Egypt. J. Agric. Res., 72 (3), 1994.

EFFECT OF STORAGE ON THE CAROTENOIDS OF *TAGETES ERECTA* L. FLOWERS

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(Manuscript received 28 December 1992)

Abstract

This investigation was conducted during two seasons of 1990 and 1991 to study the chemical changes of *Tagetes erecta* L. flower carotenoids (pigments) as affected by storage condition (temperature and period). The flowers were obtained from plants produced from the Farm of Seds Experimenal Station, Beni-Swaif Governorate, Agricultural Research Centre, Egypt.. The extract of the fresh flower petals was stored under room temperatures (25°C) refrigerator temperature (5°C) at 6 month intervals, i.e. 0, 6, 12, 18 and 24 months respectively. The obtained results cleared that T.L.C. analysis revealed the presence of 6 components which were identified as lutein (the main constituent), antheraxanthin, B. crepto-xanthin, B. carotene, carotene and phytofluence. Lutein and creptoxanthin percentages were increased while B-carotene decreased by prolonging the storage period under both room and refrigerator temperatures. The variation in such compounds was pronounced after 18 months of storage. Generally, slight changes in carotenoid percentages could be observed after 6 and 12 months and were remarkable after 18 and 24 months of storage in comparison with control (0 time). From the result, storage at refrigerator for a period not exceeding 12 months could be recommended to keep marigold carotenoids in a good quality.

INTRODUCTION

The African marigold (*Tagetes erecta* L.) plant belongs to the family Compositae (Mohi 1980). The plant is a summer annual and has different flower sizes and types with a wide spectrum in colour between lemon yellow to deep organe. Bailey (1949), Kuhn (1931), Kantor (1977) and Radwan (1988) reported that marigold flowers contain xanthophyll as major component (3, 6-9 gram/pound) with small amounts of carotenes. They added that the carotenoid compounds were identified as lutein, antheraxanthin, B-creptoxanthin, B-carotene, carotene and phytofluene. They also added that carotenoid pigments of marigold are used commercially for colouring hen eggs and skin broiler chicken. Candela *et al.* (1984) concluded that, B-creptoxanthin increased when *Capsicum annuum* fruits were stored in the dark at 15°C for 2 weeks. Lee (1986), demonstrated that during storage of carrot at 2°C and 90% (R.H.), the content of B-carotene increased slowly for 100 days and then decreased. Golias (1972), found that stored lettuce cv. Lednicky at different temperatures decreased the content of B-carotene. Doncheve (1976), stated that, storage apple fruits at 0°C slightly increased lutein content of the fruit skin. The present work was performed to study the effect of storage period and temperature on the carotenoid components of *Tagetes erecta* L. flowers. Also the study aimed to find out the optimum conditions of storage for carotenoids.

MATERIALS AND METHODS

The full opened matured flowers of *Tagetes erecta* L. cultivars Hawai were obtained from plants grown at the Farm of Seds Experimenal Station, Beni-Swaif Governorate during 1990 and 1991 seasons. The chemical analysis was done in the laboratory of Aromatic and Medicinal Plant Research Section, Agricultural Research Centre. The flower petals were picked to extract the carotenoids using pure hexane. The extraction and purification were done according to the procedure described by Key and Berry (1970). The carotenoids extract which was kept in cleaned and dried brown glass bottles which were completely filled by the extract and stoppered carefully. The bottles were stored for 24 months at two storage temperatures, room

(25°C) and refrigerator (5°C). The samples were taken every 6 months to study the effect of storage period and temperature on the percentage of each of the analysis components of marigold carotenoids. Spectroscopic was carried out colorimetrically according to the method mentioned by Hamed (1985). Chromatographic analysis (Thin layer) was done according to Davies (1976). Three systems were used to cover all the components. Also, the determination of each fraction of carotenoids was calculated using the equation mentioned by Davies (1976).

RESULTS AND DISCUSSION

1. Identification of carotenoids:

Natural carotenoids of fresh marigold flowers were identified using spectrophotometric analysis and thin layer chromatography.

