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Antioxidant activity of fresh and dry herbs of some *Lamiaceae* species

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Abstract

The herbs of lemon balm, oregano, and peppermint were analysed immediately after harvest and after drying to determine their antioxidant activity and content of total phenolics, L-ascorbic acid, and carotenoids. The strongest inhibition of linoleic acid (LA) peroxidation was found for fresh and dried oregano. For peppermint and lemon balm it was significantly lower and decreased after drying. The ability to scavenge the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was very high in almost all tested samples, exceeding 90%. The three species tested had a very high content of total phenolics and drying of oregano and peppermint resulted in their considerable increase. The highest content of ascorbic acid was determined in fresh peppermint and lemon balm and carotenoid content was at a similar level in all the species tested. Drying caused great losses of these compounds.

Keywords: Ascorbic acid; Antioxidant activity; Carotenoids; *Melissa officinalis*; *Mentha × piperita*; *Origanum vulgare*; Phenolics

1. Introduction

Chemical constituents with antioxidant activity found in high concentrations in plants (Velioglu, Mazza, Gao, & Oomach, 1998) determine their considerable role in the prevention of various degenerative diseases (Challa, Ahmad, & Mukhtar, 1997; Diplock et al., 1998; Hu & Willett, 2002). Besides the fruits and vegetables that are recommended at present as optimal sources of such components, the supplementation of human diet with herbs, containing especially high amounts of compounds capable of deactivating free radicals (Madsen & Bertelsen, 1995), may have beneficial effects (Lutomski, 2001). Although consumption of fruits and vegetables has increased in Poland recently, it is still lower than in the Mediterranean countries. Therefore the incorporation of seasoning based on herbs into everyday meals may be of crucial importance. Bearing in mind that Poland is one of the leading countries in the European production of herbal raw material (Jambor, 2001), enriching the diet with herbs should not meet any obstacles. The benefits resulting from the use of natural products rich in bioactive substances has promoted the growing interest of pharmaceutical, food and cosmetic industries as well as of individual consumers in the quality of herbal produce.

Halvorsen et al. (2002) demonstrated over a 1000-fold difference between total antioxidants in dietary plants and stressed the need for investigations on the role of processing and storage of raw materials in this respect. Among the important constituents participating in the cell defence system against free radicals are phenolic compounds and also ascorbic acid and carotenoids (Diplock et al., 1998; Gao, Ohlander, Jeppsson, Björk, & Trajkovski, 2000; Szeto, Tomlinson, &

Abbreviations: AA, L-ascorbic acid; GA, gallic acid; LA, linoleic acid; ORAC, oxygen radical absorbing capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TAA, total antioxidant activity; RSA, radical scavenging ability; f.m., fresh matter.

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Benzie, 2002). According to Kalt, Forney, Martin, & Prior (1999) the effect of ascorbic acid on antioxidant capacity of fruits is negligible. Similarly, the antioxidative properties of carotenoids seem to be controversial and under certain conditions such as high oxygen pressure in the plant tissue can be converted into prooxidant activity (Larson, 1988). Herbs, regardless of the purpose they serve, are used as fresh or dried. Enzymatic processes during drying fresh plant tissues may lead to significant changes in the composition of phytochemicals (Jambor & Czosnowska, 2002). The evaluation of antioxidant properties of the raw material allows the determination of its suitability as high quality food beneficial for human health and therefore is of considerable importance.

The aim of this study was to compare the antioxidant activity of fresh and dried herbs of some species from the *Lamiaceae* family.

2. Materials and methods

Plant material was collected from the following three perennial species of *Lamiaceae* family in their second year of cropping: lemon balm (*Melissa officinalis* L.), oregano (*Origanum vulgare* L.), peppermint (*Mentha* × *piperita* L.). Plants were grown in the Experimental Station of the Agricultural University in Krakow. Harvest was done in the second half of June 2002, when lemon balm and oregano were at the beginning of flowering and peppermint just before flowering. Immediately after harvest, one part of the herb was prepared for analysis and the second one was air-dried in a shady, well-ventilated room at the temperature of 25–32 °C for 10 days and then packed in paper bags and stored for 6 months. Analytical samples consisted of leaves and shoot tops, hard stalks being discarded.

