

## THE EFFECT OF DRYING ON ANTIOXIDANT ACTIVITY OF SELECTED LAMIACEAE HERBS

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### ABSTRACT

Antioxidant activity and total phenolics content of selected fresh and dried herbs from the *Lamiaceae* family were compared. The analysed herbs included *Thymus vulgaris*, *Origanum vulgare*, *Satureja hortensis*, *Origanum majorana*, and *Origanum heracleoticum* from the 1<sup>st</sup> and the 2<sup>nd</sup> harvests. The antioxidant activity was determined using DPPH method and the total content of phenols was analysed using the Folin-Ciocalteu reagent. Ascorbic and gallic acids were used as reference standards. All the analysed herbs had the reasonable potential to reduce the DPPH radical. The dried herbs from the 2<sup>nd</sup> harvest had the highest antioxidant activity. Oregano exhibited the highest antioxidant activity from the analyzed samples of both harvests together. The descending order of the samples was oregano > Greek oregano > marjoram > summer savory > thyme. Marjoram from the 2<sup>nd</sup> harvest had the highest antioxidant activity from the fresh samples. The lowest activity was observed in thyme from the 2<sup>nd</sup> harvest. In case of dried samples, the highest antioxidant activity was measured in sample of Greek oregano from the 2<sup>nd</sup> harvest. The lowest activity was observed in thyme from the 1<sup>st</sup> and 2<sup>nd</sup> harvest again. The descending order of total phenolics content for both harvests together was oregano > Greek oregano > marjoram > summer savory > thyme. In case of fresh herbs the highest total phenolics content was measured in oregano from the 1<sup>st</sup> harvest, the lowest content was measured in summer savory from the 2<sup>nd</sup> harvest. Greek oregano from the 2<sup>nd</sup> harvest had the highest values from dried herbs. Dried thyme from the second harvest had the lowest total phenolics content. The correlation between the DPPH values and the total content of phenols was determined (for fresh herbs: 0.4917; for dried herbs: 0.8698). According to the total content of phenols a statistically significant difference between the fresh and dried herbs from the 2<sup>nd</sup> harvest ( $p = 0.0185$ ) was found.

**Keywords:** Herb; *Lamiaceae*; antioxidant activity; DPPH method; total phenolics contents

### INTRODUCTION

People's interest in health and healthy lifestyle is increasing at present. People are mainly focused on sport and a healthy diet. For proper nutrition it is important to know not only the composition of food, but also its quantity and technological processing. This processing must be chosen so as to preserve the food nutritional value.

The use of herbs and their processing have a long tradition. Medicinal, aromatic and culinary plants, which synthesize many useful chemical compounds, are traditionally eaten in fresh and dried forms. Antioxidants belong to the biologically important compounds in herbs. They represent chemical compounds, which are able to inhibit oxidation reactions caused by free radicals. Free radicals can cause damage of biologically important molecules, cells and tissues. This process can be one of the main factors of various pathological lifestyle diseases such as cardiovascular diseases (atherosclerosis, ischemia, hypertension, etc.) (Jacob, 1995). Therefore it is important to have a sufficient amount of antioxidants in our nutrition. Moreover, antioxidants in foods prolong their shelf life and protect them against undesirable oxidation (rancidity changes of lipids and other easily oxidizing agents) (Cao et al., 1997).

Antioxidants can be classified according to the various aspects – their source and origin (natural and synthetic), chemical structure, etc. The most important antioxidants in food are vitamin C and E, carotenoids, flavonoids, and selenium. Herbs of the *Lamiaceae* family are an important source of antioxidants and other biologically active substances. Summer savory (*Satureja hortensis* L.), marjoram (*Origanum majorana* L.), Greek oregano (*Origanum heracleoticum* L.), oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) have been commonly used in households for culinary food processing for many years. They have also an irreplaceable role in the food industry. Furthermore, they are used in pharmacy and cosmetics.

