

Full Length Research Paper

The effect of concentration on the antioxidant activity of selected culinary herbs

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Antioxidant activity of selected widely used culinary herbs (oregano, Greek oregano, marjoram, summer savory, rosemary and two varieties of leafy parsley) was monitored to see the effect of increasing herb content on the inhibition of pork lard oxidation. The activity of tested dry herbs was significant (protection factors were from 1.7 to 11.4) and linearly increased at all range of concentrations from 10 to 100 g/kg. No prooxidant effect occurred under the Schaal test conditions. The antioxidant activity of plants decreased in the following order: marjoram > Greek oregano > flat parsley > rosemary > summer savory > curly parsley > oregano, which did not correspond clearly with their total phenolics content.

Key words: Antioxidant activity, *Lamiaceae*, *Apiaceae*, prooxidant effect, Schaal test.

INTRODUCTION

Plant secondary metabolites are an enormously variable group of phytochemicals with antioxidant properties that can prevent oxygen-based damage to cell structures throughout the body (Zia-Ul-Haq et al., 2008; 2011a, b). They predominantly occur as glycosides but the sugar and the corresponding aglycone are released by enzymatic and hydrolytic reactions (Basile et al., 1999). The botanical families *Lamiaceae* and *Apiaceae* are widely used as culinary herbs to enhance the flavour of food. They contain a large number of plants well known for their antioxidant properties. Oregano, marjoram, rosemary and savory have been widely studied, and the majority of their antioxidant components (rosmarinic, carnosic and caffeic acids, rosmanol, thymol, carvacrol, carnosol, luteolin etc.), have been described (Chen et al., 1992; Frankel et al., 1996; Ollanketo et al., 2002; Pizzale et al., 2002).

The antioxidant effect of parsley is mainly caused by natural antioxidants such as polyphenols, ascorbic acid,

α -tocopherol and selenium (Lachman et al., 2000). The two major phenolic compounds extracted from parsley were identified as apiin and malonyl-apiin (Cazzola et al., 2011; Hossain et al., 2011; Luthria, 2008; Yildiz et al., 2008).

Parsley also contains a high level of quercetin glycoside rutin (Lachman et al., 2000). It is thought that all these components are principally responsible for the health-promoting properties of culinary herbs and spices. Furanocoumarins, such as bergapten, xanthotoxin, isopimpinellin and psoralen, with efficient antibacterial and antifungal properties, represent another efficient group, which, however, should be consumed under control, because they could cause hepatotoxicity and injure renal function (Lachman et al., 2000; Wang et al., 2012).

The advantage of crude herbs or their extracts is that they are generally regarded as safe (GRAS) and therefore better accepted by consumers than synthetic

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antioxidants (Rizwan et al., 2012). Some dietary food supplements containing various antioxidants, can also exhibit prooxidant effects under certain conditions and when taken in high quantities.

This behaviour was seen in the case of vitamin A and E (Bowry et al., 1992; Müller et al., 2011; Traber and Stevens, 2011; Young and Lowe, 2001). Prooxidant behaviour of phenolics depends on many factors such as metal-reducing potential, chelating behaviour, pH, and solubility characteristics (Decker, 1997; Simić et al., 2007). Their possible prooxidation activity *in vivo* has been discussed by Halliwell (2008). *In vitro* prooxidant effects of some phenolics on DNA from aloe were described by Tian and Hua (2005).

On the other hand, one disadvantage of natural antioxidants is that they are available from raw materials of variable composition depending on the plant variety, growing, harvesting, postharvesting and climatic conditions, and many other factors. For example, light stimulates creation of phenolic acids and flavonoids which results in higher antioxidant activity of plants grown in the field than in greenhouses (Hunt and Baker, 1980). Comparison of results from different literature sources is therefore difficult partly because of the factors mentioned above.

In the case of herb extracts, authors also use different solvents and ways of extraction which makes results more difficult to compare (Liu et al., 2007; Mishra et al., 2003; Petersen and Simmonds, 2003; Wang et al., 2002). Therefore, our samples were grown and treated under the same conditions and applied to the tested material directly without any extraction. The antioxidant activity of plants is usually determined by various chemical methods [2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), Oxygen radical absorbance capacity (ORAC), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) etc.]. However studies that have data evaluated their potential in real food systems has been scarce.

Therefore, the aim of this work was firstly to compare the antioxidant activity of selected widely used culinary herbs in real food materials containing no other natural antioxidants (pork lard). Literature data often compare antioxidant activities of plants at one concentration, so the main reason for doing this research was to monitor the effect of increasing herb content on the antioxidant activity and to find out if possible prooxidant behaviour occurred in samples with addition of herbs at higher concentrations.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu phenol reagent, Merck, Germany; Standards: ascorbic acid and gallic acid, Sigma- Aldrich, USA.

