STUDIES OF *PHYSALIS ALKEKENGI* L. FRUITS AS A SOURCE OF XANTHOPHYLLS

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The studies reported here established that fruits of the perennial and quite hardy plant *Physalis alkekengi* grown in the conditions of the Belgorod area accumulate zeaxanthin (and β-cryptoxanthin) derivatives at levels of up to 20 mg/g of husks, which are needed for the prevention of age-related visual loss. Furthermore, attention is drawn to the berries of this *Physalis* species, which can be used in foods as a source of zeaxanthin (about 0.30 mg per berry).

The roles of lutein and zeaxanthin have been actively studied in recent years with a view to preventing age-related macular disease [1]. Preparations enriched in lutein are commercially available, though this is not the case for zeaxanthin [2]. The availability of lutein depends on the use of marigold flowers, which accumulate mainly lutein diesters and are cultivated commercially in many areas of the world [3]. These plants were used to create concentrates intended to increase xanthophylls in the yolks of chick eggs (ORO GLO, Kemin-Europa). Apart from lutein, the concentrate also contains zeaxanthin, though the proportion of zeaxanthin in xanthophyllic flowers of the common varieties of *Tagetes* species is no greater than 5 – 10%. Given that the ratio in the macula lutea is some 4:1, the imbalance in the content of these xanthophylls is obvious. This is probably why mutant marigolds were produced with increased zeaxanthin contents [4]. An alternative pathway to producing xanthophyll-balanced preparations is to use natural sources of zeaxanthin. Plants accumulating zeaxanthin (and its esters) include pepper (*Capsicum annuum* L.) fruits, i.e., orange varieties [5], desert thorn (*Lycium barbarum* L.) fruits [6], decorative physalis (*Physalis alkekengi* L.) fruits [2], and sea buckthorne (*Hippophae rhamnoides*) fruits [2].

The aim of the present work was to assess the potential for using physalis fruits grown in conditions of the Belgorod area to produce concentrates enriched with zeaxanthin.

METHODS

Studies used fruits grown in the Belgorod and Kursk areas in 2004 and 2005.

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Quantitative estimation of xanthophylls (in terms of zeaxanthin) was performed by spectrophotometry (using a KFK-3 – 01 spectrophotometer) measuring the absorption of light at (λ_{max}) 445 nm of acetone extracts using $E_{1cm}^{1\%} = 2340$ [7]. Qualitative analysis was performed by comparison of the spectra of fractions, both of the initial extracts and of the products of complete and partial saponification; the fatty acid composition of hydrolysates was also determined.

Extraction of xanthophylls from swollen bladder-like bells with fruits. A batch of sample material (about 0.2 g fresh bells or 0.050 g dried) was ground with quartz sand and the mixture was transferred to a 100-ml measuring flask; about 75 ml of acetone was added and the mixture was shaken without exposure to direct light for 30 min. The contents of the flask were then made up to the mark with acetone and the optical density of the extract was measured, diluting with acetone when necessary. Repeated extraction was generally not required - the degree of extraction of xanthophylls was greater than 99%.

The carotenoid composition of decorative physalis fruit was determined by HPLC using a chromatography system consisting of an Altex 110A pump, a injection valve with a 20-μl loop, a Spectromonitor LC/9563 (λ = 435 nm) detector, and a Shimadzu C-R3A integrator. The chromatography conditions were: 250 × 4 column containing Kromasil C18.5 μm; a mobile phase consisting of acetonitrile and acetone (10:90 by volume) at a flow rate of 1 ml/min.

RESULTS AND DISCUSSION

Decorative physalis fruits are orange "lanterns" (swollen bladder-like bells with fruits[8]) containing orange berries. The good persistence of the orange coloration and the un-

