PHENOLIC COMPOUNDS IN BASIL, OREGANO AND THYME

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Abstract
Among various abundant sources of phenolic compounds are spices such as oregano, thyme and basil. The aim of this research was to determine phenolic compounds in oregano, thyme, basil. In spices six phenolic compounds were identified – caffeic acid, rosmarinic acid, eriodyctiol, luteolin, naringenin, apigenin. The main phenolic compound in spices was rosmarinic acid, other compounds were present in less than 4% (from total phenolics). Caffeic acid was identified only in thyme. Compounds from two flavonoid classes were identified in spices: flavons (apigenin, luteolin) and flavonons (eriodictyol, naringenin). Apigenin and luteolin were detected in oregano and thyme. Eridyctiol was present in all spices, with the highest concentration determined in oregano, while naringenin was present in oregano and thyme. Flavonons eriodyctiol and naringenin were present in spices in higher concentrations compared to flavons apigenin and luteolin. Total amount of identified phenolic compounds was the highest in thyme.
Key words: phenolic compounds, aromatized oil, spices

Introduction
Several thousand molecules having a polyphenol structure (ie, several hydroxyl groups on aromatic rings) have been identified in higher plants, and several hundred are found in edible plants. Phenolics behave as antioxidants, due to the reactivity of the phenol moiety. Source of polyphenols as mentioned above are ubiquitous. Literature, however, shows that researchers have become interested in phenolic compounds in spices. Different amounts of phenolic acids and flavonoids have been detected in spices depending on growing conditions, plant part analyzed (leaves, flowers), and/or extraction conditions Spices are used for oil aromatisation either for oil enrichment or expanding the commercial advantages. Among spices frequently used for oil aromatisation are basil, thyme, oregano. Literature data show that these spices contain different amounts of phenolic compounds (Jayasingne *et al.*, 2003; Javanmardi *et al.*, 2002; Grayer *et al.*, 1996). Rosmarinic acid is a powerful antioxidant (Javanmardi *et al.*, 2002) and was identified as a main phenolic compound in oregano (Exarchou *et al.*, 2002, Pizzale *et al.*, 2002). Other phenolic compounds in oregano are caffeic acid, luteolin, apigenin, eriodictyol, dihydroxicampherol, dihydroxiquercitine (Škerget *et al.* 2005, Pizzale *et al.*, 2002, Kulevanova *et al.*, 2001). In thyme the main phenolic compounds are glycuronids of apigenin, luteolin, eriodyctiol, luteolin glycosides, rosmarinic acid, quercitine (Justesen, 2000; Guillen and Manzanos, 1998). The main phenolic compounds in basil are rosmarinic acid, lithospermic acid, vanillic acid, coumarinic acid, hydroksibenzoacid, syringic acid, ferulic acid, protocatheuic acid, caffeic acid (Jayasingne *et al.*, 2003, Javanmardi *et al.*, 2002). Plant phenolics are one of the most important primary antioxidants, and during aromatization process they could migrate from spices in oil and protect oil from oxidation. The aim of research was to detect phenolic compounds in the three spices (basil, oregano and thyme) that will be used for oil aromatisation and to evaluate two methods for extraction of phenolic compounds from spices.

Materials and Methods
The plant material for the analysis was obtained from the plant collections of Faculty of Agriculture, Latvia University of Agriculture and from Santa Maria (producer – AS Paulig Baltic, Estonia, licence – Santa Maria AB, Sweden, further – commercial sample). The following samples were analysed: commercial basil, commercial oregano, commercial thyme, basil Green (*Ocimum basilicum* L.), Greek oregano *(Oreganum vulgare* L.), thyme *(Thymus vulgare* L.). Commercial samples were supplied dry in hermetically sealed packaging. Samples from LUA Faculty of Agriculture were air dried (30 °C temperature) and packaged.

Phenolic compounds from spices were extracted using two methods.

Method 1.

