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Quantitative analysis of lutein in saponificable extracts from marigold flowers (*Tagetes erecta*) by capillary electrophoresis

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ABSTRACT

In this paper the results of quantitative analysis of lutein in saponified marigold extracts (*Tagetes erecta*) by Capillary Electrophoresis are shown.

KEYWORDS

Lutein;
Capillary electrophoresis;
Tagetes erecta;
Marigold.

INTRODUCTION

Marigold flowers (*Tagetes erecta*) are commercially cultivated in different parts of the world; its dried flowers and their extracts are used as feed supplements for poultry, to color muscles skin and egg yolks. The fact that these extracts have a pigmentation effect is due that the extract of these flowers is rich in lutein 1, a common carotenoid present in almost all green structures of plants. The extracts have found other uses: as antioxidant[1], for visual improvement and eye illness[2].

Different methods have been used to determine the carotenoid composition and lutein concentration in marigold extracts and other plants belonging to Tagetes family, HPLC and/or Gas Chromatography[3-23] are some of the methods widely used. The detection of lutein has been determined in other systems different to marigold flowers, like human plasma[24-26], food plants[27,28], *Nicotiana tabacum*[29], algae[30], paprika[31], other flower petals[32], viviparous kernels and seedlings of *Zea mays L.*[33].

The extract of marigold flowers has a high concentration of triglycerides and in order to eliminate them from the flowers several methods have been reported in the literature to saponification of the extract is used to eliminate triacylglycerides and other compounds[34-48]. The aim of the present paper was to apply a fast, reliable, economic and sensitive method to quantify lutein concentration in marigold extracts obtained applying 3 different extraction methods (saponification).

EXPERIMENTAL

Sample preparation: In order to eliminate high contents of triacylglycerides three different saponification methods reported in the literature were applied:

Method 1: 45 % potassium hydroxide at an 18 % level, 41 % propylenglycol at 65°C during 30 minutes[11].

Method 2: 45 % sodium hydroxide at 70°C, during 35 minutes[43].

Method 3: 15 % potassium hydroxide in ethyl ether during 60 minutes[4].

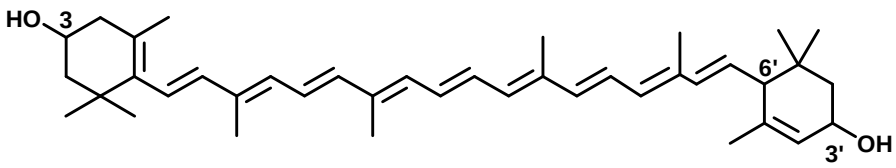
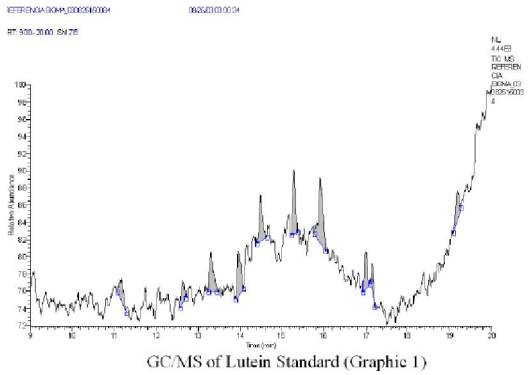
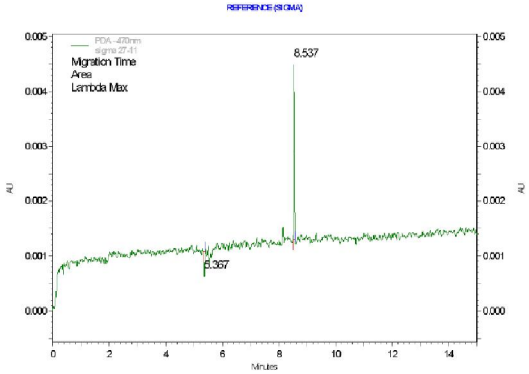


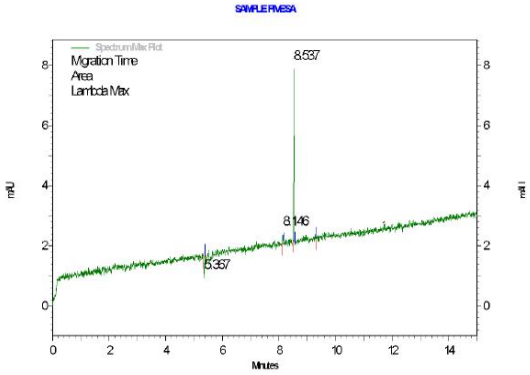
Figure 1 : Structure of lutein(3R, 3'R, 6'R-β,ε-carotene-3,3'-diol)



