375	420±46	2580±283
		dried	5790±458	7190±569	4320±359	5560±462
	2.	fresh	400±45	2280±257	340±38	2190±245
		dried	3030±258	3560±303	3010±269	3590±321

GA = gallic acid

probably did not create a greater amount of secondary metabolites. Differences in antioxidant activity and phenolic content between the first and the second harvest were statistically insignificant.

Some differences between samples were found during the assessment of the antioxidant activity by the Schaal test. Pork lard was chosen as the model edible fat material for the antioxidant activity evaluation. It does not contain significant concentrations of natural antioxidants which would affect the results. Induction period was deducted from the charts illustrating the relative weight changes of the samples (dried herbs from the first harvest) at the time of fat oxidation (Fig. 1). All the tested herbs from organic and conventional farming have a strong ability to delay the oxidation of fats. This ability was more significant in

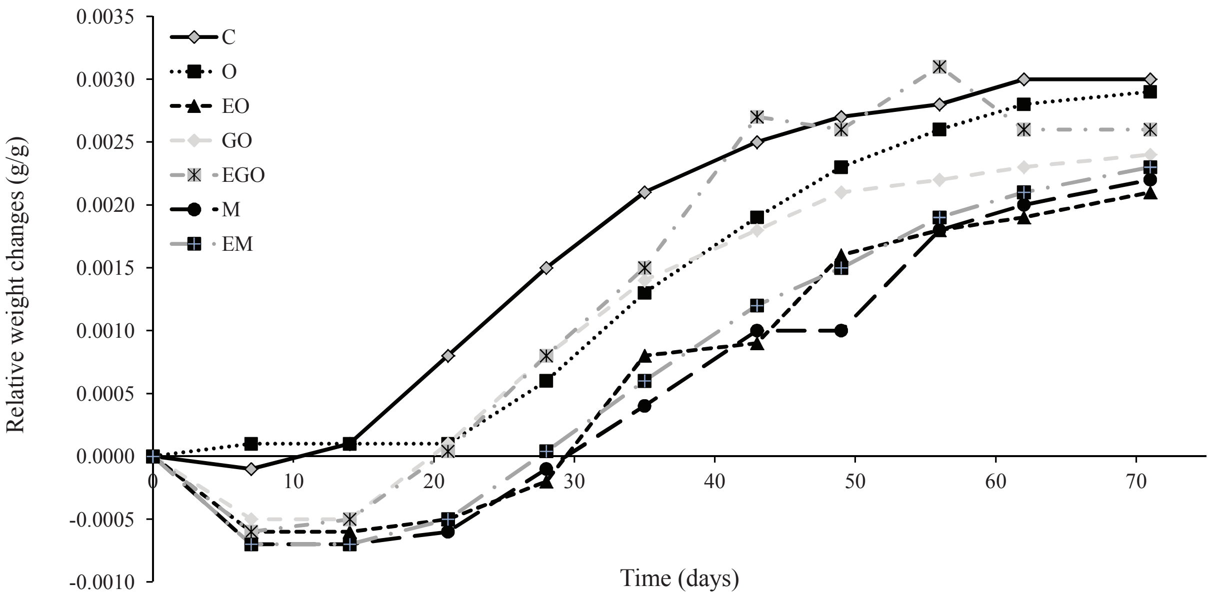

Figure 1. The time course of oxidation of herbs from conventional and organic farming

C - control (lard), GO – conventional Greek oregano, EGO – organic Greek oregano, O – conventional oregano, EO – organic oregano, M – conventional spearmint, EM – organic spearmint

Table 3. Schaal test results - induction periods (IP) and protection factors (PF) of selected herbs from two harvests and two different farming methods

Herb	Harvest	Method of cultivation	Fresh		Dried	
			IP	PF	IP	PF
Pork lard (control)	1.		10		9	
	2.		8		6	
Oregano	1.	Organic	51	5.1	12	1.3
		Conventional	60	6.0	15	1.7
	2.	Organic	41	5.1	15	2.5
		Conventional	46	5.8	17	2.8
Greek oregano	1.	Organic	56	5.6	23	2.6
		Conventional	56	5.6	24	2.7
	2.	Organic	53	6.6	32	5.3
		Conventional	40	5.0	31	5.2
Spearmint	1.	Organic	38	3.8	18	2.0
		Conventional	53	5.3	20	2.2
	2.	Organic	41	5.1	18	3.0
		Conventional	35	4.4	16	2.7

Note: Induction periods of two parallel determinations of the same sample were identical or the difference between them was not more than one day.

the case of fresh herbs. Greek oregano and oregano were the best herbal lipids antioxidants. The effect of the growing method (organic, conventional) on individual herbs was not clear and statistically insignificant.

Significant differences were found between the antioxidant activity of fresh and dried herbs. Herbs may lose up to 2/3 of valuable substances (including unstable ascorbic acid) during the drying procedure depending on its temperature and the way of processing. In our experiments drying significantly shortened the induction period of all the tested herbs. Protection factors of dried herbs were significantly ($P < 0.05$) lower than those of fresh herbs (Table 3).

Any correlation between the analytical methods used is not relevant because each method has a completely different reaction scheme. The differences between the tested herbs in the hydrophilic medium (DPPH and TPC methods) were statistically insignificant. On the other hand, the inhibitory activity of spearmint was significantly lower than the activity of oregano and Greek oregano in the liphophilic medium (Tables 1–3).

