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Contributed Paper

Determination of Major Carotenoid Constituents in Petal Extracts of Eight Selected Flowering Plants in the North of Thailand

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ABSTRACT

The composition and concentration of carotenoids in fresh and dried petal extracts of selected flowers from four families Compositae, Bignoniaceae, Apocynaceae and Cannaceae, were separated and quantified by high performance liquid chromatography with photodiode array detection. The major carotenoids were identified and quantified by comparison with standard carotenoids. There are significant differences in the distribution percentages of the various carotenoid components depending on the color, nature, and particular variety of selected flowers. The highest amount of total carotenoids within this study was found in the family Compositae, especially in *Tagetes erecta*, *Melampodium divaricatum* and *Cosmos bipinnatus*, respectively. Six major carotenoids, b-carotene, b-cryptoxanthin, neoxanthin, lutein, violaxanthin, and zeaxanthin were found in some selected flowers. Lutein was found to be the main carotenoid component. It was presented in the largest amount in fresh and dried petal extracts in family Compositae, especially in the petal extract of *T. erecta* (83% and 88% w/w of total carotenoids in fresh and dried extracts), *M. divaricatum* (76% w/w of total carotenoids in both fresh and dried extracts) and *C. bipinnatus* (77% w/w of total carotenoids in both fresh and dried extracts). *M. divaricatum* and *C. bipinnatus* were found to be as potentially good and new alternative sources of carotenoids for the food industry.

Keywords: carotenoids, flowering plant, pigment.

1. INTRODUCTION

Carotenoids have been widely accepted as safe chemicals for food supplementation and neutraceutical purposes due to their intense coloring abilities, their role as precursors of vitamin A [1], and their antioxidant activity in animals [2]. They are linked to photosynthesis, photoprotection and plastid structure [3]. Carotenoids consist of long chains of alternating double and single carbon-carbon bonds, with cyclic end groups and various keto-, hydroxyl-, and acid- functional groups in different positions [4]. They have been shown to be beneficial in preventing major health problems in developing and developed countries, including cancer, cardiovascular and coronary heart disease [5], and ophthalmological disease [6] including age-related macular degeneration [7, 8].

Data from several laboratory studies and scientific reports illustrate that over six hundred

different carotenoid compounds have been characterized from plants, algae, bacteria and animals [4, 9]. Carotenoids are found in all parts (root, leaf, flower, fruit and seed) but they are usually most noticeable in the flowers. By attracting insects and birds, plant pigments serve an important ecological function by mediating pollination and seed dispersal [10]. Several different classes of pigments are responsible for coloration but in many yellow/orange/red flowers, the pigments synthesized by the plant are carotenoids [3, 11]. They are the major pigment in marigold [12], daylily (*Hemerocallis disticha*) [13], Californian yellow poppy (*Eschscholtzia californica*) [14], and Calendula products [15], *Sandersonia aurantiaca* (Hook) [16]. The predominant carotenoids vary widely among different species.

Flowering plants have interesting potential as sources of pigments for use in food products. This research focuses on the carotenoid composition of eight commonly grown garden flowers that bloom year-round and that have adapted well to the environmental conditions in the North of Thailand and may be of commercial value as sources of carotenoids. The eight selected flowers are divided into four different families: Compositae, Bignoniaceae, Apocynaceae and Cannaceae (Table 1). The colors of the selected flowers vary from yellow to red. In this work the carotenoid pigments were extracted, separated, identified and quantified for their potential use as low-cost and abundant sources in the food industries.

2. MATERIALS AND METHODS

2.1 The Plant Materials and Reagents

The selected flowers (Table 1) were collected from plants growing wild in the North of Thailand. The fresh petals were cut into small pieces and were kept at –20°C. For the freeze-dried petals, those of the selected flowers were freeze-dried to minimize oxidative loss before grinding into fine powder with a mechanical blender. The ground petals were stored at -20°C until analysis. All solvents were HPLC grade and were purchased from Fisher (USA). The standard carotenoids, β-cryptoxanthin (0.626 mg/L), canthaxanthin (0.685 mg/L), fucoxanthin (1.310 mg/L), lutein (1.314 mg/L), neoxanthin (1.137 mg/L), violaxanthin (1.092 mg/L), zeaxanthin (0.751 mg/L) and α-carotene (0.789 mg/L) were purchased from DHI (Denmark). β-Carotene and astaxanthin were purchased from Sigma (UK).