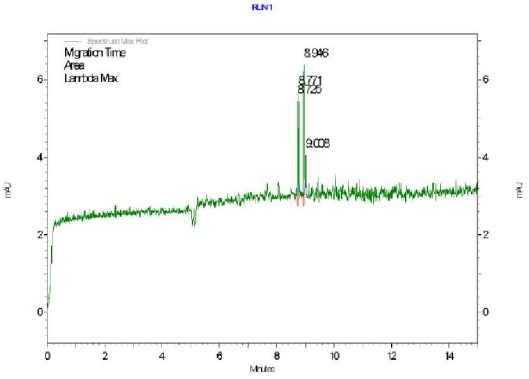
Graphic 1 : GC/MS of lutein standard



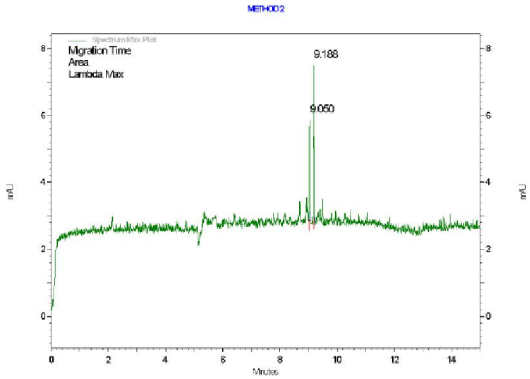
Graphic 2 : Capillary electrophoresis of lutein standard



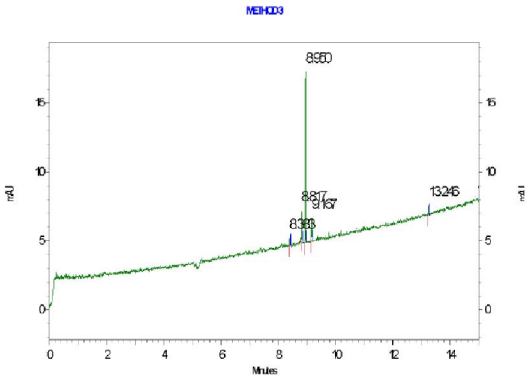
Graphic 3 : Capillary electrophoresis of comercial source



Graphic 4 : Capillary electrophoresis of extract of method 1



Graphic 5 : Capillary electrophoresis of extract of method 2



Graphic 6 : Capillary electrophoresis of extract of method 3

mediately after cleanup.

To avoid cis-trans isomerization and decomposition during the process, all procedures were performed under dimmed light and all samples were analyzed im-

Chemicals

All solvents used in the experiments were distilled

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TABLE 1 : (Commercial sample) Relatives plot results

Retention Time	Area	Area %	Height	Height %
5.367	1285	16.95	693	10.04
8.146	470	6.20	282	4.08
8.537	5696	75.13	5734	83.04
9.317	131	1.73	196	2.84
Totals	7582	100.00	6905	100.00

TABLE 2 : (Method 1) Relatives plot results

Retention Time	Area	Area %	Height	Height %
8.725	3197	30.61	2404	25.62
8.771	2744	26.27	2623	27.95
8.946	3646	34.90	3347	35.67
9.008	859	8.22	1009	10.75
Totals	10446	100.00	9383	100.00

TABLE 3 : (Method 2) Relatives plot results

Retention time	Area	Area %	Height	Height %
9.050	3083	39.35	3000	39.29
9.188	4751	60.65	4636	60.71
Totals	7834	100.00	7636	100.00

TABLE 4 : (Method 3) Relatives plot results

Retention time	Area	Area %	Height	Height %
8.383	1017	5.38	502	2.88
8.817	3369	17.83	2326	13.32
8.950	12270	64.92	12332	70.65
9.167	1727	9.14	1644	9.42
13.246	517	2.74	652	3.74
Totals	18900	100.00	17456	100.00

before use. A HEAT solution (hexane, ethyl alcohol, acetone and toluene,) (10:7:7:6) was prepared, this solution was used to dissolve the samples. A buffer solution of sodium borate 0.02M and sodium cholate 0.05M (used like surfactant) were prepared Standard Lutein crystals were obtained from Sigma Aldrich Chemicals.