500 mg of dried leaf material was pulverized (particle size 0.125–0.250 mm), suspended in 5 ml of methanol (High Performane Liquid Chromatography (HPLC) grade), and left overnight at 4 °C under dark conditions. All supernatants were decanted and filtered using syringe filter (Javanmardi *et al.*, 2002) and transferred into HPLC vials. This method is mainly used for the extraction of phenolic acids.

Method 2.

500 mg dried leaf material was pulverized, suspended in 10 ml diethyl ether (HPLC grade) and left to extract overnight at room temperature. The diethyl ether (HPLC grade) was evaporated in rotavapour (till dryness, temperature of water bath 18±2 °C). The dried residues was redissolved in 1 ml of 80% MeOH, filtered using syringe filter and transferred into HPLC vials (Grayer *et al.*, 2003).

Reversed-phase HPLC and mass detection were performed in an Agilent 1100 LC-MSD system controlled by Agilent software v. A.09.03 (Agilent Technologies, Waldbronn, Germany). A Phenomenex C18 (ODS, Octadecyl) security guard and a Phenomenex Luna C18 (2) 100 Å column (4.6 mm i.d. x 250 mm; particle size =10 µm), maintained at 35 ^{o}C, were used. Elution was performed at a flow rate of 1.0 mL/min, using as mobile phase a mixture of 0,05% formic acid (HPLC grade) in water (solvent A) with pH=3.1, acetonitrile (HPLC grade) (solvent B). The solvent gradient is changed according to the following conditions: 1) 0–5 min, B: 10–35%; 2) 5–20 min, B: 35–70%; 3) 20–40 min, B: 70–90; 4) 40–41 min, B: 90–50%; 5) 41–42 min, B: 50–25%; 6) 42–43 min, B: 25–5%; 7) 43–44 min, B: 5–0%; and 8) 44–46 min, B: 0–10% Detection was done at 280 nm and 320 nm. The mass detector was an Agilent G1946D (SL) ion-trap mass spectrometer (Agilent Technologies, Waldbronn, Germany) equipped with an electrospray ionisation (ESI) system. Nitrogen was used as nebulizing gas at a pressure of 50 psi and the flow was adjusted to 13 l/min. The full scan mass spectra of the phenolic compounds were measured from m/z 100 up to m/z 1000. Mass spectrometry data were acquired in the negative ionization mode. For identification of phenolic compounds, HPLC retention times, UV spectra, mass spectra were compared with those of standards or those phenolics identified in spices previously. Analysis of variance was performed using SPSS 11.0 for Windows. Significant differences between means were determined a level of $p<0.05$.

Results and Discussion

Total amount of quantified phenolic compounds in analysed spices differed significantly ($p<0.05$) (Fig.1). Thyme was shown to have the highest amount of phenolics whereas basil had six times lower concentration of phenolics. There were some differences between commercial samples and those obtained from the University plant collection. Besides, the obvious influence of the solvent on the amount of extracted phenolics was observed.

Out of diethylether and methanol, the latter was shown to contribute better to the recoveries of polyphenols from the matrix. Literature studies showed different methods that could be used for extraction of phenolic compounds (Lee, 2000, Jayasingne *et al*., 2003). Methanol is widely used to extract antioxidants from plant material which can be applied also for the extraction of antioxidants from spices (Kim *et al*., 2005, Jayasingne C. *et al*., 2003, Pizzale *et al*., 2002). On the other hand, for extraction of flavonoids also diethyl ether has been used (Grayer *et al*.., 2003; Grayer *et al.*, 1996).

In the present study more phenolics were extracted using methanol. Extracted amount with diethyl ether was 8.8–9.1 times lower. The highest amounts were in commercial thyme, Latvian thyme and Greek oregano, but the lowest amount in both basil samples. Phenolic compounds of spices belong to different clases: phenolic acids, flavonoids, anthocyanins.