Herb samples growing and preparation

Antioxidant capacity was monitored in the following plants: oregano (*Origanum vulgare* L.), Greek oregano (*O. heracleoticum* L.), marjoram (*O. majorana* L.), summer savory (*Satureja hortensis* L.), rosemary (*Rosmarinus officinalis* L.) and two varieties of leafy parsley (*Petroselinum crispum* (Mill.) Nyman ex A.W.Hill): curly leaf form (var. *crispum*) and flat leaf (var. *neapolitanum*). Seeds of plants were purchased from Semo Company (Smržice, Czech Republic) and seeded in greenhouses at the end of March. After one month, they were pricked out and grown in the Czech University of Life Sciences experimental field (50° 7' 22.486" N, 14° 23' 58.181" E, 196 m above sea level) without any fertilizer addition. The soil was classified as loamy sand and less permeable (pH 6.49; cation exchange capacity 171 mmol/kg; Ca, Mg, K and P content was 4.3, 0.3, 0.5 and 0.2 g/kg, respectively). Plants were cut at the end of July and dried at 40°C in a dryer with air circulation for 30 h.

Chemical analysis

Dry matter content of herbs was analysed by using infrared balances (Precisa 310M, Precisa HA300, Precisa Instruments AG, Switzerland) [drying at 102 ± 2°C, constant weight at difference less than 1 mg]. The content of total phenolics was determined spectrophotometrically by using the Folin-Ciocalteu reagent (Dorman et al., 2003). Despite the fact that many phenolic compounds are lipophilic, hydrophilic extraction was chosen as it is widely used in the literature, and because phenolic glycosides are more hydrophilic than their aglycones. Our extracts from 1.5 g of dried herb leaves were prepared by twice repeated extraction with boiling demineralised water (2 × 50 ml). Extracts were left in a water bath at 70°C for 10 min and then filtered into a 100 ml volumetric flask. These stock solutions of herb extracts were diluted to a concentration for which the absorbance was within the range of a calibration curve prepared with a gallic acid standard. Results were expressed as the content of gallic acid (GA) per unit mass of sample (Singleton et al., 1999).

Antioxidant activity determination

Antioxidant activity by the Schaal test (Chrpová et al., 2010; Kulisic et al., 2005) was based on the monitoring of the course of fat oxidation gravimetrically with free oxygen access in the dark at 60°C. Weight changes were monitored in two parallel determinations in pork lard without additive and with the addition of 0.25 to 2.5 g of dried herbs per 25 g of lard (10 to 100 g/kg of lard). The protection factor (PF) was expressed as the prolongation of induction period in comparison with the control sample (Pokorny et al., 1992; Holasova et al., 2002) and calculated by the formula: PF = Induction period (IP) with additive/IP without additive.

Statistical analysis

Linear regression equations, as well as regression coefficients (R^2), were calculated from the data using Microsoft Office Excel 2007.

RESULTS

Characteristics of the dried herb samples are given in Table 1. Dry matter content of most samples was between 86 and 87 g/100 g except summer savory where

Table 1. Characteristics of the dried herb samples monitored in the experiment.

Herb	Dry matter content (%)	Total phenolics (mg GA/100 g)
Greek oregano	86.8±0.4	5990±78
Oregano	86.7±0.2	4990±52
Marjoram	86.0±0.4	872±21
Rosemary	86.3±1.1	1380±17
Summer savory	83.6±0.4	1978±40
Curly parsley	86.5±0.9	666±32
Flat parsley	86.2±0.6	766±30

Table 2. Protection factors (PF) of monitored samples with linear regression coefficients R².

Herb	PF10	PF20	PF30	PF40	PF60	PF80	PF100	R ²
Greek oregano	3.1	3.6	4.0	4.7	5.8	6.8	8.6	0.9895
Oregano	1.8	2.2	2.5	2.6	3.4	3.7	5.3	0.9456
Marjoram	3.0	3.9	4.8	6.2	8.1	9.2	11.4	0.9918
Rosemary	2.2	2.7	3.2	4.0	4.6	5.6	7.1	0.9921
Summer savory	2.4	2.9	3.2	3.6	4.6	5.6	6.8	0.9939
Curly parsley	1.7	2.2	2.8	3.4	4.3	4.9	7.0	0.9743
Flat parsley	-	2.6	-	4.5	6.1	7.9	9.4	0.9984

PF10 to PF100 = protection factors of samples with addition of herbs at concentrations from 10 to 100 g/kg.

