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## LUTEIN IN FOOD SUPPLEMENTS AVAILABLE ON THE MARKETS OF THE VISZEGRAD COUNTRIES

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### ABSTRACT

RP-HPLC method with UV-VIS detection was implemented for determination of contents of lutein in food supplements available on the markets in the Czech Republic, Slovakia, Poland, and Hungary. Altogether, 48 samples of food supplements in three dosage forms (22 samples of tablets, 18 samples of soft capsules, and 8 samples of hard capsules) were analysed. The amounts of lutein specified by the producer complied with their real contents only in 7 samples of the food supplements. Lutein in soft capsules showed the highest stability against oxidation; lutein in tablets was more prone to oxidation and lutein in hard capsules was most susceptible to oxidation process. Out of 21 Czech products, only four fell into the category of satisfactory products, three of them were soft capsules and one was a tablet. Out of 27 products manufactured abroad, only three were evaluated as satisfactory products, all of them were soft capsules, out of 48 analysed food supplement samples just seven fell into the category of satisfactory preparations, eight were evaluated as less satisfactory preparations, five were found inadequate products and 28 samples were labelled unsatisfactory. Only one in six analyzed samples contained the amount of lutein specified by the manufacturer, almost 60% of monitored lutein containing food supplement samples fell into the unsatisfactory product category.

Keywords: Carotenoids; RP-HPLC; marigold flower; tablets; capsules

### INTRODUCTION

(systematically Lutein named  $(3R,3'R,6'R)-\beta$ , ε-carotene-3,3'-diol or 3,3'-dihydroxy-α-carotene, see Figure 1), is a yellow plant pigment that belongs to the carotenoid family, namely to xanthophylls. It occurs in many kinds of fruits and vegetables, especially in leafy vegetables. It is also found in yolk and eye tissues (Calvo, 2005; Čopíková et al. 2005). Pure lutein is a red-orange crystallic substance soluble in fats and organic solvents, but insoluble in water.

Lutein (luteus means yellow in Latin) acts as an effective antioxidant. It is also able to stop degenerative changes of macula lutea resulting in blindness. Lutein plays an important role in perception phenomenon called Heidinger's brush that enables human's determination of plane or direction of polarized light rotation.

Moreover, lutein protects the organism against heart diseases and cancer. It is soluble in fats, therefore it is transported by a form of cholesterol, namely by low density (LDL) lipoproteins. Lutein protects vitamin E against oxidation and, furthermore, it most likely improves function of immune system (Calvo, 2005). It also protects both eyes and skin against strong sun radiation and against effects of air pollutants and products of smoking.

Moreover, lutein prevents fat peroxidation that widely occurs both in blood serum and eyes. High doses of lutein decrease risk of cervical cancer (Calvo, 2005; Evans and Johnson, 2010).

All over the world, interest in healthy life-style has been increasing recently and the conceptions of disease prevention have been adopted intensively. Human organism does not show ability to synthesise lutein, which is why humans can acquire it solely by consumption of fruits, vegetables and food supplements (Calvo, 2005). Due to relatively low biological utilizability of lutein contained in natural resources, consumption of lutein enriched food or intake of food supplements are suggested (Calvo, 2005). Recommended daily dose is 6 - 10 mg of lutein. Some researchers suggest even higher daily intake up to 20 mg of lutein (Garti et al., 2003; Abdel-All et al., 2007; Bernstein et al., 2010; Cerón-García et al. 2010; Evans a Johnson, 2010; Li et al., 2011).

Lutein containing food supplements have become integral part of our common everyday nourishment. They

$$H_3C$$
 $CH_3$ 
 $CH_3$ 

Figure 1 Structural formula of lutein.

are widely available in Czech pharmacies, supermarkets or in E-shops. Many of lutein containing products available on the market declares high contents of lutein, which calls their real lutein levels, in question. Such products are sold at relatively low prices. Lutein content is usually determined by common spectrophotometric methods that are not specific, though, to find lutein content using HPLC, a chromatographic column with C18, or C30 sorbents is commonly applied; it is also suitable for separation of individual trans- and cis- isomers of lutein. To analyse substances with identical molecular mass such as lutein and zeaxanthin, cyanopropyl column with silica sorbent containing -(CH<sub>2</sub>)<sub>3</sub>-CN end group is recommended.

