LUTEIN AND ZEAXANTHIN CONTENT IN FLOWERS OF FRENCH MARIGOLD (*TAGETES PATULA* L.) HUNGARIAN VARIETIES

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Abstract

French or dwarf marigold (*Tagetes patula* L.) is a well-known ornamental plant, but also utilized in medicine, cosmetics, agriculture and food industry due to its biologically active components (xanthophylls, carotenes, terpenes, flavonoids, benzofuran derivatives, thiophenes, etc.). Three varieties of Hungarian bred *Tagetes patula* ('Csemő', 'Robuszta kénsárga', 'Orion') were transplanted into the pot experiment (5.05.2021) in the following conditions and soil mixtures: in the greenhouse in peat-based soil; outside the greenhouse in peat-based soil, outside the greenhouse in a peat-free soil, and hydroponic system in the greenhouse (University of Debrecen, Hungary). At the beginning of June 2021, whole petals were collected from each variety and condition. The lyophilised samples were extracted by ethanol and ultrasonic assisted method. The lutein and zeaxanthin content of the extracted samples were determined by the HPLC instrument.

Tagetes patula 'Csemő' was the richest source of lutein (709.9 - 1359.5 mg/kg based on dry matter), followed by 'Robuszta kénsárga' (161.4 - 429.7 mg/kg) and 'Orion' (62.0 - 135.8 mg/kg). The highest lutein concentration was measured in the peat-free soil mixture in the field, in each variety. The concentration of lutein and zeaxanthin were depending on the conditions (greenhouse, field) and the medium (peat-based, peat-free and hydroponics), but in the field, the measured lutein and zeaxanthin content was considerably higher than in the greenhouse in the same type of soil mixture (peat-based).

Introduction

Tagetes patula L. (French or dwarf marigold) belongs to the *Asteraceae* family, is commonly known as an ornamental plant. *Tagetes* species have been distributed all over the world and can be cultivated in different soils and weather conditions with low requirements and a long flowering period. *Tagetes patula*, similar to other marigold species, has been utilized in medicine, agriculture and industry [1, 2]. Leaves and shoots of *Tagetes* sp. synthesize and accumulate different terpenes, flavonoids, benzofuran derivatives, steroids, etc., due to theirs biologically active secondary metabolites used in medicines and cosmetics industry [2, 3]. Thiophenes, which are mostly concentrated in the roots of *Tagetes patula*, are used in agriculture production, due to its repellence of insects (nematicidal), bactericide, fungicide and insecticide effects [4, 5]. Petals of *Tagetes sp.* are yellow, orange, red and a mix of these colours, contain carotenoids (especially lutein, lutein fatty acid and β-carotene), used in ophthalmology as a dietary supplement and in the food industry as a natural food colorant (E161b) [6, 7]. Xanthophyll and carotenes contents of *Tagetes* species depend on the varieties, parts of plants, climate and soil conditions etc. [2, 8, 9].

Experimental

Germination and plant experiments were conducted in Biological Research and Plant Experiment Greenhouse (Biodrome), Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen (Hungary) and in the field outside the greenhouse, from February to August 2021. Three *Tagetes patula* Hungarian bred varieties were cultivated: 'Csemő' (TAG1), 'Robusta kénsárga' (TAG2), 'Orion' (TAG3) were derived by NARIC (National Agricultural Research and Innovation Centre, Hungary) and were bred by †Dr. Zoltán Kováts, a famous Hungarian ornamental plant breeder and colleagues [10].