1.A. Spectrophotometric analysis:

The maximum absorption spectra of the extract obtained from fresh flowers of marigold was done to identify the different fractions of the pigments. The obtained results are shown in Fig. 1. It could be observed that, the fresh extract of pigments (control) had six maximum absorption regions. The first region was between 346 and 348 nm., the second one between 418 and 420 nm., the third between 424 and 426 nm., the fourth between 440 and 442 nm., the fifth at 470 nm. and the sixth between 474-476 nm. These six regions indicate the presence of phytofluene, luteine, B-carotene, -carotene, antheraxanthin and B-creptoxanthin respectively. Davies (1976), reported that the maximum absorptions of phytofluene in hexane are 331, 347 and/or 366 nm., luteine are 420, 445 and/or 475 nm. B-carotene are 425, 450 and/or 477 nm., -carotene are 420, 442 and/or 472 nm., antheraxanthin are 421, 445 and/or 470 nm. and B-creptoxanthin are 412, 446 and/or 475 nm. These results were true in the two seasons of 1990 and 1991.

1.B. Thin layer chromatograhy:

The Rf values of the separated fractions were calculated, and their colours were recorded and compared with standard Rf value and colour of each fraction. The

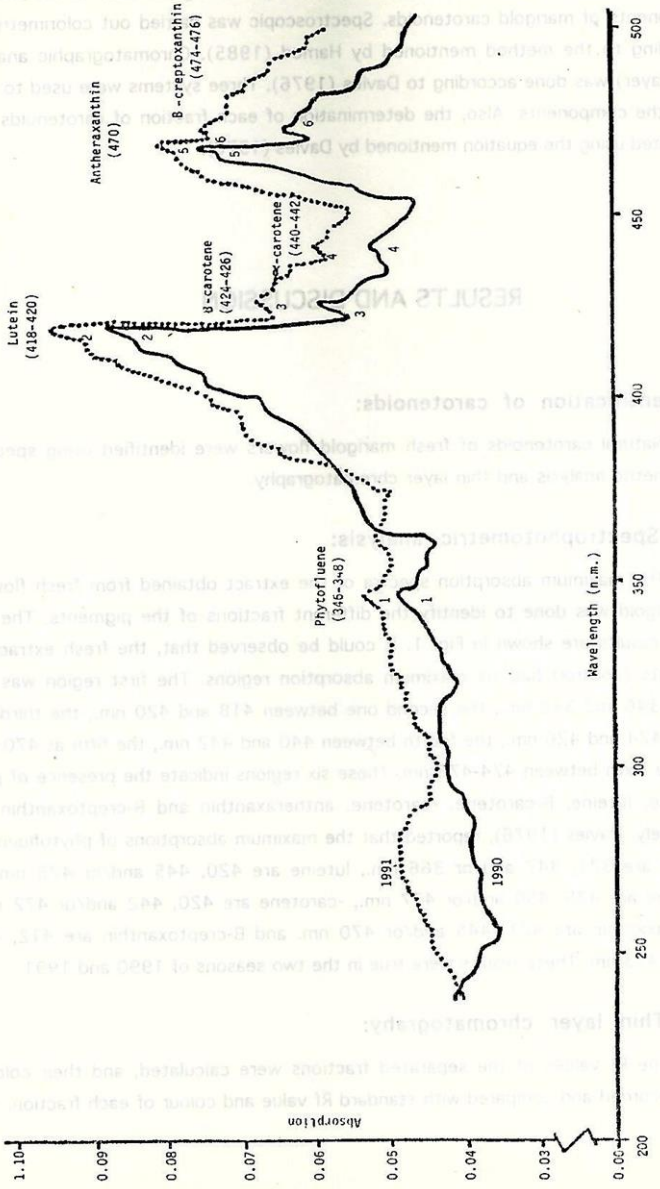

Fig. 1. Absorbances of pure hexane extract (extracted from the petals of marigold flowers) in 1990 and 1991 seasons.

btained results of the fresh extract of carotenoids are shown in Table1. The three systems used fractionated the pigments extracts into six fractions. The fractions were identified as phytofluence, B-carotene, Z-carotene, antheraxanthin, lutein and B-creptoxanthin with colours of green, o f green, light orange, light orange, yellow, light orange and having Rf values of 0.08, 0.82, 0.90, 0.38, 0.56 and 0.72 respectively. The same fractions were identified in 1991 seasons. These results confirmed the presence of the six previous components. Alam *et al.* (1968) and Radwan (1988), on *Tagetes erecta* L. identified the same components. These components were identified compared in different storage treatments used.

