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HORTICULTURE

Analysis of anthocyanins and carotenoids in two varieties of *calendula officinalis* L. flowers

Gazala Qazi^{1*}, Shoukat Ara¹, Imtiyaz Murtaza², Syed Maqbool Geelani¹ and Hina Qazi³

¹ Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, J&K-190 025, India

²Biochemistry and Molecular Biotechnology Laboratory, Biochemistry Section, Division of Post Harvest Technology, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, J&K-190 025, India

³ Division of Textile Science and Apparel Designing, SNDT Women's University, Santacruz, Mumbai-400049, India

*Corresponding author: qazigazala@gmail.com

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Abstract

Pot Marigold (*Calendula officinalis* L.) is an annual erect herb with hispidly pubescent, corymbosely branched stem cultivated in lawns. The present investigation was carried out to study the dye yielding potential of the two varieties of Pot Marigold (*Calendula officinalis* L. var. Gitana Orange and Gitana Yellow) of Kashmir valley. The stability and color quality of pigments (anthocyanins and carotenoids) were assessed by quantifying the pigments and recording their color quality at 10 days interval at 0, 10, 30, 40, 50, 60, 70, 80 and 90 days after collection under ambient and refrigerated storage conditions. Results revealed that Pot marigold (var. Gitana Orange) had higher carotenoid content (288.33 mg/100g) than the Pot marigold (var. Gitana Yellow) (205.50 mg/100g). Highest pigment content was recorded during 0 days of storage in both the varieties. With increase in days of storage pigment content decreased in both plant material as well as extract under both storage conditions. Pigments in the plant material and extract stored under refrigerated conditions lasted longer than those stored under ambient conditions. Pigment degradation was more in the plant material as compared to the plant extract. Color quality of the plant species changed with increase in days of storage. From the present study it was concluded that both varieties of Pot Marigold have good levels of total carotenoids, thus petals of both varieties can be used as a raw material for extraction of yellow to orange natural dyes.

Highlights

- The stability and color quality of pigments (anthocyanins and carotenoids) were assessed by quantifying the pigments and recording their color quality at 10 days interval at 0, 10, 30, 40, 50, 60, 70, 80 and 90 days
- Pot Marigold (var. Gitana Orange) had higher carotenoid content than the Pot Marigold (var. Gitana Yellow).
- With increase in days of storage pigment content decreased in both plant material as well as extract under both storage conditions.
- Pigment degradation was more in the plant material as compared to the plant extract.

Keywords: Calendula officinalis L., anthocyanin, carotenoids, hunter lab

Pot Marigold (*Calendula officinalis* L.) is an annual erect herb with hispidly pubescent, corymbosely branched stem cultivated in lawns. Its flowers are in terminal peduncled heads and are bright

yellow to orange in color. The wide range of petal colors originates mainly from the combination of carotenoids and anthocyanins. Carotenoids are one of the most important groups of natural pigments



(Ali, 2011). Carotenoids are lipid-soluble, yelloworange-red pigments found in all higher plants and some animals. Plant, algal and fungal carotenoids are allowed as colorants. Anthocyanins may be found in red, blue or purple flowers, fruits or vegetables and are very wide in nature. They are considered very essential as food colorants and classified as E 163 according to the colorant code. Colorants have become the most sensitive part of any commodity as they enhance consumer acceptability (Clydesdale, 1993). The color of a food substance indicates freshness and safety that are indices of good aesthetic and sensorial values (Pritam et al. 2008). In the ancient times wide varieties of food colorants were derived from natural sources-plant, animal or mineral. The British chemist Sir William Henry Perkin created the first synthetic dye, mauveine, in 1856 by oxidizing aniline. By the end of the century, eighty synthetic dyes were produced. With the discovery of synthetic dyes the use of natural food dyes declined in the middle of nineteenth century. Industrialization of the food system, including a rise in food processing, has increased the use of food additives including food dyes as synthetic food dyes are less expensive and have intense hue than natural edible dyes but have general toxicity (Carvalho, 1992). Synthetic food dyes and other additives may contribute to hyperactivity and other disturbed behavior in children (Feingold, 1975; Pollock and Warner, 1990; Weiss, 1980). Interest in anthocyaninrich foods and extracts has intensified because of their possible health benefits. Carotenoids are linked with enhancement of the immune system and decreased risk of degenerative diseases such as cancer, cardiovascular disease, age-related muscular degeneration, and cataract formation (Gerster, 1991). Anthocyanins are potent antioxidants and may be chemoprotective. The increased stability of these pigments together with their added value due to potential beneficial effects opens a new window of opportunities for use of these extracts in a variety of food applications. Anthocyanins represent an option for use, with the potential to substitute artificial red dyes, since they present brilliant, attractive colors, besides possessing several recognized functional properties (Ribeiro et al. 2005). In view of harmful impact of synthetic dyes on human health and environment and beneficial effect of natural food colorants, the present investigation was carried out to study the natural dye yielding potential of two varieties of Pot Marigold (*Calendula officinalis* L. var. Gitana Orange and Gitana Yellow) in Kashmir valley.

