

Lutein Content in Marigold Flower (*Tagetes erecta* L.) Concentrates used for Production of Food Supplements

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Abstract

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The RP-HPLC with UV-VIS DAD detection was employed for the separation, identification, and quantification of lutein content in Marigold flower (*Tagetes erecta* L.) concentrates used for the production of food supplements. The concentrates commercially available in encapsulated (“granules” – 16 samples) and powder (18 samples) forms were analysed. Only 10 samples showed lutein levels complying with lutein contents declared by the manufacturers. Marigold extract in the encapsulated form proved to be more stable against oxidation than the extract in the powder form.

Keywords: *Tagetes erecta* L.; carotenoids; RP-HPLC; UV-DAD detection

Lutein is a yellow plant pigment that belongs to the carotenoid family, namely to xanthophylls. Its structural formula is depicted in Figure 1. It occurs in many kinds of fruits and vegetables, especially in leafy vegetables, but also in the yolk and eye tissues (ŠTĚRBOVÁ *et al.* 2003; CALVO 2005; ČOPÍKOVÁ *et al.* 2005; ŠIVEL *et al.* 2013). Lutein acts as an effective antioxidant, namely in the protection of eyes, because it neutralises free radicals formed by the action of ultraviolet radiation on eye retina. Humans are not able to synthesise lutein, so they can acquire it solely by the consumption of fruits, vegetables, and/or food supplements (CALVO 2005). Plant materials contain all-*trans*-isomer of lutein; nevertheless, *cis*-isomers of lutein are regenerated, apart from other agents, also by the actions of light and temperature, and other factors were also detected during extraction

and sample analysis (HUMPHRIES & KHACHIK 2003; UPDIKE & SCHWARTZ 2003; CALVO 2005; ČOPÍKOVÁ *et al.* 2005; KHACHIK & CHANG 2009; ŠIVEL *et al.* 2013). In plants, lutein is present either in the form of free lutein in leafy vegetables such as spinach, cabbage, and broccoli, or in the form of esters with fatty acids in the following fruits and vegetables: mango, orange, papaya, red or green pepper, yellow corn etc. (KHACHIK *et al.* 1986; BREITHAUPT & BAMEDI 2001; CALVO 2005). The content of lutein in natural sources depends on their kind, variety, level of maturity, part of fruit, and also on the way of processing by heat, preservation, or storage (CALVO 2005).

Marigold flower (*Tagetes erecta* L.) represents a rich source of lutein. It is grown for business purposes in Mexico, Peru, Ecuador, Spain, India or China. Dried Marigold flowers contain 0.1–0.2% dry matter (DM)

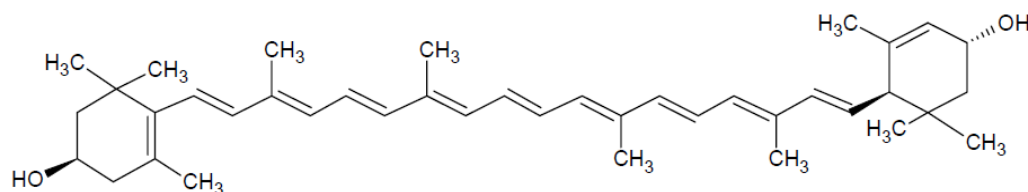


Figure 1. Structural formula of lutein

of carotenoids, out of which 80% are lutein diesters. By the extraction of dried and ground flowers, a non-polar oleoresin extract is acquired. In the process of the technological treatment, conversion of *trans*-lutein into *cis*-lutein is catalysed by heat, light, air oxygen, and acids. Lutein ester is acquired by purification of the non-polar extract; its subsequent saponification leads to the isolation of free lutein. Both lutein ester and free lutein in the form of powder, oil, or as beadlets are used for the manufacture of dietary supplements or for the enrichment of foods and drinks with carotenoids.