2. Quantitative determination of carotenoid fractions:

The percentage of each component (fraction) for the different storage treatments are listed in Table 2. It is clear that a predominance of xanthophylls (B-creptoxanthin, antheraxanthin and lutein) and only a small amount of carotenes (Phytofluence, -carotene and B-carotene) in the carotenoid extract of different treatments. The same findings were obtained by Kuhn *et al.* (1931), Alam *et al.* (1968), who found that a predominance of xanthophylls and small amount of carotenes in *Tagetes erecta* L. flowers Kantor (1977) demonstrated that the petals of marigold contained xanthophylls. Also, Radwan (1988) on *Tagetes erecta* L. confirmed these results. The results indicated that in most cases, lutein (the main component) and B-creptoxanthin percentages were by prolonging the storage period to 18 months, then still nearly constant up to the end of the storage period. On the other hand α-carotene, phytofluence and antheraxanthin compounds did not show remarkable chemical changes so that their values were nearly constant during the storage period.

Data also showed that, the storage temperature had little effect on the carotenoid components than the storage period. Moreover, the storage in refrigerator decreased the effect of storage period. In addition, during the first three periods of storage, (0, 6 and 12 months), the variations in the percentages of carotenoid components were little and then increased. The same trend of results was obtained when the experiment was repeated in the second season. These findings are in accordance with those obtained by Candela *et al.* (1984), on *Capsicum annuum* and Lee (1986), with carrot plant. Golias (1972), reported that stored lettuce at different temperatures decreased B-carotene. Doncheve (1976), stated that lutein content increased when apple fruits were stored at $0^{o}C$. The decrease in B-carotene was nearly equal with the increase of B-creptoxanthin and lutein percentages, it may be due to the ef-

Table 1. Identifaction of carotenoids extracted with hexane from the fresh petals of marigold flowers by thin layer chromatography in 1990 and 1991 seaons

T.L.C system	Standard fractions			Indentified fraction in 1990 season (Control)			Indentified fraction in 1991 season (Control)		
	Name of fraction	R_f value	Colour	Name of fraction	R_f value	Colour	Name of fraction	R_f value	Colour
1	Phytofluene	0.10	Green	Phytofluene	0.08	Green	Phytofluene	0.09	Green
2	B-Carotene	0.84	Light orange	B-Carotene	0.82	Light orange	B-Carotene	0.82	Light orange
	- carotene	0.88	Light orange	- carotene	0.90	Light orange	- carotene	0.89	Light orange
	Antheraxanthin	0.40	Yello	Antheraxanthin	0.38	Yello	Antheraxanthin	0.40	Yello
3	Lutein	0.57	Light orange	Lutein	0.56	Light orange	Lutein	0.56	Light orange
	B-creptoxanthim	0.70	Light organe	B-creptoxanthim	0.72	Light organe	B-creptoxanthim	0.71	Light organe

System 1 - Adsorbant : Deactivated silica gel G- Solvent system : Petroleum ether
System 2 - Adsorbant : Ca (OH)2 silica gel G (6/1) - Solvent system : Pertoleum ether / benzene (98/2)
System 3 - Adsorbant : Activated silica gel G-Solven system : Benzene / ethyl acetate/ methanol (75/20/5).

Table 2. Effect of storage period and temperature on the components % of carotenoids extracted from the petals of marigold flowers in the two seasons of 1990 and 1991.