Essential oils in herbs

According to the literature statements, from the substances contained in essential oils only thymol and carvacrol may show a practically significant antioxidant activity (Boskou, 2006; Politeo et al., 2006; Chrpová et al., 2010). In the group of the herbs tested, these compounds occur only in Greek oregano. On the other hand, it was found out that the concentration of other substances in essential oils can significantly vary depending on whether the plant was grown organically or conventionally.

Composition of essential oils (Table 4) of organically and conventionally grown Greek oregano was mainly different in the ratio of carvacrol:thymol (organic Greek oregano 14:1, conventional Greek oregano 40:1). Composition of essential oils present in oregano and Greek oregano differed even though they both belong to the same genus, *Origanum* L.

In case of organic and conventional variants of oregano, various ratios were found between the (+)-linalool and (-)-β-caryophylene contents (organic oregano 2.1:9.3, conventional oregano 4.4:5.9) and also between the contents of (-)-β-phellandrene and (E)-β-ocimene (Table 4). But in general the differences in the content and composition of essential oils did not depend on the method of cultivation (organic and conventional farming) (Table 4). The most typical component of essential oils of *Mentha spicata* L. is (-)-carvone, which was found in the prevailing concentration in all samples of this herb. *Mentha spicata* does not contain menthol which is typical for *Mentha x piperita* and has a significant antioxidant activity.

Harvest time has a great effect on the composition of essential oils as it is seen in the essential oils of spearmint. The content of (+)-limonene significantly decreased during the second harvest. Essential oils from the second harvest had significantly lower content of (-)-carvone and significantly higher content of its precursors (+)-*trans*-carveol and dihydrocarvone. This fact could indicate a slowing of metabolic processes in the autumn, when the second harvest was realized.

Table 4. Composition of essential oils in tested herbs

Coumpound	Content (%)						
	GO	EGO	O	EO	M	EM 1	EM 2
myrcene	3.0	3.8	2.9	3.0	1.3	1.1	0.8
(*E*)-β-ocimene	-	-	18.3	21.2	-	-	-
p-cymene	8.7	8.2	-	-	-	-	-
(+)-limonene	-	-	-	-	11.0	10.6	7.1
α-terpinene	2.7	3.4	-	-	-	-	-
γ-terpinene	15.3	15.7	-	-	-	-	-
(-)-β-phellandrene	-	-	25.0	21.0	1.0	0.9	0.7
α-pinene	1.5	1.9	0.7	0.5	1.9	1.7	1.4
β-pinene	-	-	0.5	0.7	1.8	1.7	1.4
p-cymene	-	-	2.0	1.8	-	-	-
ocimene	-	-	8.0	7.2	-	-	-
2-thujene	1.4	1.5	0.3	0.4	-	-	-
(3*Z*,6*E*)-α-farnesene	-	-	1.9	3.5	-	-	-
(-)-β-bisabolene	1.6	1.6	-	-	-	-	-
(-)-germacrene D	-	-	11.2	16.2	-	-	-
(-)-β-caryophyllene	3.5	4.4	5.9	9.3	3.9	2.9	4.1
β-bourbonene	-	-	-	-	5.0	3.9	4.3
(+)-linalool	-	-	4.4	2.1			
1,8-cineol	-	-	2.0	1.5	4.8	4.9	3.6
(+)-terpinen-4-ol	1.1	1.6	-	-	-	-	-
(+)-*trans*-carveol	-	-	-	-	-	-	5.9
(-)-carvone	-	-	-	-	52.0	54.8	42.3
dihydrocarvone	-	-	-	-	12.7	13.4	25.0
3-octanol	-	-	-	-	2.5	2.3	2.1
1-octen-3-ol	-	-	2.5	2.0	-	-	-
carvacrol	59.8	54.2	-	-	-	-	-
thymol	1.5	3.8	-	-	-	-	-
elixene	-	-	2.9	-	-	-	-
cis-jasmone	-	-	-	-	2.1	1.9	1.2
δ-cadinene	-	-	1.1	1.6	-	-	-
caryophyllene oxide	-	-	3.4	2.3	-	-	-
germacren-4-ol	-	-	1.4	1.3	-	-	-
(-)-spathulenol	-	-	3.9	2.3	-	-	-
α-cadinol	-	-	1.8	2.4	-	-	-

GO – conventional Greek oregano, EGO – organic Greek oregano, O – conventional oregano, EO – organic oregano, M – conventional spearmint, EM1 – organic spearmint from the first harvest, EM2 – organic spearmint from the second harvest

CONCLUSION

Determination of antioxidant activity using the DPPH method, TPC method, and the Schaal test showed that herbs grown under organic conditions do not have statistically significantly higher antioxidant capacity than conventional plants. Significantly lower values of antioxidant activity monitored by the DPPH and TPC methods for fresh plant was caused by larger disruption of tissues of the dried plant material. The effectiveness of herbs as natural antioxidants is in correlation with the composition of their essential oils and the content and structure of phenolic compounds. The composition of essential oils of oregano and Greek oregano depends on conventional or organic farming method, while the composition of spearmint essential oils was much more affected by the harvest time.

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