Our study had three goals: i) determination of lutein contents in products available on Czech, Polish, Hungarian and Slovak markets; ii) comparison of lutein quality in various dosage forms like tablets and soft or hard capsules; iii) comparison of quality of lutein containing food supplements manufactured by various producers.

### MATERIAL AND METHODOLOGY

### Sample selection

Selected food supplements available on Czech, Slovak, Polish and Hungarian markets were subjected to study. Contents of lutein ranging between 0.25 to 25 mg in one tablet or capsule were declared in food supplements manufactured by the producers from the Czech Republic, Denmark, Finland, Canada, Hungary, Germany, Poland, Austria, Slovakia, Sweden, Switzerland, and USA. We monitored samples of food supplements produced in the form of tablets, soft capsules or hard capsules. Their characteristics are listed in Table 1. All the food supplement samples were analysed before expiration date stated by their producers.

#### Lutein standard

Standard solution of lutein was prepared by dissolution of accurate amount (0.50  $\pm$ 0.01 mg; Extrasynthese, France) of lutein standard in acetone-methanol solvent (50 ml; 1:1 v/v). Calibration curve was plotted based on signals of various volumes of standard lutein solution injected into an HPLC column: 1, 2, 3, 5, 8, and 10  $\mu$ L. Calibration was always implemented on the day of analysis.

### HPLC determination of lutein content

Contents of food supplement samples in the form of a tablet or a capsule with average mass of 1 g was dissolved in 50 ml of acetone-methanol solvent (1:1 v/v). For 10 minutes, the sample was treated in an ultrasound

**Table 1** Dietary supplements: lutein content (mg per tablet or mg per capsule) and their percent rate found in tablets (D1-D22), soft capsules (D23-D40) and hard capsules (D41-D48).

Sample	Product expiration	Lutein content				Product	Lutein content		
		Declared	Determined mean ±S.D.	%	Sample	expiration	Declared	Determined mean ±S.D	%
D1	II/2013	0.25	$0.01 \pm 0.00$	4.0	D25	V/2013	10.0	$12.5 \pm 0.09$	125
D2	IX/2013	2.0	$3.07 \pm 0.03$	15.4	D26	VI/2012	10.0	$9.45 \pm 0.05$	94.5
D3	IX/2012	3.8	$0.02 \pm 0.00$	5.3	D27	III/2013	12.0	$11.4 \pm 0.12$	95.0
D4	I/2013	5.0	$0.08 \pm 0.01$	1.5	D28	VIII/2012	12.0	$6.13 \pm 0.07$	51.1
D5	III/2013	5.0	$0.04 \pm 0.00$	0.8	D29	VII/2013	12.0	$10.2 \pm 0.11$	85.0
D6	V/2013	5.5	$0.08 \pm 0.00$	1.5	D30	III/2013	15.0	$13.4 \pm 0.13$	89.3
D7	II/2013	15.0	$0.04 \pm 0.00$	0.3	D31	V/2014	15.0	$14.8 \pm 0.14$	98.7
D8	III/2013	3.0	$0.09 \pm 0.00$	3.0	D32	VI/2012	15.0	$5.66 \pm 0.20$	3.7
D9	IV/2014	3.0	$2.44 \pm 0.07$	81.3	D33	IX/2012	6.0	$0.10 \pm 0.01$	2.2
D10	VIII/2012	6.0	$5.71 \pm 0.09$	95.2	D34	IX/2013	4.0	$6.52 \pm 0.09$	161
D11	II/2013	6.0	$0.02 \pm 0.00$	0.3	D35	I/2014	4.0	$7.64 \pm 0.11$	190
D12	V/2013	10.0	$0.02 \pm 0.00$	0.2	D36	IX/2012	8.0	$4.52 \pm 0.09$	56.8
D3	III/2013	12.0	$1.19 \pm 0.00$	9.9	D37	V/2014	6.0	$2.33 \pm 0.07$	38.2
D14	IX/2012	0.3	$0.01 \pm 0.00$	3.3	D38	II/2013	10.0	$1.22 \pm 0.04$	12.2
D15	III/2013	6.0	$0.05 \pm 0.01$	0.8	D39	IX/2012	10.0	$0.99 \pm 0.06$	9.9
D16	VI/2013	6.0	$0.04 \pm 0.00$	0.7	D40	II/2014	20.0	$23.3 \pm 0.18$	116
D17	VIII/2013	12.0	$10.0 \pm 0.11$	83.3	D41	III/2014	3.0	$0.02 \pm 0.00$	0.7
D18	IV/2013	0.5	$0.12 \pm 0.01$	24.0	D42	V/2013	6.0	$0.05 \pm 0.00$	0.8
D19	II/2013	1.0	$0.16 \pm 0.01$	16.0	D43	IV/2014	6.0	$0.10 \pm 0.01$	1.7
D20	VIII/2012	6.0	$0.16 \pm 0.01$	2.7	D44	I/2014	11.0	$0.07 \pm 0.01$	0.6
D21	VII/2012	3.0	$0.02 \pm 0.00$	0.7	D45	IX/2012	5.0	$0.20 \pm 0.01$	4.0
D22	V/2014	0.25	$0.11 \pm 0.01$	44.0	D46	X/2012	25.0	$0.09 \pm 0.01$	0.4
D23	VI/2014	3.0	$6.44 \pm 0.08$	205	D47	IV/2013	0.8	$0.09 \pm 0.00$	11.3
D24	III/2013	3.0	$4.10 \pm 0.10$	137	D48	III/2013	20.0	$0.05 \pm 0.00$	0.3