Caroteniod compounds	1990 season					1991 season				
	Storage period (months)					Storage period (months)				
	0 (Control)	6	12	18	24	0 (Control)	6	12	18	24
Phytofluene	3.29	3.24	3.26	3.19	3.19	2.96	2.93	2.85	2.80	2.27
-carotene	5.97	5.77	5.72	5.69	5.10	5.45	5.60	5.51	5.22	5.18
B- carotenes	8.95	7.47	5.54	4.05	4.07	7.91	7.55	6.18	3.61	3.43
Total carotenes	18.03	16.48	14.52	12.93	12.36	16.05	16.08	14.54	11.63	10.33
B- creptoxanthin	12.33	13.41	14.47	16.57	16.43	13.68	13.66	14.58	16.78	17.08
Anrheraxanthin	22.81	22.92	22.64	22.58	22.14	22.54	22.43	22.34	22.30	22.19
Lutein	46.19	46.69	47.38	47.37	48.73	47.12	47.37	48.12	48.75	49.50
Total Xanthophylls	81.33	83.02	84.49	86.52	87.30	83.34	83.46	85.04	87.83	88.77
Phytofluene	3.29	3.24	3.32	3.29	3.21	2.96	2.96	3.00	2.88	2.41
-carotene	5.97	6.01	5.81	5.75	5.75	5.45	5.39	5.30	5.35	5.26
B- carotene	8.95	8.53	7.30	6.65	6.34	7.91	7.84	6.90	5.30	5.43
Total carotenes	18.21	17.78	16.43	15.69	15.30	16.05	16.19	15.20	13.53	13.10
B- creptoxanthin	12.33	12.63	13.35	14.03	14.53	13.68	13.63	13.69	14.59	14.94
Antheraxanthin	22.81	22.75	22.66	22.60	22.33	22.54	22.51	22.64	22.41	22.55
Lutein	46.19	46.34	46.79	47.09	47.29	47.12	47.14	47.82	48.87	48.96
Total Xanthophylls	81.33	81.72	82.80	83.72	84.15	83.28	83.28	84.15	84.10	86.45

fect of storage on enhancement the oxidation process of B-carotene to B-creptoxanthin and lutein (carotenes to xanthophylls). In general, an increase of the total oxygenated carotenoids (xanthophylls) was observed and such increase was associated by a reduction of the non oxygenated carotenoids (carotenes) (Table 2). In this regard, it may be concluded that the adversed relationship between total carotenes and total xanthophylls could be attributed to the auto oxidation which took place during the storage period. The obtained results are supported by the findings reported by Goodwin and Goad (1970), who mentioned that xanthophylls are formed with simultaneous disappearance of carotenes.

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تأثير التخزين على كاروتنويدات أزهار نبات القطيفة

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أجرى هذا البحث خلال موسمى ١٩٩٠ ، ١٩٩١ لدراسة التغيرات الكيماوية لكاروتنويدات أزهار نبات القطيفة نتيجة لظروف التخزين المختلفة (درجات حرارة وفترات تخزين). وقد تم الحصول على الأزهار من النباتات المزروعة بمزرعة محطة بحوث سدس بمحافظة بنى سويف التابعة لمركز البحوث الزراعية. وقد تم تخزين مستخلص كاروتنويدات الأزهار فى كل من درجة حرارة الغرفة (٢٥°م) ودرجة حرارة الثلاجة (٥°م) وأخذت منها عينات كل ٦ شهور (صفر ، ٦ ، ١٢ ، ١٨ ، ٢٤ شهر).

وتشير النتائج المتحصل عليها الى:

١ – أظهر التحليل الكرماتوجرافى بإستخدام الطبقة الرقيقة وجود كل من مركبات الليوتين – الانثرازانثين – بيتا كربتوزانثين – بيتا كاروتين – الفاكاروتين – الفيتوفلاوين.

٢ – كان تأثير درجات الحرارة على مكونات الكاروتنويدات اقل من تأثير فترات التخزين.

٣ – زاد كل من مركبى الليوتين والبيتاكربتوزانثين بينما قل مركب بيتا كاروتين بزيادة فترات التخزين فى كل من درجة حرارة الغرفة والثلاجة. وكانت هذه الفروق أكثر وضوحا بعد مضى ١٨ شهرا من التخزين.

٤ – لم تتأثر مركبات الانثرازانثين والفيتوفلاوين بمعاملات التخزين المختلفة.

٥ – كانت الإختلافات فى النسب المئوية لمكونات الكاروتنويدات ضئيلة خلال الثلاث فترات الاولى من التخزين (صفر ، ٦ ، ١٢ شهر) بينما اصبحت اكثر وضوحا عند زيادة فترات التخزين الى ١٨ ، ٢٤ شهر.

٦ – دل تحليل كاروتنويدات القطيفة على وجود الزنثوفيلات بكميات كبيرة والكاروتينات بكميات قليلة.

ومن النتائج المتحصل عليها يمكن التوصية بتخزين كاروتنويدات ازهار نبات القطيفة فى الثلاجة لمدة لا تزيد عن ١٢ شهر وذلك للحفاظ على جودتها.