The antioxidant activity of the material produced from plants of the family *Lamiaceae* depends on many factors, including the plant cultivation conditions (conventional and organic farming methods, soil composition, irrigation and plant protection, harvest period, place of growing), collection method, plant processing (drying method and conditions - natural drying, oven drying, lyophilisation, exposure and intensity of light, temperature, humidity, etc.), the way of sample processing and extraction, and the selection of the antioxidant activity method.

Phenolics are a heterogeneous group of compounds with antioxidant activity found in many food resources (Halliwell, 1995). They are often part of plant essential oils and are responsible for characteristic aroma of individual foods.

## MATERIAL

### Herbs

Lamiaceae family herbs: thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), summer savory (*Satureja hortensis*), marjoram (*Origanum majorana*) and Greek oregano (*Origanum heracleoticum*) were planted and analyzed. The seeds were pre-grown in flowerpots, sown on 7<sup>th</sup> April 2013 and replanted to bed on 21<sup>st</sup> May 2013. All herbs were planted on the sunny, unfertilized plot of sandy loam medium soil in the locality Malá Hraštica (49° 48' N, 14° 16' E, district Přeborn).

The seeds purchased from different companies were sowed in the following depths:

- thyme, oregano and marjoram (NOHEL GARDEN): 0.5 cm,
- summer savory (MORAVO SEED): 0.5 cm,
- Greek oregano (SEMO) 0.4 cm.

Plant parts were harvested on 18th July 2013 (1st harvest) and on 16th September 2013 (2nd harvest). The proportion of herbs was then dried.

Herb samples were taken before flowering when they should have the highest content of bioactive compounds. Fresh and dried leaves from herbs were used for the analysis.

### Chemicals

- Methanol, p. a., CH<sub>4</sub>O, M = 32.04 g/mol, Lachner, PP/2011/08626
- Sodium carbonate anhydrous, Na<sub>2</sub>CO<sub>3</sub> p. a., M = 105.99 g/mol, Lachner, PP/2012/08988
- DPPH 2,2-difenyl-1-pikrylhydrazyl, Sigma Aldrich, USA
- Folin & Ciocalteu's phenol reagent, Merck, UN 3264
- Ascorbic acid p. a., Penta, batch 1507080710, M = 176.13 g/mol
- Gallic acid p. a., Sigma Aldrich.

### Equipment

- Spectrophotometer UV- 2900 PC, Tsingtao Unicom-Optics Instruments Co., Ltd., China
- Analytical balances AND, ER- 180A, max. 180 g, d = 0.01 mg
- Ultrasonic bath Tesla

- Balances with infrared dryer, Precisa HA 300, Precisa Instruments, Switzerland
- Thermostat Memmert 54853, Germany

## METHODOLOGY

### Drying

Herbs were dried in the open air at 25 °C for one week.

### Determination of total dry matter

Infrared balances Precisa HA 300 were used for dry matter content determination. Fresh and dried herbs (0.5 g) from both harvests were ground and spread on aluminium foil. Program for vegetable drying was applied: maximum temperature 105 °C, constant value if the weight difference was less than 2 mg for 30 s. Samples were measured in triplicate and the average was calculated.

### Herb extraction

Fresh herbs (6 g) or the equivalent amount of dried herbs (calculated from total dry matter of individual herbs, **Table 1**) were taken for the preparation of water extracts. Herb samples were extracted twice by 50 mL of hot demineralized water in the ultrasonic bath for 10 min. Samples were then filtered into 100mL volumetric flasks and filled up to the mark after cooling. The extracts were analysed on the same day.