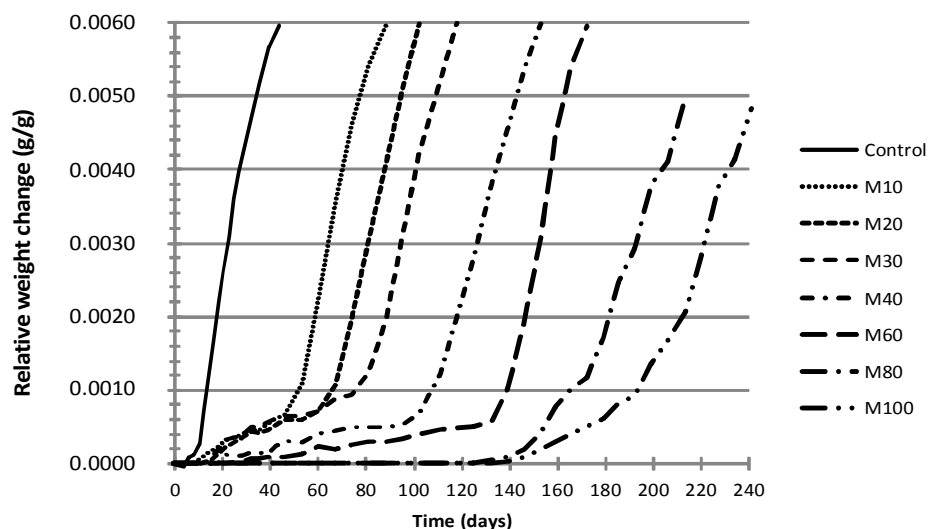


Figure 1. Relative weight changes of marjoram samples under the Schaal test conditions. Control = lard without any herb; M10 to M100 = lard with addition of marjoram at concentrations from 10 to 100 g/kg.

it was a little lower. An example of the course of oxidation of marjoram samples during the Schaal test is given in Figure 1. The addition of herb was able to prolong the induction period from 17 days (control sample: lard without any herb) to 50 days (10 g of marjoram per 1 kg of lard) or 194 days (100 g/kg).

The effect of concentration on the protection factors(PF) of all monitored samples was determined by

linear regression, and the coefficients (R²) are given in Table 2. The examples of linear regression plots curve for the most effective and the least effective herbs are shown in Figures 2 and 3.

The antioxidant activity of plants decreased in the following order: marjoram > Greek oregano > flat parsley > rosemary > summer savory > curly parsley > oregano. This order does not reflect the total phenolics content

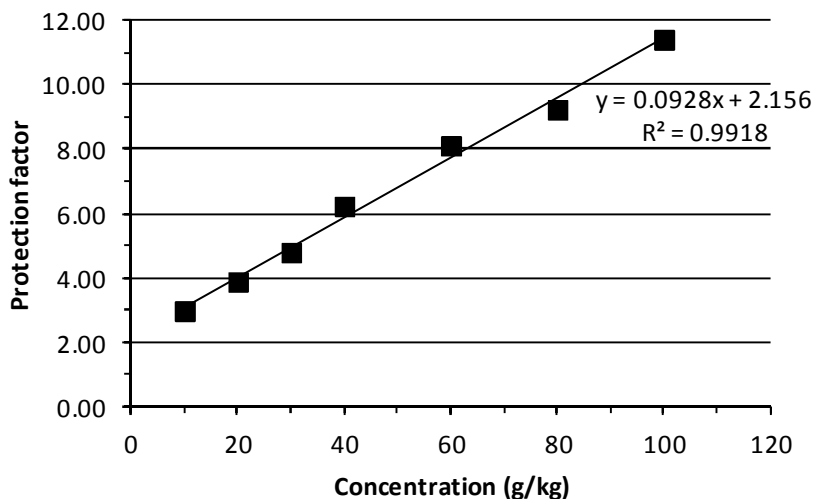


Figure 2. The effect of concentration on the protection factor of marjoram added to lard.

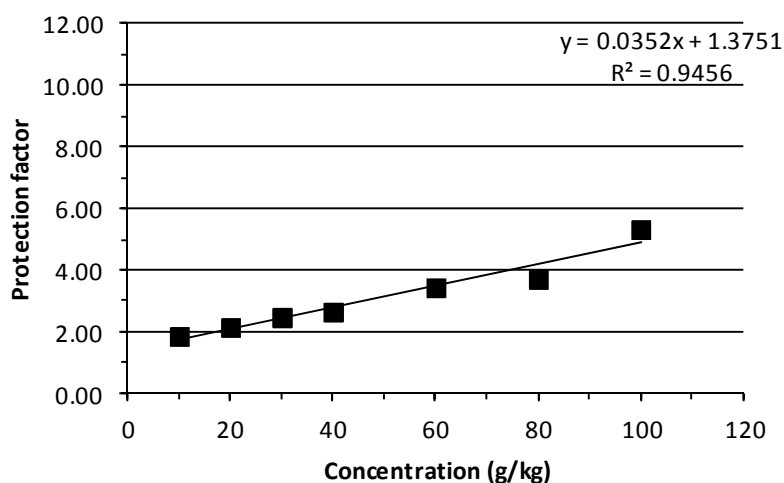


Figure 3. The effect of concentration on the protection factor of oregano added to lard.