Many studies deal with the determination of lutein content in fresh or dried Marigold flowers or in non-polar concentrates (EMENHISER *et al.* 1996; DELGADO-VARGAS & PAREDES-LÓPEZ 1997; DELGADO-VARGAS *et al.* 1998; HADDEN *et al.* 1999; KHACHIK *et al.* 1999; BREITHAAPT *et al.* 2002; KHACHIK 2003; NAVARRETE-BOLANOS *et al.* 2004; LI *et al.* 2007; BHATTACHARYYA *et al.* 2010). Prior to an HPLC analysis of lutein, the implementation of its quantitative extraction is essential. The extraction with organic polar or non-polar solvents and their combinations is the most common procedure and, at the same time, the least demanding instrumental method employed. Depending on the nature of the sample, sole extraction can be done or it might be preceded by sample saponification (CALVO 2005). The solubility and stability of lutein in individual extraction agents differ widely. The combination of various organic solvents can exert either positive or negative effects on the results of HPLC analysis. The effects of organic solvents on the environment and their safe use were also monitored (KHACHIK *et al.* 1988; CRAFT 1992; ISHIHA & CHAPMAN 2009). Supercritical fluid extraction with CO₂ did not show satisfactory results (NARANJO-MODAD *et al.* 2000; FERNÁNDEZ-SEVILLA *et al.* 2010; GUEDES *et al.* 2011; HSU *et al.* 2011). This extraction method, however, is recommended for the separation of lutein stereoisomers (KHACHIK 2003).

To determine lutein content using HPLC, chromatographic columns with C₁₈, or C₃₀ sorbents are commonly applied (ŠTĚRBOVÁ *et al.* 2003); the latter packing is also suitable for the separation of individual *trans*- and *cis*-isomers of lutein. To analyse the substances with identical molecular masses such as lutein and zeaxanthin, cyanopropyl column with the packing containing –(CH₂)₃-CN end group is recommended. None of the articles dealing with determination of lutein and other carotenoids in Marigold flower concentrates reported on the subjection of the concentrates used for the production of food supplements to HPLC analysis.

Our study had the following goals: (i) HPLC determination of lutein contents in Marigold flower concentrates that are commercially used to manufacture dietary supplements or to enrich foods or drinks with carotenoids; (ii) comparison of the lutein stability in encapsulated (beadlet) and powder extract forms.

MATERIAL AND METHODS

Sample selection. Marigold flower concentrates in the forms of granules (samples L1–L16) and powder (samples L17–L34) used for the production of food supplements were analysed at least three months before their expiration time. The concentrates were purchased directly from the producers (from China, India, Israel, and Mexico). These declared the content of lutein to range from 5% to 80%. The respective characteristics are listed in Table 1.

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

HPLC determination of lutein content. The sample of each concentrate (in the form of granules or powder, 5–10 ± 0.01 mg) was dissolved in 50 ml of acetone-methanol solvent (1 : 1 v/v) and was subsequently treated in an ultrasound bath for 10 min followed by spinning at 6000 g for 5 minutes. Two separate solutions of a sample were always prepared.

The separation was carried out using an HPLC 1100 instrument with UV-VIS DAD detector at 30°C (all from 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 × 4.6 mm, 3.5 µm) column with mobile phase flux of 0.7 ml/min (3.153 g/l of ammonium formate in water – A and methanol – B). The signal was recorded upon the injection of the analysed sample (1–5 µl) at 446 nm with the band width of 16 nm. The reference signal was taken at λ = 600 nm with the bandwidth of 100 nm. The HPLC analysis of each sample was implemented in triplicate. The recorded results were processed by ANOVA variance analysis using both Unistat 5.1 statistical software and Office Excel[®] Microsoft program (SNEDECOR & COCHRAN 1967). Figure 2 shows an example of the

Table 1. Lutein contents (mg/g DM) in samples of Marigold flower concentrates (beadlets L1–L16; powder L17–L34)