Equipment

Capillary Electrophoresis: The separations were carried out using a P/ACE System MDQ (Beckman-Coulter capillary electrophoresis), with a capillary of fused silica with the following dimensions, effective length 50 cm; total length 60 cm (5 cm to detector) X 50 m and added with a PDA detector coupled to a personal computer, controlled by 32 Karat software ver 5.0 from Beckman-Coulter Inc. All experiments were conducted with an applied voltage of +30.0 KV.

Gas chromatography-Mass spectrometry

The experiments were performed in a Trace GC 2000 Gas Chromatography, Thermo Quest Ce Instrument; RTX-5ms column, cross bond 5% diphenyl-95%dimethylpolysiloxane 30 meters, 0.25 mm id, 0,25 microns. Max temp. Programmable: 350° C, Minimal Bleed at 330° C RESTEK xti Technology. And Finnigan Mat GCQ /, GC/MS System, GCQ Plus Thermo Quest Finnigan. Using Xcalibur Home Page software, Version 1.0. Gas Chromatography/Mass Spectrometry analysis were carried out as a comparison standard method used widely to analyze these extracts.

Methodology

Capillary electrophoresis

Solution A: 5.0 mg of each sample were taken from the extract and were dissolved in 4 ml of HEAT solution. After the heating period the solution was made up to volume (5ml), the sample obtained stood under refrigeration and protected from the light during 5 hours.

After the second standing period a drop from Solution A was dissolved in 1.5 ml of the borate/cholate buffer, shaking the mixture until a homogeneous sample was obtained.

The samples were run by Capillary Electrophoresis (CE) and GC/MS following the method and parameters mentioned in the previous paragraphs.

Before running any sample the equipment was rinsed with 0.1 M sodium hydroxide, water and the sodium borate/sodium cholate (0.02M/0.05M) buffer solution. Between analyses the capillary was washed with methanol and then with one volume of separation medium.

RESULTS AND DISCUSION

Lutein bears two different ionone rings, one of them with β-configuration and the other one with the ε-configuration. The double bond of ε-configuration ring is isolated by the method applied. This carotenoid shows a high instability and is very sensitive to light and heat, so when the different extracts obtained applying the different saponification processes were analyzed by gas chromatography mass spectrometry the chromatogram obtained shows more than one peak

for lutein, which is a sign of decomposition (graphic 1) this behavior was shown by all the samples, including the lutein standard, therefore, GC-MS is not a recommended method to quantify the lutein content in marigold extracts, due to their heat instability. The CE analysis of the Sigma Lutein reference showed one peak in m.t. 8.537 min (λ_{max} = 470 nm) with a 83.04 % concentration, as well as other peaks corresponding mainly to lutein esters, cis lutein and other carotenoids like zeaxanthine, cryptoxanthine (Graphic 2). In other hand, CE analysis of commercial source showed, main peak at 8.537 min. that corresponding to lutein (Graphic 3, TABLE 1).

When the 3 extracts obtained applying 3 different saponification procedures were analyzed, the peak corresponding to lutein in different concentrations was found, as well as the other peaks found in the standard corresponding as mentioned before to other lutein forms and other carotenoids. Sample one (Method 1) showed its lutein peak at 8.946 min with 35.67% of the total concentration (Graphic 4, TABLE 2). Sample 2 (Method 2) showed its lutein peak at 9.188 min. with a 60.70 % concentration of lutein of the total extracted carotenoids (Graphic 5, TABLE 3). Sample 3 (Method 3) showed the best result between the different saponification methods, as its lutein peak was obtained at 8.95 min. with a 70.65 % concentration from the total carotenoids (Graphic 6, TABLE 4). The slight differences found in the retention time of the lutein may be due to the saponification procedure applied to the extract.

CONCLUSIONS

Capillary Electrophoresis was a simple, reliable and economic method to identify lutein in saponified extracts of marigold flowers.

From the data obtained it can also be concluded that the saponification procedure chosen is very important in the preparation of marigold extracts, as the renderings obtained vary considerably according to the method.

The high instability of the lutein and other carotenoids present in the saponified extract can be seen when a Gas Chromatography/Mass Spectrometry method is applied.

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