Tagetes patula seeds were sown on 19th February 2021, in perlite (particle size: 2-6 mm, Magyar Perlit Kft., Hungary), in a germination chamber (humidified 4 times per day / 15 minutes, EasyGreen, USA). After 4 days, seedlings were transferred to peat-free and peat-based soil mixtures (COMPO, OBI, Hungary) into the cell seedling tray with a volume of 60 cm^3, and Grodan Rockwool cubes (25x25x40 mm, Playgrowned.com, Hungary). Terra Aquatica Tripart advanced nutrient solutions (Micro, Grow and Bloom 3-Part Flora Series, General Hydroponics Europe – GHE) were utilized with reverse osmosis water for Deep Water Culture (DWC) hydroponic production, according to the user guide of products and development phases of seedlings [11]. Three varieties of plantlets were transplanted into the pots (diameter of 14 cm, 1800 cm^3) on 5th May 2021 for pot experiments in the following conditions and soil mixtures: in the greenhouse in peat-based soil; outside the greenhouse in peat-based soil, outside the greenhouse in a peat-free soil, and hydroponic system in the greenhouse.

The first flowers appeared at the beginning of June 2021, whole petals were collected (Figure 1) and frozen in the freezer before lyophilisation. The samples were stored in the dark at room temperature before extraction and HPLC measurements.

Figure 1. Flowers of *Tagetes patula* 'Csemő' (TAG1), 'Robuszta kénsárga' (TAG2), 'Orion' (TAG3)

The lyophilised samples were grounded with a Retsch MM200 ball mill for 15 minutes. 0.1 g of the powdered samples were sonicated (NEY, Ultrasonik 3 QT HEAT, 175W) with 0.5 mL water for 15 minutes. After that 7 mL of absolute ethanol was added to the sample and sonicated at 30 minutes. Finally, the samples were filled up to 10 mL with ethanol and 0.45 µm pore size PVDF membrane filters were used for the filtration of the samples.

Extracts should be analyzed with HPLC as soon as possible, certainly within 24 hr, and must be stored at 4 ^{o}C. The lutein and zeaxanthin content of the samples was determined with an ECOM s.r.o. ECS05 gradient analytical HPLC system. This system consisted of an ECP2000 pump, ECB2004B gradient box with a degasser, ECO2080 column oven, ECDA2800 UV/Vis PDA detector and L-3320 autosampler made by RIGOL. A 10 µL sample was injected into the HPLC and eluted with acetonitrile 63.0 %, methanol 8.60 %, water 10.4 % and ethyl-acetate (18.0 %) on a C18 HPLC column (Phenomenex, 250x4.6 mm; 3 µm) at a flow rate of 1.0 mL/min. The measurements were done at 446 nm. The applied HPLC determination method was modified version of Habib method [12]. With these parameters, the elution times were close to 7.2 (lutein), 7.6 (zeaxanthin) minutes. The chromatograms were processed with Clarity 8.7 software.

Results and discussion

Flower petal of *Tagetes patula* ’Csemő’ (TAG1) was the richest source of lutein, it was between 709.9-1359.5 mg/kg (based on dry matter) depending on the conditions and medium. In the peat-based medium, the lutein content of ’Robusta kénsárga’ (TAG2) was double in the field (321.6 mg/kg) than in the greenhouse (161.4 mg/kg). The highest lutein concentration of ’Orion’ (TAG3) variety were measured in the peat-free soil in the field (135.8 mg/kg) and in the hydroponic systems (121.6 mg/kg) in the greenhouse. Lutein concentration of ’Orion’ (TAG3) were considerably lower than other varieties (Figure 2).
Zeaxanthin content of ‘Csemő’ (TAG1) was also the highest among the varieties, in the field was similar (approx. 117 mg/kg) in the different soils, followed by the hydroponics and peat-based soil in the greenhouse (86.1 and 71.1 mg/kg, consequently). In the greenhouse, in case of different medium, the zeaxanthin content of ’Robusta kénsárga’ (TAG2) and ’Orion’ (TAG3) was almost similar (15.0-19.4 mg/kg). In the field, zeaxanthin concentration of ’Orion’ (TAG3) was much lower than other varieties (Figure 2).