(Table 1) which may be due to non-phenolic compounds contributing to the total antioxidant effect (Motamed and Naghibi, 2010) or perhaps some phenolics are more active than others.

DISCUSSION

The high content of total phenolics in oregano and Greek oregano samples, mainly because thymol and carvacrol are major components, is consistent with the literature data (Dzamic et al., 2008). Parsley samples and other *Lamiaceae* herbs contained lower concentrations of phenolics than *Origanum vulgare* and *O. heracleoticum* (syn. *O. vulgare* ssp. *hirtum*) herbs. It is difficult to make

valid comparisons of the total antioxidant capacity and phenol content of herb samples based on different literature sources because of variation in horticulture and extraction methods. Variables in horticultural practice include factors such as location, climate, growing conditions etc., which affect both total antioxidant activity and the content of antioxidants (Hamouz et al., 1999). Nevertheless, significantly higher values of the total antioxidant capacity and total phenolics content for Greek oregano and oregano in comparison with other *Lamiaceae* herbs and other plants (Chrpová et al., 2010) have been widely reported.

The relatively low activity of oregano could reflect the very low activity of thymol and carvacrol observed under the same conditions (Chrpová et al., 2010). This finding is

consistent with the “polar paradox” effect which describes the observation that slightly polar compounds such as esters of phenolic acids, flavonoids and their glycosides show higher antioxidant activity than nonpolar lipophilic compounds in nonpolar medium (for example, in pork lard).

Exarchou et al. (2002), Yanishlieva et al. (2006) and other studies monitoring the antioxidant effect of herbs and their extracts also reported the significant activity of marjoram samples. A protection factor higher than 4 was considered as a very significant value and PF more than 10 as an extremely high value. In our study, we found a PF higher than 4 in marjoram samples at concentration 30 g/kg and in all samples at concentrations 100 g/kg. In the case of marjoram, the PF at 100 g/kg was even higher than 10. Very significant antioxidant activity (but lower than in the case of marjoram) was also found for samples of rosemary, which is very effective for stabilizing the fat in meat products (Rohlik et al., 2010).

Significant antioxidant activity was observed in the case of flat parsley samples. Parsley contains several important redox-active compounds. Besides thermolabile ascorbic acid and carotenoids, it also contains phenolic compounds with an antioxidant potential; mainly the flavones apigenin and luteolin (usually in the form of 7-*O*-glycosides) and gallic acid (Hossain et al., 2011; Luthria, 2008). Phenylpropenoic acid derivatives apiol and myristicin also have antioxidant activity, but they are in high concentration mainly in seeds. The lower activity of curly parsley is consistent with its lower total phenolics content (Table 1). The most important result from our data is that even at high concentrations of herb samples in lard, no prooxidant activity was observed. Liu and Ng (2000) described a slight prooxidant effect of the water extracts of some Chinese medicinal herbs. Motamed and Naghibi (2010) found prooxidant activity of the water extract of *O. vulgare* in the deoxyribose degradation test. Kilicgun and Altiner (2010) reported that *Rosa canina* L. from the Roseaceae family may act not only as an antioxidant, but also as a prooxidant depending on its concentration. This effect did not occur in our *Lamiaceae* and *Apiaceae* dry herb samples under the Schaal test conditions where they were used directly in lard without any extraction. According to Rødtjer et al. (2006), the prooxidant effect of the extracts increased with the polarity of the extraction solvent. The prooxidant activity of some antioxidants in biological systems could also be eliminated by the presence of vitamin C through its regeneration of glutathione and α -tocopherol (Jacobs, 1995).

Conclusion

The antioxidant activity of tested dry herbs from *Lamiaceae* and *Apiaceae* families was significant and linearly increased in the range of concentrations from 10 to 100 g/kg. This research shows that these natural herbs do not exhibit a prooxidant effect under the Schaal test

conditions in lard as a real food material. Besides phenolics, there are several other types of phytochemicals which could be effective in preventing lipid peroxidation. Their synergistic effect must be taken into account when herb samples are tested. Our results also confirm the known fact that, the activity of natural antioxidants in real food material cannot be clearly predicted on the basis of the results from chemical screening tests for antioxidant activity evaluation.

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