### Determination of antioxidant activity by the DPPH method

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method is one of the commonly used methods for antioxidant activity assessment. The principle of this method is based on the reduction of stable DPPH radical to DPPH-H by compounds with antioxidant activity and the measurement of the intensity of the violet DPPH radical solution at 522 nm. The method was calibrated with ascorbic acid and the results were expressed as equivalents of ascorbic acid per unit mass of sample. This method was taken from **Chrprová et al. (2010)** and **Buřičová et al. (2011)**. Samples were kept in dark and measured after 1, 2 and 3 hours to reach the reaction maximum.

### Determination of total phenolic compounds (TPC)

The content of total phenolic 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. The method was taken from **Dorman et al. (2003)** and **Stratil et al. (2008)**.

### Statistical evaluation

**Table 1** Weight of dried herbs for extraction.

Herb	1 <sup>st</sup> harvest	2 <sup>nd</sup> harvest
Oregano	1.2265 g	1.8887 g
Greek oregano	1.2039 g	1.1279 g
Marjoram	1.7091 g	0.6223 g
Thyme	1.1047 g	1.9311 g
Summer savory	1.2467 g	1.2104 g

**Table 2** Herbs dry matter content [%]

Harvest	Fresh herbs		Dried herbs	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Oregano	20.2 ±0.6	28.0 ±0.3	98.6 ±0.2	88.5 ±0.1
Greek oregano	18.1 ±0.4	16.5 ±0.2	90.2 ±0.2	87.9 ±0.3
Marjoram	25.8 ±0.5	9.2 ±0.5	90.5 ±0.1	89.2 ±0.1
Thyme	16.8 ±0.2	28.5 ±0.4	91.3 ±0.3	88.6 ±0.2
Summer savory	19.7 ±0.3	17.4 ±0.5	94.8 ±0.1	86.3 ±0.2

Using the software STATISTICA 12 (StatSoft Inc.), statistical evaluation of values recalculated to dry matter content was performed. Statistical evaluation of the difference between the fresh and the dried samples was done by paired t-test at a probability level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Dry matter content

Dry matter content results of analysed herbs are presented in Table 2.

### Antioxidant activity

Results of antioxidant activity of herbs per 100 g of sample or dry matter are shown in Tables 3 and 4. It is seen that the herbs in the dried state have a higher antioxidant activity than fresh herbs.

### Total phenolics content

Each measurement was repeated four times. Total phenolics content was then recalculated to 100 g of the extracted sample as well as to 100 g of dry matter of herbs. From the results shown in Table 5 and 6 is clear that the herbs have a reasonable content of phenolics. Higher

values can be observed in samples of dried plants. The only exception was the Greek oregano from the first harvest in Table 6.

### Statistical evaluation

There was a statistically significant difference between fresh and dried samples, in terms of the potential to quench free radical DPPH (1<sup>st</sup> and 2<sup>nd</sup> harvest) and total phenols (second harvest). A statistically significant difference was not detected in TPC between fresh and dried herbs from the first harvest (Table 7).

Furthermore, the correlation between TPC and DPPH results was analysed (Table 8). It was found that there is a strong correlation between the values from dried herbs and a medium correlation in case of fresh herbs.

## DISCUSSION

### Dry matter content of herbs

Dry matter content results of dried and fresh herbs (Table 2) are consistent with **Vidovic et al. (2014)** and **Sabolová (2012)**. Some variability of dry matter content in fresh herbs can be observed, which can be affected by

**Table 3** Antioxidant activity of herbs in grams of AA per 100 g of sample.

Harvest	Fresh herbs		Dried herbs	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Oregano	1.80 ±0.16	1.84 ±0.01	11.53 ±0.06	12.03 ±0.01
Greek oregano	1.35 ±0.17	1.85 ±0.07	6.54 ±0.01	12.46 ±0.05
Marjoram	0.86 ±0.07	1.07 ±0.01	5.63 ±0.05	11.69 ±0.04
Thyme	0.64 ±0.08	0.74 ±0.02	5.38 ±0.01	5.31 ±0.07
Summer savory	0.63 ±0.08	0.92 ±0.14	6.30 ±0.02	8.20 ±0.06