shaking apparatus and then spun at 6,000 g for 5 minutes. Separation was performed using an HPLC 1100 instrument with UV-VIS DAD detector at 30 °C (all instruments produced by Agilent Technologies, Waldbronn, Germany) with linear gradient elution (0 min 30% A, 10 min 0% A and 15 min 30% A) on a ZORBAX SB CN (75 mm x 4.6 mm, 3.5  $\mu m$ ) column with mobile phase flow of 0.7 ml/min (3.153 g/L of ammonium formate in water - A; and methanol - B). The signal was recorded at 446 nm with bandwidth of 16 nm upon the injection of analysed samples (1-10  $\mu L$ ). The reference signal was monitored at  $\lambda=600$  nm with bandwidth of 100 nm. Figure 2 illustrates an example of a chromatogram of a lutein containing food supplement sample.

### **HPLC** Metod validation

Accuracy, precision, and recovery were evaluated (n = 6 - 10) with model solutions and samples spiked with lutein standards (concentrations varying from 0.5 to 3.0  $\mu$ g/g). Intraday and interday repeatability were verified by analyzing standard solutions and lutein samples using the same procedure as in **Šivel et al. (2015)**.

The limit of detection (LOD, S/N=3) was  $0.22~\mu g/g$  and the limit of quantification (LOQ, S/N=10) was  $0.73~\mu g/g$ . Two independent sample solutions were always prepared. The HPLC analysis of each of them was determined in triplicate. The recorded results were processed by ANOVA variance analysis using both statistical Unistat 5.1 software and Office Excel®Microsoft program (Snedecor and Cochran, 1967).

### RESULTS AND DISCUSSION

### Lutein content in food supplements

Altogether, the following 48 lutein containing food supplement samples were analysed: 22 supplements produced in the form of tablets (D1 - D22 samples), 18 food supplements manufactured in the form of soft capsules (D23 - D40), and 8 food supplements produced in the form of hard capsules (D41 - D48). The declared and detected contents of lutein in samples are specified in Table 1; their percent rate found in tablets is shown in Figure 3.