Both fresh and dried plant material were subjected to the same analyses. The weight of analytical samples were equivalent, i.e. the water losses during drying and storing of herbs were taken into account. This allowed the comparison of results expressed as per fresh matter (f.m.) of plant tissue regardless of herb character. The content of L-ascorbic acid (AA) was determined by the iodometric method of Tillmans (Krełowska-Kułas, 1993), that of total carotenoids – spectrophotometrically, after the extraction of plant material in 80% acetone, basing on the equations by Lichtenthaler & Wellburn (1983). For the test of total phenolics and two assays of antioxidant capability the herb extracts were prepared using 80% methanol. Total soluble phenolics were estimated by a photometric method with the Folin–Ciocalteu reagent using gallic acid (GA) as a standard (Slinkard & Singleton, 1977; Zheng & Wang, 2001). Total antioxidant activity (TAA) was determined by the method given by Toivonen & Sweeney (1998). The products of peroxidation of exogenous linoleic acid (LA), initiated by ferrous-EDTA, were measured spectrophotometrically at 232 nm in the solutions of herb extracts (final concentration was 0.82%) and control. A control consisted of the above reaction mixture with 80% methanol in place of the herb extract. TAA was expressed as a percentage of inhibition of LA peroxidation by herb extracts in comparison to the oxidation level in the control. Radical scavenging activity (RSA) was tested according to the method with DPPH as a stable free radical (Pekkarinen, Stockmann, Schwarz, Heinonen, & Hopia, 1999). The ethanolic solution of DPPH (0.1mM) was used as a point of reference for monitoring the decrease of its absorbance at 516 nm after addition of herb extracts (final concentration was 0.41%). The control was prepared without herb extract, ethanol being used for a baseline correction. RSA was expressed as the percentage of DPPH elimination after 1 and 5 min since the start of reaction, calculated according to the following formula: [absorbance of control sample – absorbance of sample with herb extract]/absorbance of control sample × 100%.

The data were subjected to ANOVA, means being compared by Duncan's test at $p = 0.05$.

3. Results

Table 1 presents the results of two assays of antioxidant capability and the content of total phenolics, ascorbic acid, and carotenoids in the fresh and air-dried herbs of the three species tested. The highest antioxidant ability, expressed as inhibition of LA peroxidation (TAA), was found for extracts from both fresh and dried oregano. The TAA value for peppermint and lemon balm was significantly lower. In the case of these two species, the process of drying decreased their total antioxidant activity.

The ability of scavenging DPPH – free radical measured after 5 min was very high in the extracts from all the tested herbs, exceeding 90%. The only exception was dried oregano for which the RSA value was lower (84%). Comparison of RSA measurements after 1 and 5 min allowed estimation of the rate of DPPH neutralisation. In the case of peppermint and lemon balm extracts, obtained both from fresh and dried plant material, this parameter reached its maximum level after 1 min, while the oregano extracts reacted with DPPH much more slowly. In oregano extracts, a significant difference of RSA between fresh and dried herbs was found. Both after 1 and 5 min the DPPH scavenging ability of extracts from dried herb was lower than that from fresh plant material.

In the three species tested the content of total soluble phenolics was very high, and ranged between 1400 (fresh

Table 1
Comparison of fresh and dry herb of three species of *Lamiaceae* with respect to antioxidant activity

Tested parameter		Herb character	Species		
			Peppermint	Lemon balm	Oregano
TAA (%)		Fresh	62b	69b	95c
		Dry	50a	50a	90c
RSA (%)	After 1 min	Fresh	93de	93de	64b
		Dry	94ef	95f	41a
	After 5 min	Fresh	93de	93de	92d
		Dry	95f	95f	84c
Total phenolics (mg 100 g^{-1} f.m.)		Fresh	1954b	2253c	1406a
		Dry	2580d	2183c	2221c
L-Ascorbic acid (mg 100 g^{-1} f.m.)		Fresh	52.6c	53.2c	23.1b
		Dry	4.5a	3.3a	4.2a
Carotenoids (mg 100 g^{-1} f.m.)		Fresh	58.1d	46.3c	51.0cd
		Dry	32.7b	21.0a	25.5ab

Means followed by the same letters are not significantly different.

oregano) and 2600 mg GA 100 g^{-1} f.m. (dried peppermint). Drying resulted in a considerable increase of total phenolics in the case of oregano and peppermint, while for lemon balm no significant difference was observed between freshly harvested and dried plant material.