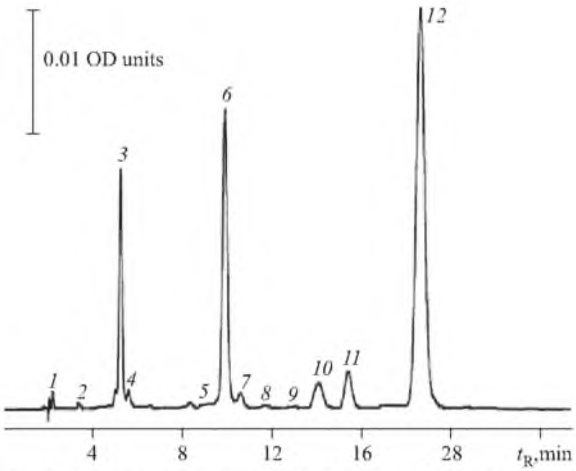

Fig. 1. Separation of xanthophylls from an acetone extract of *Physalis alkekengi* L. fruits. Column: 250 x 4 mm, Kromasil C18.5 μm. Mobile phase: 10% acetonitrile in acetone (by volume) at 1 ml/min. Detection at 445 nm. *1*) Zeaxanthin; *2*) β-cryptoxanthin; *3*) zeaxanthin monopalmitate; *4*) β-carotene; *6*) β-cryptoxanthin palmitate; *9*) zeaxanthin dimyristate; *11*) zeaxanthin myristate palmitate; *12*) zeaxanthin dipalmitate; *5, 7, 8, 10*) unidentified carotenoids.

TABLE 1. *Physalis alkekengi* Fruit Xanthophylls.

Xanthophylls	Decorative physalis fruit samples					
	No. 1	No. 2	No. 3	No. 4	No. 5	Fruit
Total xanthophylls, mg (zeaxanthin) per g raw material	20.2	12.5	16.2	9.8	10.5	> 0.3
Zeaxanthin derivatives	Content from peak areas, mol% (± 0.5%)					
unesterified	1.7	1.2	0.8	0.7	1.2	1.1
monopalmitate	7.4	8.4	4.9	4.0	7.0	10.6
myristate palmitate	6.1	5.5	8.7	9.5	9.8	4.7
dipalmitate	58.2	60.9	51.7	57.3	58.3	53.7
dipalmitate	73.4	76.0	66.1	71.5	76.3	70.1
β-Cryptoxanthin derivatives	Content from peak areas, mol% (± 0.5%)					
unesterified	Trace	Trace	Trace	Trace	Trace	Trace
palmitate	18.8	19.4	24.7	23.1	17.7	22.5
Unidentified carotenoids	Content from peak areas, mol% (± 0.5%)					
	7.8	4.6	9.2	5.4	6.0	7.4

usual shape of the "lanterns" make the plant highly decorative and allow it to be used in "dry" bouquets.

Chromatograms of extracts of the bells and fruits were virtually identical (Fig. 1, Table 1) – all showed the same components present at quite similar ratios. The main component of the xanthophyll complex was zeaxanthin dipalmitate, while significantly smaller quantities of zeaxanthin myristate palmitate were present; the peak with second largest relative area corresponded to β-cryptoxanthin palmitate, and minor quantities of zeaxanthin monopalmitate were present; a compound whose retention coincided with that of β-carotene eluted later, in minor quantities. Unesterified zeaxanthin and β-cryptoxanthin were usually present in trace amounts and their positions on chromatograms were identified by using products of partial saponification of the extract.

Since the spectral characteristics of the major components of the extract – diesters of zeaxanthin and the ester of β-cryptoxanthin – were similar, their levels were assessed quantitatively by spectrophotometry. Xanthophyll contents in the bells of the fruits of *P. alkekengi* were significantly greater than in the petals of marigold flowers, reaching 20 mg (in terms of zeaxanthin) per g of raw material, with slightly lower levels in fruits with poor development of the orange coloration. The quite high stability of the xanthophylls was demonstrated by results obtained from sample No. 5 (Table 1), which had been used as a decorative bouquet for several months, though the initial xanthophyll content in this case was unknown. We also note that complete and rapid extraction of xanthophylls requires initial careful grinding of the starting material, which might take several days for complete extraction.

Exhaustive extraction of xanthophylls from fresh fruits was particularly difficult. Addition of acetone (20 ml per berry) and malaxation of the berries beneath the solvent layer results in release of a small proportion of the xanthophylls. After the residue was dried and ground with quartz sand, a significant quantity of xanthophylls can be extracted, though complete decolorization of the residue could not be achieved even after five rounds of extraction (portions of 20 ml per berry) with infusion for 30 min at each stage. Therefore, the quantitative characteristics of xanthophyll accumulation in the fruits (0.3 mg/berry) is a minimum estimate. However, it is clear that processing of the edible (which has a pleasant, slightly bitter, taste) berries for the coloration is unnecessary consumption of 3 – 6 berries per day is sufficient to provide the daily zeaxanthin dose [9].

Thus, the fruits of the perennial and quite hardy plant *P. alkekengi* can be used for extraction (from the bladder-like bells) of zeaxanthin (and β-cryptoxanthin) derivatives required for the prophylaxis of age-related visual loss. In addition, the berries could be a valuable addition to the available imported strawberry physalis fruits which have only a pleasant taste and aroma (these can be cultivated in Belgorod and the region even by growing of self-sown plants).

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