Out of seven tablet samples of Czech food supplements,

only one (D2) demonstrated lutein level higher than declared by the producer. We found that the D2 sample contained 3.07 mg/tablet; the manufacturer stated that it included just 2.00 mg/tablet. The contents of lutein in the other tablet products (D1, D3 - D7) were below 10% of the level declared by the producers (Figure 3). None of 15 tablet samples produced abroad (D8 - D22) showed lutein content given by the producers. In three samples, the detected contents of lutein were almost the same as the levels declared by the producers (D9, D10, and D17). The other analyzed tablet samples contained less than 50% of lutein level indicated by their producers (Figure 3).

The structure of tablets in four food supplement samples (D2, D9, D10, and D17) that contained more than 80% of declared lutein amount suggests the use of encapsulated form of lutein in the manufacture. For soft capsule samples, Figure 4 depicts the percent proportion of detected lutein contents to levels declared by producers (Table 1).

Out of ten Czech soft capsule samples, three (D23 - D25) contained lutein levels higher than indicated by producers. Other five Czech samples (D26, D27, D29 - D31) included above 85% of lutein amounts specified by producers (see Figure 5). Out of eight soft capsule samples produced abroad, three (D34, D35 and D40) showed higher contents of lutein than declared by producers.

The D23 sample contained 6.14 mg/capsule, which corresponds to almost 205% of the amount specified by its producer, that is 3.00 mg per soft capsule. Figure 5 plots percent proportion of lutein contents detected in hard capsules to levels declared by producers (values stated in Table 1).

Four hard capsule samples produced by the Czech manufacturers (D41 - D44) included less than 2% of the declared lutein contents, out of which three even less than 1%. Four samples of hard capsules made abroad (D45 - D48) contained less than 12% of the specified lutein amounts; two samples showed even contents lower than 1%.

### Quality of lutein in food supplements

To compare quality of lutein in various dosage forms of food supplements (tablets; soft and hard capsules),

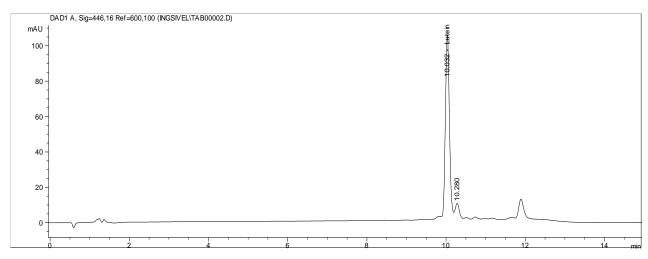


Figure 2 Chromatogram of a dietary supplement sample D2 containing lutein.

categorization according to proportion of detected to declared lutein contents was employed: i) satisfactory (~ 100%), ii) less satisfactory (75 - 100%), iii) inadequate (25 - 75 %), and iv) unsatisfactory (<25%).

Three samples of soft capsules and one sample of tablets of Czech producers were evaluated as satisfactory products. Five samples of soft capsules were labelled less satisfactory; six tablet samples and all four samples of hard capsules were found unsatisfactory goods. Three samples of soft capsules of food supplements produced abroad were evaluated as satisfactory; three samples of tablets were evaluated as less satisfactory preparations; eleven samples of tablets, three samples of soft capsules and all four samples of hard capsules were found unsatisfactory

goods.

Comparing three dosage forms such as tablets, soft or hard capsules in reference to lutein resistance against oxidation by air oxygen, we can conclude that soft capsules are the most resistant dosage form followed by tablets and hard capsules, the least resistant ones.

