FLOWERS OF MARIGOLD (*Tagetes*) SPECIES AS A SOURCE OF XANTHOPHYLLS

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The accumulation of xanthophylls in flowers of marigold (*Tagetes*) species cultivated under conditions of the Belgorod region has been studied. Five cultivars representing three marigold species were investigated, including *T. erecta* (Rhodes and Orange Snow cultivars), *T. patula* (Bolero and Harmony) and *T. tenuifolia* (Red Gem). The overall xanthophyll content in the petals of flowers has been determined spectrophotometrically, while the composition of lutein diesters in each *Tagetes* species as well as the composition of anthocyanins in the flowers with claret spots have been studied by reverse phase HPLC. It is established that the total content of xanthophylls and their composition are close to the published data for analogous species growing in other regions of the world. It is shown that more than 90% of xanthophylls in flowers are retained upon drying and the content of lutein diesters in the dry material can exceed 15 mg/g.

The high biological activity of β-carotene stimulates the search for new natural sources of carotenoids and new methods for their synthesis [1]. The class of natural carotenoids includes compounds whose biological role is not restricted to provitamin activity. For example, special interest in recent years is devoted to lutein – a compound the deficit of which (together with zeaxanthin) is probably responsible for the age-related impairment of vision [2]. In many countries, the natural source of luteins is provided by cultivating marigold (*Tagetes* species) flowers, the petals of which contain predominantly lutein diesters [3 – 7]. Xanthophylls extracted from these plants are used to obtain a concentrated material for use in a new direction of nutrient design, in particular, for the production of chicken eggs with xanthophyll-pigmented yolks [4, 7, 8]. These results increased the interest in marigold species as medicinal plants, which have been used for a long time in preparing drinks, seasonings, and tinctures, the secondary metabolites of which have been proved in experiment to exhibit antiviral properties [9].

This study was aimed at evaluating the potential of using various marigold species cultivated in the Belgorod region as a source of lutein.

EXPERIMENTAL PART

The plants of marigold (*Tagetes*) species were grown in the botanic garden at the Belgorod State University. Freshly collected flowers were studied shortly (within one to two hours) after harvesting. The air-dry material was obtained by drying petals in a thermal box without access to direct sunlight. The quantitative analysis for xanthophylls in acetone extract was performed using spectrophotometry (KFK-3-01 instrument), by measuring the optical absorption at $\lambda_{max} = 435$ nm and recalculating for lutein with $E^{1\%}_{1cm} = 2550$ [10].

Extraction of xanthophylls. An accurately weighed sample (about 0.2 g for fresh petals or 0.050 g for air-dry material) was triturated with fine quartz sand. The mixture was placed into a 100-ml measuring, diluted with 75 ml of acetone, and extracted by shaking for 30 min in a box without

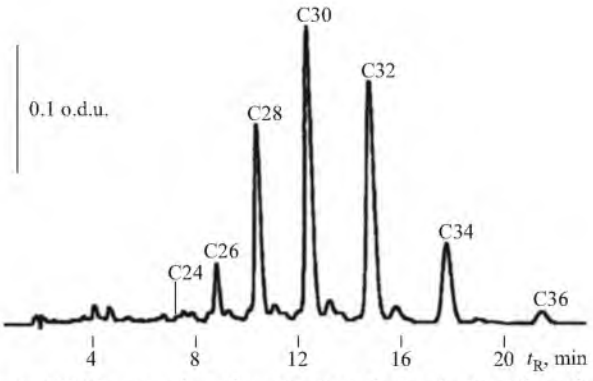

Fig. 1. HPLC separation of xanthophyll diesters isolated from flowers of *Tagetes* species (figures at the peaks indicate the total number of carbon atoms in the corresponding acid radical).

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TABLE 1. Total Content of Xanthophylls (Recalculated for Lutein) in Fresh Petals of Flowers of Various Marigold Species

Species	Cultivar	Color	Content of xanthophylls, mg/g (Date of collection)			
			11.07.2005	28.07.2005	29.09.2005	06.10.2005
T. erecta	Rhodes	Orange	5.24	3.95	2.70	2.330
	Orange Snow	Orange	2.05	2.09	3.15	–
T. patula	Bolero	Orange-red	1.84	1.47	1.66	–
		Orange	2.74	3.06	1.79	–
	Orange-red	Orange	2.36	1.77	1.44	–
T. tenuifolia	Red Gem	Orange-red	–	1.72	–	–

access to direct sunlight. Then, the flask was filled with the same solvent to the mark, the solution of xanthophylls was separated by filtration, and the optical density of this extract was measured (where necessary, upon additional dilution with acetone). It was established that no repeated extraction was usually necessary, since the degree of isolation of xanthophylls exceeded 99% while the scatter of their content in parallel runs was within or even greater than 5 – 10%.

The extraction of anthocyanins was effected using an analogous procedure with an aqueous $HClO_4$ solution (pH 0.5 – 1.0) as the extractant. The total content of anthocyanins was evaluated by recalculation to cyanidin-3-glucoside with $\varepsilon = 26900$ at $\lambda_{max} = 510$ nm [11]. The qualitative analysis of anthocyanins was performed as described elsewhere [12, 13].

