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Effect of development stage at harvest on the content of flavonoids and phenolic acids in aerial parts of thyme (Thymus vulgaris)

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Introduction
Thyme (Thymus vulgaris) has been used in the Mediterranean countries since antiquity for its culinary qualities, as well as for its preservative and medicinal properties. Thyme is well known for its flavor, which is mainly due to volatile terpenes. In addition, thyme has antimicrobial and antioxidant activity. The antioxidant properties of thyme are mainly due to flavonoids and phenolic acids. The content of polyphenols in plants may, however, depend on many factors such as growing conditions, development stage at harvest, and genotype.

The aim of the present study was to investigate how the concentration of flavonoids and phenolic acids in thyme, depends on the development stage of the plant during the growing season in cool temperate climate.

Plant material
Thyme plants were cultivated in a field experiment over two years. Aerial parts (upper 10 cm of shoots) were harvested at five different development stages during the growing season (from beginning of June to mid July). The yield of fresh weight plant material (FW) and dry matter content (DM) was determined at each harvest and samples were stored at -24°C until analysis.

Extraction & Analysis
Fresh frozen aerial parts of thyme (3g) were immediately homogenized in a centrifuge tube (40 mL) for 1 min together with 20 mL 80% sq. methanol followed by extraction for 2h at room temperature. After extraction, the samples were centrifuged for 10 min and the supernatant analysed for flavonoids and phenolic acids by HPLC-PDA. The polyphenols were quantified using external standards and analyses were performed in triplicate.

Polyphenols
Flavonoids and phenolic acids were identified from thyme methanol extracts by LC-MS/MS (Fig. 1). Major polyphenols identified were apigenin 6,8-di-C-β-glucopyranosyl, apigenin-7-O-glucoronic, luteolin-7- O-β-glucopyranoside, luteolin-7-O-glucoronic, and rosmarinic acid. All these components have previously been reported in thyme [1, 2].

Results and conclusion
Concentration of total flavonoids and phenolic acids in aerial parts of thyme varied from 2.3–6.8 mg/g DM (year 1) to 4.7–11.5 mg/g DM (year 2), and total phenolic acids from 5.4–8.2 mg/g DM (year 1) to 15.4–22.9 mg/g DM (year 2) (Fig. 2). The highest content of flavonoids was obtained at 1st harvest (pre-flowering stage) in both years. The content of phenolic acids was more variable being 3 times higher in year 2 compared to year 1 (results not shown). In year 1, the content of phenolic acids was not significantly affected by harvest time but in year 2 the highest content was at 1st harvest. Such time dependent variations were also previously observed for flavonoids and phenolic acids in oregano [3].

The conclusion of the present study is that the development stage has a significant impact on the concentration of polyphenols in thyme and that an optimal harvest time is around the pre-flowering stage.

References