Table 1. Botanical description of the selected flowering plants.

Botanical name	Common name	Appearance	Country of origin
Family Compositae			
Tagetes erecta Linn	Marigold	Deep orange	America
Melampodium divaricatum	Little Yellow Star	Yellow	America
Cosmos bipinnatus	Maxican Aster	Yellow-orange	Mexico
Family Bignoniaceae			
Pyrostegia venusta	Orange Trumpet	Deep orange	Brazil
Tabebuia chrysantha	Golden Tree	Yellow	Mexico
Family Apocynaceae			
Allamanda cathartica	Golden Trumpet	Yellow	South
America *Theretia peruviana*	Yellow Oleander	Yellow	Mexico
Family Cannaceae			
Canna indica Linn.	Indian Shot	Red	America

2.2 Extraction of Carotenoids

The extraction of carotenoids was performed by following Chen and Yang (1992) [17]. The fresh and freeze-dried petals (~1.0 g) were extracted with 30 mL extractant (hexane-acetone-ethanol-toluene, 10:7:6:7, v/v/v/v containing BHT in 100 mL volumetric flask, and then were saponified with 2 mL of 40% methanolic KOH. The mixture was left standing in the dark at room temperature for 16 h. Then, 30 mL hexane was added to the flask and was swirled gently for 1 min and then diluted to volume with 10% Na_2SO_4. The solution was shaken vigorously for 1 min and stored in the dark for 1 h until two phases separated. The upper hexane phase was used for further analysis.

2.3 Separation of Carotenoids into Carotene and Xanthophyll Fractions

A sample of the hexane layer was pipetted onto a column containing an adsorbent (activated magnesium oxide : diatomaceous earth, 1:1). Carotenes and xanthophylls were eluted separately with hexane:acetone (90:10, v/v) and hexane : acetone: methanol (80:10:10, v/v/v), respectively. The absorbance of carotenes and xanthophylls were measured at 436 and 474 nm, respectively, with a U-2000 UV/VIS spectrophotometer. The total carotenoids were expressed as the sum of carotene and xanthophyll concentrations.

2.4 Analysis of Carotenoids in the Fresh and Dried Petal Extracts by HPLC Method

The petal extracts (3 mL) were filtered through a 0.45 μm membrane filter. The carotenoid extracts were analyzed using a Hewllet-Packard 1100 HPLC with diode array detector (DAD). The carotenoid analyses were carried out by using reversed phase C_{30} column (4.6 × 250 mm, ODS 5 μm) operating at 22°C, detected at 450 nm. The eluent for isocratic separation was a mixture of acetonitrile and methanol (65:35, v/v) with a flow rate of 1.0 mL/min. The injection volume was 25 μL. For β-carotene and astaxanthin standards, the solid compounds were dissolved in chloroform. Other standards were obtained from the manufacturer (in 100% ethanol).

Carotenoids in the petal extracts were identified by their retention times in HPLC and by their UV/Visible absorption spectra compared to reference standards. For measurements and spectral determination, the diode array measurements of spectral properties for the individual peaks (from 300 to 600 nm) were determined at the up slope, apex and down slope for checking of peak purity (Figure 1). The concentrations of carotenoids in petal extracts were determined by an external standard method using standard carotenoids (Table 2).

Table 2. The UV-visible maximum absorption and HPLC retention time of individual standard carotenoids.