**Table 4** Antioxidant activity of herbs in grams of AA per 100 g of dry matter.

Harvest	Fresh herbs		Dried herbs	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Oregano	8.92 ±0.78	6.56 ±0.03	11.70 ±0.06	13.59 ±0.01
Greek oregano	7.47 ±0.92	11.16 ±0.40	7.25 ±0.01	14.18 ±0.05
Marjoram	3.32 ±0.26	11.59 ±0.07	6.22 ±0.05	13.11 ±0.05
Thyme	3.80 ±0.50	2.61 ±0.60	5.89 ±0.01	6.00 ±0.08
Summer savory	3.20 ±0.40	5.26 ±0.80	6.65 ±0.02	9.50 ±0.07

**Table 5** Total phenolics content of herbs in grams of GA per 100 g of sample.

Harvest	Fresh herbs		Dried herbs	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Oregano	0.84 ±0.10	0.61 ±0.01	4.66 ±0.04	4.28 ±0.01
Greek oregano	0.64 ±0.03	0.58 ±0.01	2.71 ±0.01	4.26 ±0.01
Marjoram	0.62 ±0.10	0.48 ±0.01	3.24 ±0.05	3.57 ±0.01
Thyme	0.40 ±0.01	0.19 ±0.01	2.21 ±0.02	2.09 ±0.01
Summer savory	0.45 ±0.10	0.23 ±0.02	3.06 ±0.02	2.41 ±0.03

**Table 6** Total phenolics content of herbs in grams of GA per 100 g of dry matter.

Harvest	Fresh herbs		Dried herbs	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Oregano	4.15 ±0.48	2.18 ±0.01	4.73 ±0.04	4.83 ±0.01
Greek oregano	3.51 ±0.11	3.49 ±0.06	3.00 ±0.01	4.84 ±0.02
Marjoram	3.15 ±0.38	2.77 ±0.05	3.42 ±0.06	4.13 ±0.01
Thyme	1.44 ±0.02	2.09 ±0.03	2.44 ±0.02	2.35 ±0.01
Summer savory	2.68 ±0.49	0.81 ±0.09	3.36 ±0.02	2.72 ±0.04

many factors, including watering, temperature and humidity. Dried herbs results are not so variable because majority of the water was lost during the drying process.

**Antioxidant activity**

There are many studies describing significant antioxidant activity of plants of the Lamiaceae family (Srinivasan, 2014). Herbs and spices are therefore important sources of natural antioxidants. Chrpová et al. (2010) presented significant activity of selected Lamiaceae herbs. Another important activity and content of phenolic compounds were observed by Katalinic et al. (2006) in lemon balm *Melissa officinalis* L. Significant antioxidant activity was demonstrated also in the analysed samples. From Table 4 it is clear that all herbs from the first and the second harvests have the potential to quench free DPPH radical on a level comparable with presented references.

Drying process may cause some changes in antioxidant activity, total phenolics content as well as composition of

essential oils. There is higher concentration of stable phenolic compounds involved in the antioxidant activity in dried herbs. Our results by DPPH method are in line with these findings (Hossain et al., 2010). Antioxidant activities of analysed samples of thyme (T), oregano (D), summer savory (S), marjoram (M) and Greek oregano (G) from the first (1), the second and (2) both harvests were in the following descending order:

- a) fresh: M2>G2>D1>G1>D2>S2>T1>M1>S1>T2
- b) dried: G2>D2>M2>D1>S2>G1>S1>M1>T2>T1
- c) both harvests together: D>G>M>S>T

From this comparison it is clear that among fresh herbs the highest antioxidant activity was showed by marjoram from the second harvest. The important antioxidant activity of marjoram, which has potential to be used as a natural antioxidant, was also mentioned by Roby et al. (2013). In case of dried herbs, the highest values were detected in Greek oregano from the second harvest. This herb also showed the highest antioxidant activity by the