Ascorbic acid content in fresh plant material differed significantly depending on the species tested. The highest content was determined in peppermint and lemon balm while in oregano it was lower by half. Drying caused a great reduction of this compound in all the examined species. In the case of lemon balm and peppermint over a 10-fold decrease was noted.

A similar level of carotenoids was observed in fresh plant material in all species studied. Drying caused their significant decrease, approximately one half of the amount determined immediately after harvest was found in dried herbs.

4. Discussion

Present results confirm the unique antioxidant properties of all the three examined species. Zheng & Wang (2001), who examined 39 herbal taxa, reported that various species of oregano showed high levels of phenolic compounds that strongly correlated (0.984) with ORAC values. This led those authors to declare that phenolics are major components of the antioxidant system in plants. The present results concerning the particularly strong inhibition of LA peroxidation by oregano extracts confirm the findings of Zheng & Wang (2001). Nevertheless, it is worth noting that in this species significantly higher TAA was accompanied either by the lower (fresh material) or by the similar (dried material) content of phenolics in comparison with peppermint and lemon balm.

The high content of phenolics affected also the strong capability of the species examined to deactivate free radical DPPH, especially fresh and dried peppermint and lemon balm as well as fresh oregano. Lagouri & Boskou (1996) & Kähkönen et al. (1999) stated that among plant phenolics responsible for antioxidant activity the prominent role was played by phenolic acids and flavonoids. A very important compound in herbs of *Lamiaceae* family is rosmarinic acid, showing high scavenging DPPH potential (Chen & Ho, 1997), this being related to the presence of four hydroxyl groups in its molecule (Fecka, Mazur, & Cisowski, 2002). The rosmarinic acid content in oregano extracts is very high (Chen & Ho, 1997). Oregano is particularly rich (55,000 ppm) in this compound and peppermint and lemon balm also contain high amounts (about 30,000 and 37,000 ppm, respectively). In the DPPH scavenging test, Chen & Ho (1997) showed that besides rosmarinic acid, other phenolic acids (i.e. caffeic, chlorogenic, and ferulic) participate in the neutralisation of this free radical. A detailed quality analysis of phenolic compounds occurring in the plant material might elucidate the question of significant differences noted with respect to TAA and RSA between the species, either fresh or dried.

In dried plant material the significant losses of both ascorbic acid and carotenoids were found in comparison with the fresh herbs. Ascorbic acid is easily decomposed during post-harvest treatment of leafy tissue. A considerable decrease (by 90%) of its level was observed in spinach stored for 3 days at ambient temperature (Diplock et al., 1998) as well as during short-term storage of lettuce leaves at room temperature (Rożek, Leja, Myczkowski, & Mareczek, 1995). It has been long known that carotenoids as a labile substances, sensitive to oxidation, radiation, etc. (Davies, 1976), could be destroyed during

drying processes. High AA content noted in fresh peppermint and lemon balm corresponded to a very high DPPH scavenging ability, whereas lower AA content in oregano was accompanied also by lower RSA. A contradictory dependence was found, however, in the case of TAA. A distinct decrease in both AA and carotenoid contents in all samples examined after their drying was not accompanied by a reduction of RSA either in lemon balm or peppermint, but in the case of oregano corresponded to a decrease in this parameter. On the other hand, the TAA value was lowered as the result of drying of peppermint and lemon balm, but remained on the same level with oregano.

Ambiguous connections between the content of particular antioxidants and antioxidant activity are difficult to explain only on the basis of quantitative analysis. Milos, Mastelic, & Jerkovic (2000) & Zheng & Wang (2001) suggested that not only the level of antioxidants but also a synergy occurring between them and the other plant constituents might influence the differences in the antioxidant ability of plant extracts. A similar suggestion concerning the mechanism of action of phytonutrients introduced in human organisms was put forward by Tran (2001).

Antioxidant activity, expressed as the ability to inhibit LA peroxidation as well as to scavenge free radicals, was very high in the case of all the examined herbs. Lower TAA values measured in the extracts of peppermint and lemon balm seemed to be compensated by very high RSA values. With oregano, the lower rate of DPPH scavenging was observed together with very high inhibition of LA peroxidation. Regardless of a significant decrease in ascorbic acid and carotenoid contents after drying of plant material, it may be noted that both fresh and dried herbs of the three species examined are rich sources of antioxidants, in particular from the group of phenolic compounds.