Standard carotenoids	UV-visible (l_{max})	R_T(min)
Fucoxanthin	427,450,476	11.90
Neoxanthin	416,437,466	21.10
Violaxanthin	420,443,471	22.95
Astaxanthin	474	33.25
Lutein	420,445,475	42.65
Canthaxanthin	467	57.35
Zeaxanthin	424,449,476	60.89
Echinenone	466	100.21
b-cryptoxanthin	422,445,475	104.21
a-carotene	420, 443,472	119.21
b-carotene	426,448,476	174.01

*C_{30} column (4.6 ´ 250 mm, ODS 5 ¼m), acetonitrile and methanol (65:35, v/v), flow rate 1.0 mL/min, 450 nm.

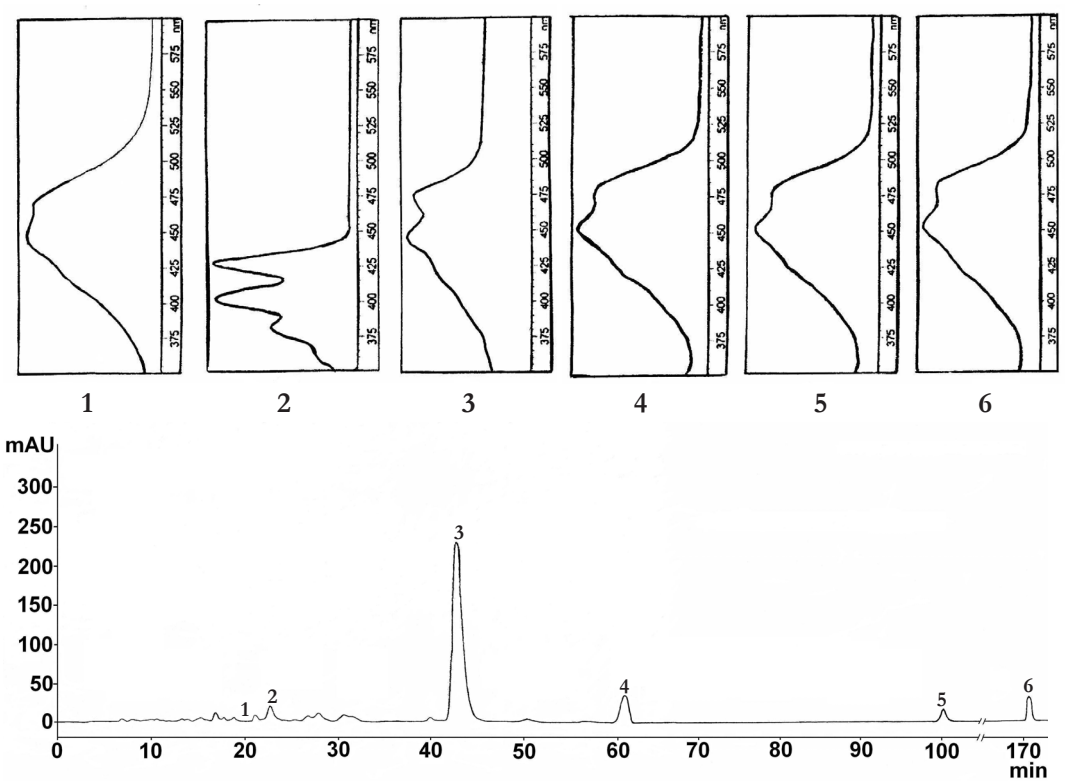

Figure 1. The HPLC profile and DAD spectra of carotenoids in the petal extract of *M. divaricatum*. (1=neoxanthin, 2=violaxanthin, 3 = lutein, 4 = zeaxanthin, 5 = β-cryptoxanthin, 6 = β-carotene.

3. RESULTS AND DISCUSSION

The production of carotenoids in fresh and dried petals of the selected flowers was investigated. This work focuses on eight commonly grown garden flowers that bloom year-round and that have adapted well to the environmental conditions in the North of Thailand. The selected flowers were divided into four different families: Compositae (*Tagetes erecta* Linn, *Melampodium divaricatum* and *Cosmos bipinnatus*), Bignoniaceae (*Pyrostegia venusta* and *Tabebuia chrysantha*), Apocynaceae (*Allamanda cathartica* and *Theretia peruviana*) and Cannaceae (*Canna indica* Linn.) (Table1). The colours vary from yellow to red. The dried petals were prepared by using the freeze-drying method. Freeze-drying is one of the most effective ways of preserving carotenoids of the plants, such as vegetables and fruits [18]. The conversion of the fresh flowers to the dried petal powder by freeze-drying reduced the oxidation of the carotenoids caused by heat, light and oxygen [13].