Sample	Lutein content			Sample	Lutein content		
	declared	determined ($\bar{x} \pm SD$)	(%)		declared	determined ($\bar{x} \pm SD$)	(%)
L1	67.7	73.6 ± 0.55	108.7	L18	54.2	51.0 ± 0.94	94.1
L2	65.0	68.7 ± 0.69	105.7	L19	55.2	30.1 ± 0.48	54.5
L3	56.8	59.4 ± 0.75	104.6	L20	54.3	38.3 ± 0.57	70.5
L4	54.5	55.1 ± 0.52	101.1	L21	50.0	3.0 ± 0.12	6.00
L5	64.0	51.3 ± 0.48	80.2	L22	74.0	63.9 ± 0.56	86.4
L6	50.0	41.6 ± 0.74	83.2	L23	68.9	46.2 ± 0.96	67.1
L7	89.0	2.02 ± 0.07	2.27	L24	217.2	194 ± 2.74	89.3
L8	116.4	127 ± 0.98	109.1	L25	203.0	161 ± 2.52	79.3
L9	104.9	91.7 ± 1.03	87.4	L26	200.5	1.75 ± 0.06	0.87
L10	114	80.5 ± 0.80	70.6	L27	200.0	5.33 ± 0.13	2.66
L11	215.4	224 ± 2.39	104.0	L28	289.6	276 ± 1.93	95.3
L12	235.0	239 ± 2.18	101.7	L29	720.2	2.21 ± 0.11	0.31
L13	204.3	189 ± 2.62	92.5	L30	700.0	6.68 ± 0.18	0.95
L14	215.0	23.8 ± 0.25	11.1	L31	750.0	573 ± 4.57	76.4
L15	272.1	293 ± 2.47	107.7	L32	750.0	531 ± 4.17	70.8
L16	265.0	271 ± 1.36	102.3	L33	802.3	677 ± 6.00	84.4
L17	55.0	58.7 ± 0.64	106.7	L34	800.0	730 ± 6.06	91.3

in bolt - samples with the highest contents of Lutein

sample extract chromatogram. A small portion of zeaxanthin (up to 0.5 % with RT = 10.31 min) was present in all samples tested.

HPLC method validation. The accuracy, precision, and recovery were evaluated ($n = 6-10$) with model solutions and concentrates spiked with lutein standards (concentrations varying from 0.5 µg/g to 3 µg/g). Intraday repeatability was verified by analysing the standard solutions and lutein concentrates during 1 day (spiked with four different concentrations); interday repeatability was verified in a 6-day period with four standard solutions of different concentrations (lutein concentrates were analysed in one sequence during a the period of < 24 h).

The limit of detection (LOD, $S/N = 3$) was 0.22 µg/g and the limit of quantification (LOQ, $S/N = 10$) was 0.7 µg/g. The calibration curve was linear in an appropriate concentration range with the correlation coefficient 0.9982. Repeatability was determined for 0.5, 1, 2, and 3 µg/g standard solutions and for real plant concentrates spiked with the same concentrations of analytes ($n = 6$). For standard solutions, RSD was 1.4% with recoveries of 99.0–101%. For spiked concentrates, RSD was 2.5% with recoveries of 97.9–102%. As for intraday variation, RSD was 2.6% and recovery was 98.8–101% for standard solutions. For spiked lutein concentrates, RSD was 2.8% and recovery was 98.3–101%.

RESULTS AND DISCUSSION

Lutein content in Marigold flower concentrates.

We analysed 16 samples of Marigold flower the concentrates (L1–L16) in the encapsulated form and 18 samples in the powder form (L17–L34). The contents of lutein indicated by the manufacturers' certificates and the determined levels of lutein found in the samples are listed in Table 1.

Table 1 also illustrates the percentage of the real to the declared levels of lutein in the encapsulated form of samples. Nine samples showed even higher amounts of lutein than declared by the manufacturers, which demonstrates the high quality of the raw material and also proves outstanding mastering of the encapsulated form production; encapsulated products usually contain 5, 10, 20, and 25% of lutein in the dry product. The lowest levels of lutein, 2.02 and 23.8 mg/g, were found in L7 and L14 samples, respectively, in spite of the producer having specified lutein contents as 89.0 mg/g in L7 sample and 215 mg/g in the L14 sample.