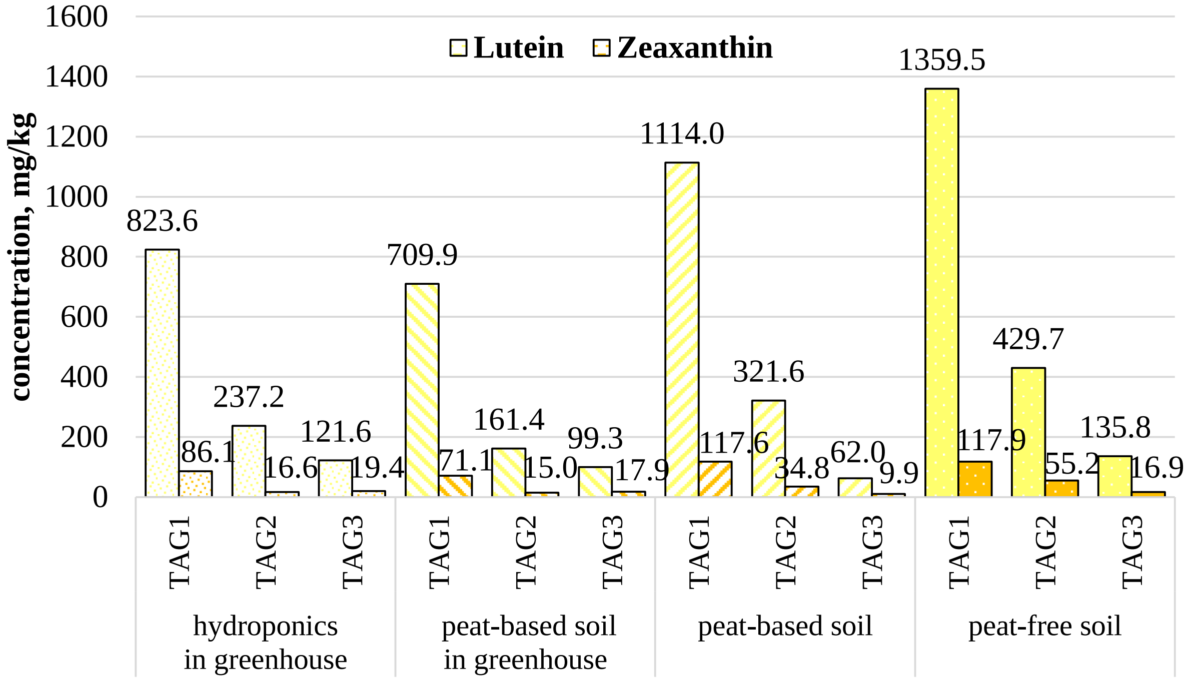

Figure 2. Lutein and zeaxanthin content (mg/kg, based on dry matter) of flowers of *Tagetes patula* Hungarian varieties in different medium and conditions (University of Debrecen, 2021)
Abbreviation: TAG1: *Tagetes patula* ’Csemő’, TAG2: *Tagetes patula* ’Robuszta kénsárga’, TAG3: *Tagetes patula* ’Orion’.

Based on our HPLC measurements and other authors references [2, 6, 8, 9] confirmed that the varieties, the types of the medium, the climate conditions and the fields may also affect the lutein and zeaxanthin concentration of *Tagetes patula* flowers. In our experiments, the highest lutein concentration was measured in the peat-free soil mixture in the field, in each variety. In case of peat-based soil mixtures, the measured lutein and zeaxanthin content were considerably higher in the field than in the greenhouse.

Conclusion

Tagetes patula has important chemical compounds which are considered for chemical and biological activity and they have a definite useful effect against various diseases. This

conference paper is the preliminary reports of HPLC measurements of lutein and zeaxanthin concentration from flowers of Hungarian bred *Tagetes patula* varieties. The applied HPLC method has fast, simple and cheap sample preparation.
We will continue this investigation of the *Tagetes patula* varieties because the growth of these plants at reduced use of peat and fertilizers is important for the commercial exploitation of these plant-produced compounds.

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