3.1 Determination of Total Carotenoids in Fresh and Dried Petal Extracts

The extraction and separation of carotenoids into two fractions: carotene (measured at 436 nm, $E^{1\%}_{1\,cm}$ = 2,620 mg/L-cm) and xanthophylls (measured at 474 nm, $E^{1\%}_{1\,cm}$ = 2,550 mg/L-cm) were used to determine total carotenoids [17]. The results were summarized in Table 3. The total contents carotenes, xanthophylls and carotenoids in the dried petal extracts of selected flowers were higher than in fresh petal extracts by 2.1-3.4-folds, on a mass basis. The highest yields were found in the extract of the dried petals of *T. erecta* at 1,954±0.35 mg/kg of total carotenes, 2,443±0.20 mg/kg of total xanthophylls and 4,397±0.25 mg/kg of total carotenoids. The

fresh petal extract of *T. erecta* also contained the highest content of total carotenoids, total carotenes and total xanthophylls. However, in the same family Compositae, *M. divaricatum* and *C. bipinnatus* extracts contained a high content of total carotenoids in fresh and dried petal extracts (1,240±0.30 mg/kg in fresh and 3,840±0.35 mg/kg in dried petals of *M. divaricatum* and 769±0.25 mg/kg in fresh and 2,260±0.50 mg/kg in dried petals of *C. bipinnatus*). The total carotenoid contents in families Bignoniaceae, Apocynaceae and Cannaceae showed lower levels.

Table 3. The total carotenes, xanthophylls and total carotenoids contents* of fresh and dried petal extracts from selected flowers in the North of Thailand.

Botanical name	Fresh flowers			Dried flowers		
	Total carotenes (mg/kg)	Total xanthophylls (mg/kg)	Total carotenoids (mg/kg)	Total carotenes (mg/kg)	Total xanthophylls (mg/kg)	Total carotenoids (mg/kg)
Family Compositae						
Tagetes erecta L.	619±0.50	685±0.30	1,304±0.40	1,954±0.35	2,443±0.20	4,397±0.25
Melampodium divaricatum	593±0.40	647±0.20	1,240±0.30	1,825±0.25	2,015±0.40	3,840±0.35
Cosmos bipinnatus	305±0.25	464±0.25	769±0.25	748±0.60	1,512±0.40	2,260±0.50
Family Bignoniaceae						
Pyrostegia venusta	80±0.35	107±0.30	187±0.30	198±0.50	270±0.70	461±0.60
Tabebuia chrysantha	98±0.25	147±0.40	245±0.35	231±0.70	285±0.60	516±0.65
Family Apocynaceae						
Allamanda cathartica	102±0.20	176±0.45	278±0.35	215±0.25	446±0.30	661±0.30
Theretia peruviana	147±0.40	178±0.40	325±0.40	313±0.55	472±0.45	785±0.50
Family Cannaceae						
Canna indica Linn	121±0.40	189±0.30	310±0.35	426±0.70	628±0.50	1,054±0.60

*Values are mean and standard deviation of triplicate determination

3.2 Determination of Major Carotenoid Contents in Petal Extracts of Selected Flowering Plants

The quantitative determination of the individual carotenoid concentration was carried out by comparing the peak area of the individual components to those of the standard carotenoids (Table 2).