Only one sample (L17) of the powder extract contained more lutein than indicated by the manufacturer. The lutein levels in the L28 and L18 samples did not differ substantially from the declared values; L28 and L18 samples contained 276 and 51.0 mg/g of lutein, respectively, although the producers speci-

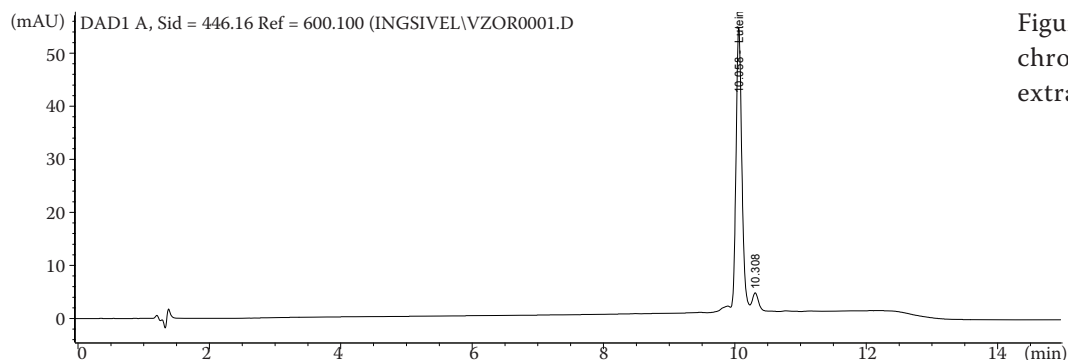


Figure 2. An example chromatogram of an extract sample

fied them to contain 290 mg/g of lutein in the case of L28 sample and 54.2 mg/g of lutein in L18 sample. Fourteen samples (L18–L27 and L31–L34) contained less lutein than specified by the manufacturers. Half of them contained even less than 75% of the declared content. The smallest amounts of lutein were found in L26 and L29 samples; instead of 201 mg/g we detected only 1.75 mg/g of lutein in L26 sample and, supposing to find 720 mg/g, we estimated only 2.21 mg/g in L29 sample.

Quality of Marigold flower concentrates. To compare the quality of Marigold flower concentrates in the encapsulated (L1–L17) and powder (L18–L34) forms, the following categorisation according to the percentage of the detected to the declared lutein content was used: (i) satisfactory (~ 100%); (ii) less satisfactory (75–100%); and (iii) inadequate (< 75%). The results show that beadlets provided substantially higher protection of lutein against oxidation by air oxygen; this finding emerges from the analyses labelling 56% of lutein beadlet samples satisfactory compared to only 6% of the powder concentrates falling into the same category. Accordingly, just 19% of the encapsulated samples were found inadequate in comparison with 50% of the powder concentrates while 25 and 40%, respectively, were found less satisfactory.

To conclude, only 10 out of 34 encapsulated or powder concentrates were evaluated as satisfactory samples. Twelve lutein concentrates were found less satisfactory and twelve samples were labelled inadequate. The concentrates from the manufacturers from India were found to be of the highest quality.

CONCLUSIONS

Based on the results of analyses, we can conclude that the producers of lutein-containing dietary supplements have to select thoroughly the concentrates of lutein for their production. A reliable and trustworthy supplier may rank among the most important

manufacturing factors. The suppliers are supposed to provide high quality lutein and to employ reliable analytical methods for the detection of its content; thereby they guarantee the declared content of lutein in the given Marigold flower extract.

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

Nine out of sixteen encapsulated concentrates were evaluated as satisfactory samples. Only one in eighteen powder concentrates was labelled satisfactory. Lutein contained in the encapsulated extracts proved to be substantially more stable than lutein in the powder extract.

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