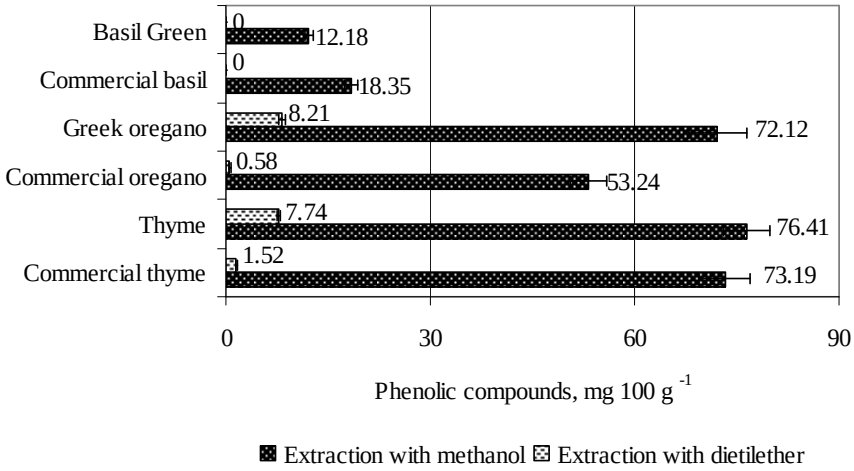

Figure 1. Total phenolic compounds in spices

In the analysed spices, 6 phenolic compounds, belonging to different classes were identified: phenolic acid (caffeic acid), phenolic acid derivative (caffeic acid dimmer – rosmarinic acid), flavons (apigenin, luteolin) and flavonons (eriodictyol, naringenin). Rosmarinic acid is the main compound in all analysed spices and content of other phenolic compounds is very low (till 4%).

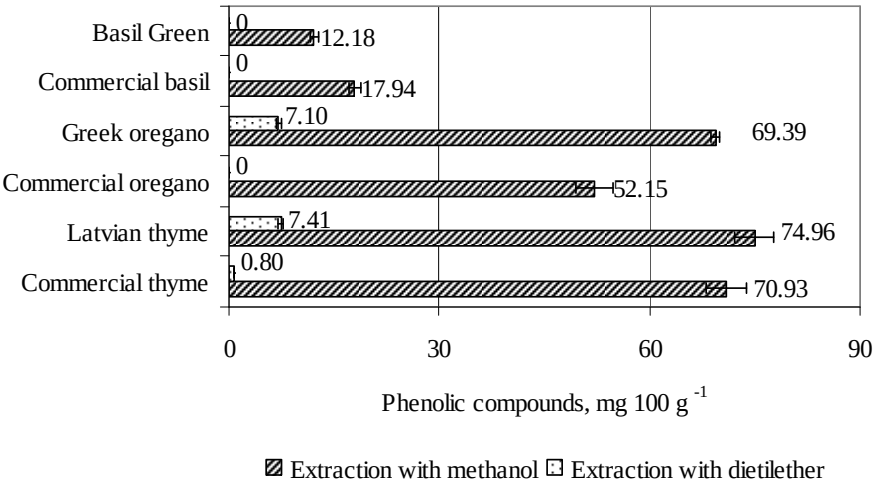

Figure 2. Rosmarinic acid in spices

The highest amount of rosmarinic acid was in Latvian thyme, commercial thyme and Greek oregano (Fig.2), but the lowest amount in basil samples. Rosmarinic acid was the only phenolic in basil Green. Extracted amount of rosmarinic acid was dependant on the used solvent, and higher amounts were obtained using methanol. There are big differences between chromatograms of samples obtained with both methods (Fig.3).

In the chromatogram of Greek oregano samples extracted with methanol (Fig.3.a) the main peak is rosmarinic acid, others are significantly smaller. On the other hand, in the chromatogram of sample extracted with diethyl ether (Fig.3.b) the main peaksare flavonoids (eriodictyol and naringenin) peaks, followed by rosmarinic acid peak. Of phenolic acids in spices, caffeic acid was the only one identified. This compound presented was present in both thyme samples: in the Latvian thyme 0.26 mg 100 g $^{-1}$, in the commercial thyme 0.38 mg 100 g $^{-1}$. Furthermore, four flavonoids were identified and their content differed significantly ($p<0.05$) between spices (Table 1). None of the flavonoids was identified in basil Green.