Table 4 summarizes the carotenoid composition of the various flowers studied in this work, grouped by families. Within families there are many similarities in terms of the presence of certain carotenoids, but considerable variation in the amounts of each. The deep orange Compositae, *T. erecta* (marigold) extract was used for comparison of the carotenoid content in other selected flowering plant extracts. A relative standard deviation (RSD) of 1% for most replicates was achieved. The results show that lutein, zeaxanthin and violaxanthin were present in all the petal extracts. Within families there were many similarities in terms of the presence of certain carotenoids, but considerable variation in the amounts of each. For example, in the family Compositae (*T. erecta, M. divaricatum* and *C. bipinnatus*), the fresh and dried petal extracts showed that lutein, β-carotene, β-cryptoxanthin, neoxanthin, zeaxanthin and violaxanthin were found in samples. The fresh and dried petal extracts in family Compositae contained lutein as the main component, while β-carotene is predominating in fresh petals. The highest yields of lutein was found to be 1,062±0.05 mg/kg in fresh petal extract (3,869±0.02 mg/kg in dried petal extract or 88% of total carotenoids) in *T. erecta* extract, while zeaxanthin was found to be 5% of total carotenoids in both fresh and dried petals of *T. erecta* extracts. Lutein content in *M. divaricatum* extract also showed

Table 4. The quantitative distribution of carotenoids in petal extracts of the selected fresh and dried flowers.

The selected flowers	Carotenoids content (mg/ kg)							Total carotenoids (mg/kg)
	b-car.	b-cryp.	Lutein	Neo.	Viola.	Zea.	Uniden.	
Compositae								
Tagetes erecta Linn.	85.5±0.01	31.6±0.02	1,062±0.05	-	43.7±0.05	53.7±0.08	34.0±0.07	1,304±0.02
	(65.0±0.03)	(78.2±0.01)	(3,869±0.02)	(-)	(92.8±0.04)	(166±0.04)	(126±0.06)	(4,397±0.08)
Melampodium divaricatum	116±0.03	48.9.±0.05	854±0.02	52.4±0.03	49.7±0.03	94±0.03	25.0±0.02	1,240±0.03
	(97±0.03)	(83.9±0.05)	(2,900±0.02)	(189±0.03)	(154±0.03)	(330±0.03)	(86.1±0.02)	(3,840±0.03)
Cosmos bipinnatus	84.5±0.04	23.3±0.02	540±0.02	15.5±0.04	45.8±0.03	32.5±0.02	24.8±0.04	701±0.02
	(78.5±0.04)	(69.9±0.02)	(1,730±0.02)	(59.1±0.04)	(154±0.03)	(90.2±0.02)	(78.3±0.04)	(2,260±0.02)
Bignoniaceae								
Pyrostegia venusta	17.5±0.01	10.3±0.03	52.4±0.02	7.80.±0.04	9.10±0.02	8.90±0.03	81±0.01	187±0.02
	(14.5±0.01)	(48.3±0.03)	(152±0.02)	(24.2±0.04)	(22.7±0.02)	(10.0±0.03)	(189±0.01)	(461±0.02)
Tabebuia chrysantha	34.1±0.05	19.5±0.04	64.7±0.03	5.50±0.05	13.6±0.02	12.0±0.01	95.6±0.01	245±0.03
	(21.0±0.05)	(25.3±0.04)	(168±0.03)	(46.3±0.05)	(20.0±0.02)	(44.1±0.01)	(191±0.01)	(516±0.03)
Apocynaceae								
Allamanda eathartica	23.4±0.02	-	41.5±0.02	5.20±0.02	17.5±0.01	16.4±0.01	174±0.02	278±0.05
	(15.4±0.02)	(-)	(128.6±0.02)	(18.7±0.02)	(22.9±0.01)	(45.9±0.01)	(429±0.02)	(661±0.05)
Theretia peruviana	36.8±0.01	14.5±0.02	84.5±0.02	13.0±0.02	21.4±0.05	19.8±0.02	135±0.02	325±0.02
	(34.2±0.01)	(14.6±0.02)	(228±0.02)	(28.3±0.02)	(34.2±0.05)	(24.7±0.02)	(421±0.02)	(785±0.02)
Cannaceae								
Canna indica	15.9±0.10	5.60±0.05	64.5±0.01	-	25.8±0.03	15.2±0.07	183±0.03	310±0.02
	(-)	(-)	(199±0.01)	(-)	(85.1±0.03)	(48.6±0.07)	(721±0.03)	(1,054±0.02)

high amount (2,900±0.02 mg/kg in dried petals extract) and made up 76% of total carotenoids. The *C. bipinnatus* extract indicated that lutein was found to be the main carotenoid component (77% of total carotenoids in dried petal extract). Violaxanthin and neoxanthin in dried petal extracts were also present in significant amounts. The unidentified pigments were present in small amounts.