**Table 7** Statistical comparison of fresh and dried herbs samples.

Method	Compared samples	p
DPPH	1 <sup>st</sup> harvest - dry x fresh herbs	0.0264
	2 <sup>nd</sup> harvest - dry x fresh herbs	0.0135
TCP	1 <sup>st</sup> harvest - dry x fresh herbs	0.1934
	2 <sup>nd</sup> harvest - dry x fresh herbs	0.0185

**Table 8** Correlations between TPC and DPPH methods.

Material	Correlation coefficient
Fresh herbs	0.4917
Dry herbs	0.8698

DPPH method when both harvests were calculated together. These results are comparable with **Chrpová et al. (2010)** who found significantly higher antioxidant activity in mint and oregano comparable to that of green tea.

A statistically significant difference between fresh and dried herbs from the 1<sup>st</sup> and the 2<sup>nd</sup> harvests was detected. Dried herbs from the second harvest had the highest antioxidant activity.

### Total phenolics content

Higher TPC values (Table 5) were observed in dried herbs. The only exception was the Greek oregano from the first harvest which shows slightly higher value in fresh state. TPC of analysed samples were in the following descending order:

- a) fresh: D1>G1>G2>M1>M2>S1>D2>T2>T1>S2  
 b) dried: G2>D2>D1>M2>M1>S2>G2>S2>T1>T2  
 c) both harvests together: D>G>M>S>T

Among the fresh herbs, the highest total phenolics content was detected in oregano from the first harvest, the lowest content was detected in summer savory. Among the dried herbs, the highest total phenolics content was detected in Greek oregano from the second harvest, which also showed the highest antioxidant activity for DPPH. Dried thyme from the second harvest had the lowest total phenolics content. Comparing both harvests together, the highest values were detected in oregano sample.

As far as the TPC is concerned, statistically significant difference was found between fresh and dried herbs from the second harvest. Dried oregano, Greek oregano and marjoram showed the highest values in case of the 2<sup>nd</sup> harvest, thyme and summer savory in the first harvest.

### Comparison of fresh and dried herbs

Our results confirmed higher antioxidant activity and TPC in dried herbs. There are many different enzymatic and non-enzymatic reactions taking place during the drying process as well as tissue decomposition. As a result of these processes, different substances (including phenolics) are created.

A statistically significant difference between dried and fresh herbs was also confirmed by **Sefidkon et al. (2006)**, and **Pirbalouti et al. (2013)**, who analysed two varieties of basil. **Rhim et al., (2009)** investigated the influence of drying on the antioxidant activity. Their results were significantly higher for dried samples too. Results of **Hossain et al. (2010)** again showed significantly higher content of TPC, rosmarinic acid and higher antioxidant capacity of the air dried samples versus fresh samples.

There was a strong correlation between DPPH and TPC (Table 8) which is in line with the study of **Mechergui et al. (2010)**. **Yesiloglu et al. (2013)** also presented strong antioxidant activity correlating with the content of phenols and flavonoids.

### CONCLUSION

Selected herbs of the Lamiaceae family exhibited a reasonable antioxidant potential and phenolics content in both fresh and dried form. According to the DPPH method results a statistically significant difference between fresh and dried herbs from the 1<sup>st</sup> and the 2<sup>nd</sup> harvests was

determined. High correlation between TPC and DPPH values was found. Dried herbs from the 2<sup>nd</sup> harvest had the highest antioxidant activity.

TPC results showed a statistically significant difference between fresh and dried herbs from the 2<sup>nd</sup> harvest. Dried *Origanum vulgare*, *Origanum heracleoticum*, and *Origanum majorana* had the highest results from the 2<sup>nd</sup> harvest, *Thymus vulgaris* and *Satureja hortensis* had the highest results from the 1<sup>st</sup> harvest.

Both antioxidant activity and the total content of phenols increased after the drying process.

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