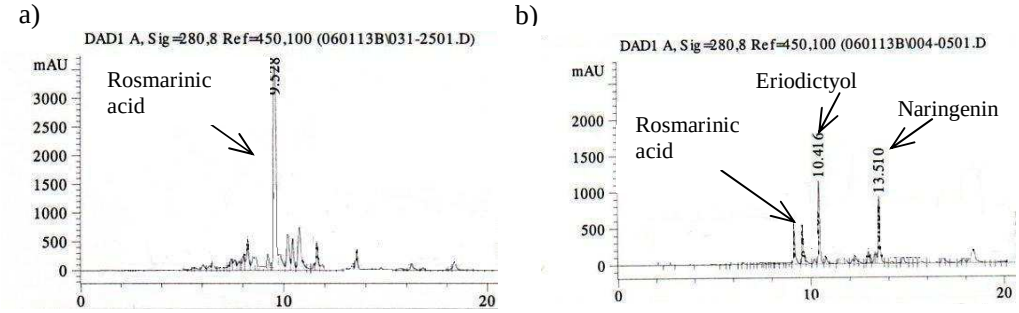

Figure 3. Chromatograms of Greek oregano phenolic compounds extracted with a) methanol, b) diethyl ether

Apigenin was identified in oregano and thyme with the highest amount in Greek oregano and Latvian thyme (Table 1) and higher amounts of apigenin were extracted with methanol. The second flavonoid – luteolin, was present in lower amounts, comparing to apigenin. It was not possible to identify apigenin and luteolin in basil samples.

Table 1

Flavonoids in spices, mg 100 g $^{-1*}$

Compounds	Extraction solvent	Spices			
		Greece oregano	Commercial oregano	Thyme	Commercial thyme
Apigenin	Methanol	0.282±0.014	0.105±0.006	0.376±0.019	0.209±0.01
	Dietilether	0.081±0.005	0.040±0.003	0.088±0.006	0.119±0.006
Luteolin	Methanol	n.d.	n.d.	n.d.	0.160±0.008
	Dietilether	0.027±0.002	0.064±0.004	0.047±0.003	0.043±0.003
Eriodictyol	Methanol	1.382±0.069	0.273±0.014	0.382±0.022	0.446±0.026
	Dietilether	0.518±0.037	0.104±0.007	0.059±0.004	0.122±0.006
Naringenin	Methanol	1.069±0.053	0.717±0.036	0.433±0.022	1.073±0.054
	Dietilether	0.481±0.024	0.372±0.019	0.124±0.001	0.432±0.022

*Results are given as mean±standard deviation
n.d. – not detected

This data are in accordance with those obtained for basil produced in Denmarks, where none of these compounds were identified (Justesen, 2001).
The highest amount of luteolin was in commercial thyme. Unlike apigenin, it was possible to extract higher amounts of luteolin with diethyl ether. Exception is the commercial thyme, from which luteolin was better extracted with methanol. The highest amount of eridyctiol was in Greek oregano, whereas in other analysed spices it was 3 to 5 times lower. Naringenin was identified in all oregano and thyme samples, with the highest amount in Greek oregano. Eridyctiol and naringenin were reported as characteristic compounds of thymes grown in Macedonia (Marin *et al.*, 2003) which is geographical close to Greece and Greece cultivars.

Conclusions

Total amount of identified phenolic compounds was the highest in the Latvian thyme, followed by the commercial thyme and the Greek oregano. In the spices six phenolic compounds were identified – caffeic acid, rosmarinic acid, eriodyctiol, luteolin, naringenin, apigenin. It is possible to extract more caffeic acid, rosmarinic acid, and apigenin using methanol as a solvent, whereas diethyl ether is a better solvent for extraction of – eriodyctiol, luteolin and naringenin. The main compound in all analysed spices was rosmarinic acid.

Literature data showed that this compound is a strong antioxidant, and therefore it can be suggested that these spices could contribute to the prevention of the oxidation of aromatised oils besides giving them nice aroma.

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