Within the Bignoniaceae, *P. venusta* and *Ta. chrysantha* were selected for separation and identification. The fresh petal extracts in all families contained lutein as the main component, followed by β-carotene. In the family Bignoniaceae, lutein in dried petal extracts was about 2.6-2.9-folds higher than in fresh petal extracts. In Apocynaceae (*A. eathartica* and *Th. peruviana*), the main carotenoid was lutein (41.5±0.02 mg/kg in fresh and 128.6±0.02 mg/kg in dried petal extracts of *A. eathartica* and 84.5±0.02 mg/kg in fresh and 228±0.02 mg/kg in dried petal extracts of *Th. peruviana*). *C. indica* extracts contained lutein (21% and 19% of total carotenoids in fresh and dried petal extracts, respectively), while violaxanthin was obtained (8% of total carotenoids) in both fresh and dried petals extracts. β-Carotene and β-cryptoxanthin were present in 5% and 2% of total carotenoids in fresh petal extract, respectively, but they were not found in dried *C. indica* extract.

The total amounts of carotenoids in dried flowers varied from 461±0.02 mg/kg dry weight in the yellow Bignoniaceae, *P. venusta,* to as much as 4,397±0.08 mg/ kg dry weight in the yellow Compositae, *T. erecta*. The total carotenoid contents in fresh petal extracts were in the range of 187±0.03 mg/kg wet weight in the yellow *P. venusta* to 1,304± mg/kg wet weight in the yellow Compositae, *T. erecta*. The total carotenoid content (mg/kg) of identified peaks in family Compositae is higher than in families, Bignoniaceae, Apocynaceae and Cannaceae.

The difference in the petal colour among the varieties examined (yellow, orange or red) is associated with the variation in the carotenoid levels. In general, the lemons and yellow flowers contain a large amount of xanthophylls, while the deeper orange petals have increasing amounts of β-carotene and lycopene [19]. The compounds of interest for commercial use as additives to poultry feed to improve pigmentation were lutein and zeaxanthin from the extracts of marigold flowers (*T. erecta*) [12, 20, 21]. Other researchers studying the stems, leaves, petals and pollens of *C. officinalis* demonstrated that the flavoxanthin and auroxanthin were the main carotenoids in the petals and pollens, as determined by HPLC analysis [15]. The pigments responsible for the golden orange flower color of *S. aurantiaca* (Hook) have been characterized and the major carotenoids were zeaxanthin and β-cryptoxanthin [16]. The petals of the Compositae, *M. divaricatum* and *C. bipinnatus*, contain lutein as the major component and may be a potential good source of carotenoids for the food industry, complementing that derived from the marigold flower, *T. erecta*.

4. Conclusion

This study demonstrates the presence of high levels of a number of important carotenoids in petals of easily grown flowering plants in the north of Thailand. The highest amount of total carotenoids within this study was found in the family Compositae, especially in *T. erecta*, *M. divaricatum* and *C. bipinnatus*, respectively. The petals of the Compositae, *M. divaricatum* and *C. bipinnatus*, contain lutein as the major component and may be potentially good and new alternative sources of carotenoids for the food industry.

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REFERENCES

[1] Römer, S., Fraser, P.D., Kiano, J.W., Shipton, C.A., Misawa, N., Schuch, W., Bramley, P.M., Evaluation of the provitamin A content of transgenic tomato plants, *Nat. Biotechnol.*, 2000; 18: 666-669.

[2] Stahl, W., Sies, H., Antioxidants activity of carotenoids, *Mol. Asp. Med.*, 2003; 24: 345-351.