The composition of carotenoids extracted from marigold flowers was determined by HPLC using a system comprising an Altex 11A pump, a Rheodyne 7100 dosing valve with a 20-μl sampling loop, a Spectromonitor LC/9563 detector (tuned to $\lambda_{max} = 435$ nm), and a Shimadzu C-R3A integrator. A 250 × 4 mm column was filled with Cromasil C18 (5 μm) and eluted with an acetonitrile – acetone (10 : 90, v/v) mixture at a rate of 1 ml/min.

RESULTS AND DISCUSSION

The results of our preliminary investigation performed in 2004 showed that the maximum amount of xanthophylls was accumulated in the flowers with orange (or orange with claret spots) petals, where the content of carotenoids (recalculated for lutein) exceeded 5 mg per gram of fresh petals

TABLE 2. Total Content of Xanthophylls (Recalculated for Lutein) in Fresh and Dry Petals of Flowers of Various Marigold Cultivars

Cultivar (color)		Sample No.	Content of xanthophylls, mg/g					
			wet mass			dry mass		
			X_i	mean	*SD*	X_i	mean	*SD*
Rhodes (of 06.10.05)	Fresh	1	2.47	2.33	0.19	—		
		2	2.43					
		3	2.27					
		4	2.05					
		5	2.42					
		6	2.56					
		7	2.14					
	Dried	1	2.20	2.18	0.10	11.4	11.0	0.47
		2	2.35			11.3		
		3	2.10			10.7		
		4	2.15			10.3		
		5	2.10			11.2		
Rhodes (of 29.10.05)			2.70			17.0		
Orange Snow			2.97			13.9		
Bolero (orange)			1.74			10.2		
Bolero (orange-red)			1.46			8.73		
Harmony (orange)			1.50			8.60		
Harmony (orange-red)			2.10			12.9		

against 1 mg/g for yellow and 0.2 mg/g for lemon-yellow flowers. For this reason, the main effort in 2005 was devoted to the marigold species with orange flowers. Among various available plants, we selected five cultivars representing three *Tagetes* species: *T. erecta* (Rhodes and Orange Snow cultivars), *T. patula* (Bolero and Harmony) and *T. tenuifolia* (Red Gem).

Table 1 presents the results of investigations performed on fresh petals of marigold flowers grown and harvested in 2005. The content of xanthophylls in this material in that year was significantly smaller than in 2004. Not all samples collected in 2004 were exactly identified, except for several belonging to *T. patula*, for which the decrease in the content of xanthophylls amounted to 25 – 50%. Probably, the decrease in productivity with respect to xanthophylls was related to some unfavorable weather conditions. Nevertheless, the advantage of species with orange flowers was confirmed: yellow flowers still contained a significantly lower amount of xanthophylls (below 1 mg/g). It should be noted that we did not observe significant differences in the qualitative composition of xanthophylls between the flowers of three *Tagetes* species under consideration. In 2005 (in contrast to 2004), the orange flowers with claret spots contained about 25% less xanthophylls than the orange flowers without spots.

Using the proposed mobile phase with 10 vol.% of acetonitrile, it is possible to separate all the main lutein diesters, which account for more than 85% of area under peaks in the HPLC pattern (Fig. 1), and the impurities (zeaxanthin diesters, etc.) contained in the extract. HPLC analysis of nonderivatized fatty acids in the products of saponification of the extract [14] showed that the main acids of isolated esters were myristic and palmitic acids (accounting for 85 – 90% of the total sum of acid radicals); smaller fractions represented stearic and lauric acid radicals. It should be noted that the identification of lutein diesters encounters no difficulties because the distances (retention time increments) between the peaks are approximately equal throughout the sequentially eluted diesters from laurate and myristate to the most lipophilic (eluted last) lutein distearate (Fig. 1). The content of dilaurate was relatively small in all samples: this ester could be detected in the group of accompanying impurities using the increment difference known for the given chromatographic system.

The marigold flowers of orange color with claret spots were characterized by a high content of anthocyanins. According to the HPLC data, the main component was cyanidin-3-glucoside; some samples also contained a significant amount (10 – 50%) of another cyanidin derivative (while the products of hydrolysis contained only cyanidin). The chromatographic behavior of this derivative is identical to that of cyanidin-3-glucoside acylated with malonic acid, which is one of the main (depending on the color) pigment components of *Callistephus chinensis* L. – a plant of the *Compositae* family [12]. The content of anthocyanins (150 mg/g fresh petals) in the flowers with claret spots was approximately the same as that in the fruits of *Ribes nigrum* L.

From the standpoint of commercial production technology, it is important to ensure standardization of the initial material, which can be provided by preliminary drying of the raw plant material. In this context, it was of interest to study the retention of xanthophylls on drying. For this purpose, we have determined the content of xanthophylls in the fresh petals, then dried known amounts of the initial material at room temperature without access to direct sunlight, and repeated the analysis. As can be seen from the data presented in Table 2, the loss of xanthophylls on drying did not exceed 5 – 10% even for material dried and stored without special precautions (e.g., with exposure to scattered light). The content of xanthophylls in air-dry samples can exceed 15 mg/g. This is an important circumstance, since the saponification of diesters (for obtaining nonesterified xanthophylls) will be accompanied by unavoidable losses [5]. However, this stage is probably not necessary, since it is known that xanthophyll diesters can be saponified in the human organism [[15].

In conclusion, we have established that marigold species cultivated under conditions of the Belgorod region offer a promising source of natural xanthophylls.

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