References

Challa, A., Ahmad, N., & Mukhtar, H. (1997). Cancer prevention through sensible nutrition (Commentary). *International Journal of Oncology, 11*, 1387–1392.

Chen, J. H., & Ho, C. T. (1997). Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *Journal of Agricultural and Food Chemistry, 41*, 2374–2378.

Davies, B. H. (1976). Carotenoids. In *Chemistry and Biochemistry of Plant Pigments* (pp. 38–165). New York: Academic Press.

Diplock, T. A., Charleux, J. L., Crozier-Willi, G., Kok, F. J., Rice-Evans, C., Roberfroid, M., Stahl, W., & Viña-Ribes, J. (1998). Functional food science and defence against reactive oxidative species. *British Journal of Nutrition, 80*, 77–112.

Fecka, I., Mazur, A., & Cisowski, W. (2002). Kwas rozmarynowy, ważny składnik terapeutyczny niektórych surowców roślinnych. *Postępy Fitoterapii, 8*(1–2).

Gao, X., Ohlander, M., Jeppsson, N., Björk, L., & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *Journal of Agricultural and Food Chemistry, 48*, 1485–1490.

Halvorsen, B. L., Holte, K., Myhrstad, M. C., Barikmo, I., Hvattum, E., Remberg, S. F., Wold, A. B., Haffner, K., Bauger, Æd. H., Andersen, L. F., Moskaug, J. Æ., Jacobs, D. R., & Blomhoff, R. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition, 132*, 461–471.

Hu, F. B., & Willett, W. C. (2002). Optimal diets for prevention of coronary heart disease. *Journal of American Medical Association, 2888*, 2569–2578.

Jambor, J. (2001). Kierunki rozwoju krajowego rynku surowców i przetworów zielarskich. *Herba Polonica, XLVII*(2), 103–121.

Jambor, J., & Czosnowska, E. (2002). [Herbal medicines from fresh plants]. *Postępy Fitoterapii, 8*(1–2), 2–5.

Kalt, W., Forney, C. F., Martin, A., & Prior, R. L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry, 47*, 4638–4644.

Kähkönen, M. P., Hopia, A. I., Vourela, H. J., Rauha, J.-P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*, 3954–3962.

Krełowska-Kułas, M. (1993). *Badanie jakości produktów spożywczych* (pp. 163–169). Warszawa: PWE.

Lagouri, V., & Boskou, D. (1996). Nutrient antioxidants in oregano. *International Journal of Food Sciences and Nutrition, 47*, 493–497.

Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry, 27*, 4969–4978.

Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochemical Society Transactions, 603*, 591–593.

Lutomski, J. (2001). Znaczenie ziółw terapii i dietetyce. *Wiadomości Zielarskie, 10*, 4–5.

Madsen, H. L., & Bertelsen, G. (1995). Spices as antioxidants. *Trends in Food Science and Technology, 6*, 271–276.

Milos, M., Mastelic, J., & Jerkovic, I. (2000). Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano (*Origanum vulgare* L. ssp. *hirtum*). *Food Chemistry, 71*, 79–83.

Pekkarinen, S. S., Stockmann, H., Schwarz, K., Heinonen, I. M., & Hopia, A. I. (1999). Antioxidant activity and partitioning of phenolic acids in bulk and emulsified linoleate. *Journal of Agricultural and Food Chemistry, 47*, 3036–3043.

Rożek, S., Leja, M., Myczkowski, J., & Mareczek, A. (1995). The effect of fertilization with nitrate and urea forms of nitrogen on quality and storage ability of lettuce grown in a foil tunnel. I. Content of certain nutritive compounds. *Folia Horticulturae, 7/1*, 91–105.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*, 49–55.

Szeto, Y. T., Tomlinson, B., & Benzie, I. F. (2002). Total Antioxidant and Ascorbic Acid Content of Fresh Fruits and Vegetables: Implications for Dietary Planning and Food Preservation. *British Journal of Nutrition, 87*, 55–59.

Toivonen, P. M. A., & Sweeney, M. (1998). Differences in chlorophyll loss at 13 °C for two broccoli (*Brassica oleracea* L.) cultivars associated with antioxidant enzyme activities. *Journal of Agricultural and Food Chemistry, 46*, 20–24.

Tran, T. L. (2001). Antioxidant supplements to prevent heart disease. *Postgraduate Medicine, 109/1*, 109–114.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomach, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*, 5165–5170.