[3] Bartley, G.E., Scolnik, P.A., Plant carotenoids: pigments for photoprotection, visual attraction and human health, *Plant Cell.*, 1995; 7: 1027-1038.

[4] Goodwin, T.W., Nature and properties, in Goodwin, T.W., ed., *The biochemistry of the carotenoids*, Chapman and Hall, London., 1980; 1: 1-32.

[5] Kritchevsky, S.B., b-carotene, carotenoids and the prevention of coronary heart disease, *J. Nutrition.*, 1999; 129: 5-8.

[6] Mayne, S.T., b-carotene, carotenoids and disease prevention in humans, *FASEB J.*, 1996; 10: 690-701.

[7] Landrum, J.T., Bone, R.A., Kiburn, M.D., The macular pigment: a possible role in protection from age-related macular degeneration, *Adv. Pharm.*, 1997; 38: 537-556.

[8] Hennekens, C.H., Current knowledge and future directions of research on antioxidant vitamins in prevention of cancer, cardiovascular and eye diseases, *Pure Appl. Chem.*, 1997; 69: 2141-2144.

[9] Olson, J.A., Krinsky, N.I., Introduction: the colorful fascinating world of the carotenoids: important physiologic modulators, *FASEB J.*, 1995; 9: 1547-1550.

[10] Camara, B., Hugueney, P., Bouvier, F., Kuntz, M., Monger, R., Biochemistry and molecular biology of chromoplast development, *Int. Rev. Cytol.*, 1995; 163: 175-247.

[11] Schoefs, B., Chlorophyll and carotenoid analysis in food products: properties of the pigments and methods of analysis, *Trends Food Sci. Tech.*, 2002; 13: 361-371.

[12] Hadden, W.L., Watkins, R.H., Levy, L.W., Regalado, E., Rivadeneira, D.M., van Breemen, R.B., Schwartz, S.J., Carotenoid composition of marigold (*Tagetes erecta*) flower extract used as nutritional supplement, *J. Agric. Food Chem.*, 1999; 47: 4189-4194.

[13] Tai, C,-Y., Chen, B.H., Analysis and stability of carotenoids in the flowers of daylily (*Hemerocallis disticha*) as affected by various treatments, *J. Agric. Food Chem.*, 2000; 48: 5962-5968.

[14] Maoka, T., Fujiwara, Y., Hashimoto, K., Takeda, S., Takaragaki, S., Ida, K., A new retro-carotenoid from the petals of the californian yellow poppy *Eschscholtzia californica., J. Nat. Prod.*, 2000; 63: 1288-1289.

[15] Bakó, E., Deli, J., Tóth, G., HPLC study on the carotenoid compositions of *Calendula* products, *J. Biochem. Biophys. Meth.*, 2002; 53: 241-250.

[16] Nielsen, K.M., Lewis, D.H., Morgan, E.R., Characterization of carotenoid pigments and their biosynthesis in two yellow flowered lines of *Sandersonia aurantiaca* (Hook), *Euphytica.*, 2003; 30: 25-34.

[17] Chen, B.H., and Yang, S.H., An improved analytical method for the determination of carotenoids and xanthophylls in dried plant materials and mixed feeds, *Food Chem.*, 1992; 44: 61-66.

[18] Cinar, I., Carotenoid pigment loss of freeze-dried plant samples under different storage conditions, *Lebensen-Wiss. u,-Technol.*, 2004; 37: 363-367.

[19] Goodwin, T.W., Britton, G., Distribution and analysis of carotenoids, in Goodwin, T.W., ed., *Plant pigments*, Academic Press, London., 1988; 62-132.

[20] Khachik, F., Process for extraction and purification of lutein, zeaxanthin and rare carotenoids from marigold flowers and plants, *U.S. Patent.*, 2001; 6: 262-284.

[21] Navarrete-Bolaños, J.L., Jiménez-Islas, H., Botello-Alvarez, E., Rico-Martínez, R., Paredes-López, O., Improving xanthophylls extraction from marigold flower using cellulolytic enzymes, *J. Agric. Food Chem.*, 2004; 52: 3394-3398