### Quality of lutein containing food supplements

Out of all 21 Czech product samples, four were classified as satisfactory preparations, five samples fell into the less satisfactory product category, two products were found inadequate, and ten were evaluated as unsatisfactory products. Out of all 27 products manufactured abroad, three samples fell into the satisfactory preparation

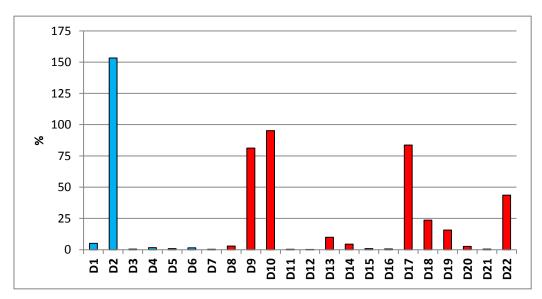
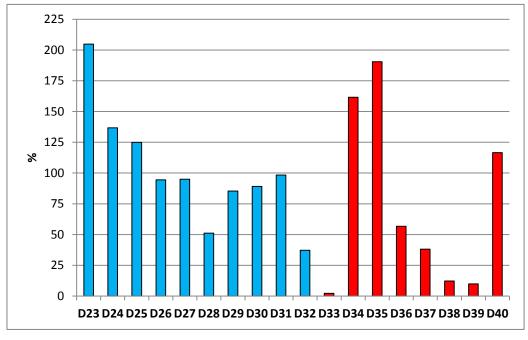


Figure 3 The percent proportion of lutein contents detected in samples to levels declared by producers in tablets.



**Figure 4** The percent proportion of lutein contents detected in the sample to levels declared by producers in soft capsules.

category, three samples were found less satisfactory preparations, three samples were labelled inadequate, and 18 samples were included in the unsatisfactory products category.

Comparing the quality of Czech and foreign products, the Czech ones ranked higher; 19% of them were evaluated as satisfactory products, while just 11% of the products made abroad fell into the same category. Moreover, only 48% of Czech products fell into the unsatisfactory category compared to 67% of products manufactured abroad.

### **CONCLUSION**

Before manufacture, producers of lutein containing food supplements have to implement a thorough selection of raw materials. Reliable and trustworthy supplier might rank among the most important manufacturing factors. Suppliers are supposed to provide high quality lutein and to employ reliable analytical methods used for detection of its content; thereby they guarantee declared content of lutein in the given marigold plant extract.

The food supplement manufacturers should implement input analyses of ingredients and output analyses of products. Using cheap ingredients like lutein containing extracts, that are widely available on the market, they should also consider possible negative effects.

The worldwide trend leads food supplement producers to make products with 5 - 15 mg of lutein per a tablet or a capsule; the above amount corresponds to recommended daily intake of lutein. Using HPLC method, some researchers (Aman et al., 2004; Young et al., 2007; Kroll et al., 2008; Thomas et al., 2012) tested 20 samples of lutein containing food supplements. To conclude, we can recommend production of soft gelatine capsules with declared content of lutein ranging between 3 - 15 mg per one capsule that is the dosage form that enables the highest protection of lutein against oxidation.

Based on the analyses of ingredients and food supplements, the following findings can be arrived at: i) out of 22 samples of food supplements produced in the form of tablets, only one showed the qualities of a satisfactory product; ii) out of 18 samples of food supplements produced in the form of soft capsules, six samples fell into the category of satisfactory products; iii) out of eight samples of food supplements produced in the form of hard capsules, no sample was evaluated as a satisfactory preparation; iv) concerning stability of lutein in all three dosage forms, we arrived to the following conclusions: lutein contained in soft capsules showed the highest stability against oxidation; lutein in tablets was more prone to oxidation and lutein in hard capsules was most susceptible to oxidation process; thus soft capsules proved to be the most suitable application form followed by tablets and hard capsules; v) out of 21 Czech products, only four fell into the category of satisfactory products, three of them were soft capsules and one was a tablet; vi) out of 27 products manufactured abroad, only three were evaluated as satisfactory products, all of them were soft capsules; vii) out of 48 analysed food supplement samples just seven fell into the category of satisfactory preparations, eight were evaluated as less satisfactory preparations, five were found inadequate products and 28 samples were labelled unsatisfactory; viii) only one in six analyzed samples contained the amount of lutein specified by the manufacturer; ix) almost 60% of monitored lutein containing food supplement samples fell into the unsatisfactory product category.

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