Materials and Methods

The experimental material, comprising of the petals of Pot Marigold (*Calendula officinalis* L. var. Gitana Orange and Gitana Yellow) were collected from Department of Floriculture, Plant Introduction Section, Cheshmashahi Srinagar, Kashmir.

Total anthocyanin content (mg/100g)

Anthocyanins were calculated as per the method of Rangana (1986).

Calculations

The total anthocyanin content was calculated using following equations :

Total optical density =
$$\frac{\text{Optical density } \times \text{Volume}}{\text{made}} \times 100$$

Weight of sample
Total anthocyanin content (mg/100 g) = $\frac{\text{Total optical}}{\frac{\text{density}}{98.20}} \times 100$

Total carotenoid content (mg/100g)

Total carotenoids were estimated as per Arya (1981). To remove the interference of the chlorophyll during carotenoid estimation, the filtrate obtained at the end of the above procedure was evaporated to dryness and the residue was dissolved in the minimum quantity (10 ml) of ethanol. Afterwards, 60% of KOH (1ml for 10ml) was added to it. The whole mixture was boiled for about 5-10 minutes. The equal amount of distilled water was added to it and the solution obtained was partitioned twice with petroleum ether. The combined ether layers were evaporated and the residue obtained was dissolved in 25 ml hexane. A pinch of anhydrous sodium sulphate was added to it and resultant mixture was filtered through Whatman No.1 filter paper and the optical density (O.D) at 449 nm was recorded using hexane as blank.

Calculations

The total carotenoid content was calculated using following equation:

Total carotenoid content (mg/100 g) =
$$\frac{\text{Optical density} \times \text{Volume made}}{250 \times \text{Weight of}} \times 100$$

sample

Evaluation of CIE L*a*b* values

CIE L*a*b* values of the dyed and undyed fabrics was determined by chromometer (Model CR-2000, Minolta, Osaka, Japan) equipped with 8 mm measuring head and AC illumination (6774 K) based on CIE system (International Commission on Illumination). The meter was calibrated using the manufacturer's standard white plate. L*, a* and b* coordinates, Chroma (C*) and hue angle (h°) values were calculated by the following equations:

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Chroma = (a^{*2} + b^{*2})^{1/2}
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Hue $(h^{\circ}) = \tan^{-1} (b^*/a^*)$

Total color change was calculated from the L*, a* and b*coordinates by applying the following equation:

Total Color change (ΔE) = [(ΔL^*)² + (Δa^*)² + (Δb^*)²]^{1/2}

Where,

 $\Delta L^* = L^*$ sample - L* standard

 $\Delta a^* = a^*$ sample - a^* standard

 $\Delta b^* = b^*$ sample - b*standard

Results and Discussion

Anthocyanin content (mg/100g)

Anthocyanin content in Pot Marigold (Calendula officinalis L. var. Gitana Orange) ranged between 0.34 to 16.29 mg/100g (Table 1; Fig. 1a & b). Every storage interval was significantly different from each other except the values recorded under refrigerated conditions for plant material on 50th to 60th day of storage and for plant extract on 10th and 20th day of storage. Highest pigment content (16.29 mg/100g) was recorded on day 0 of storage. These results are contradictory to those reported by Kishimoto et al. (2007) who could not detect total anthocyanin content in the four orange flowered cultivars of C. officinalis L. (Alice Orange, Orange Star, Orange Gem, Ponpon Orange). The variety of Pot Marigold used in the present study showed good amount of total anthocyanin content and thus it can be assumed that

orange petal color might be due to red anthocyanins and yellow carotenoids. Total anthocyanin content in the Pot Marigold (Calendula officinalis var. Gitana yellow) ranged between 1.69 to 10.01 which is lower than the value 159.3 μ g/g or 15.93 mg/100 g reported by Kishimoto et al. (2007) in the yellow flowered C. officinalis L. cv. Alice Yellow (Table 2; Fig. 2a & b). Under ambient storage conditions no anthocyanin content was recorded after 30th day of storage in plant material and after 60th day of storage in plant extract. Under refrigerated storage conditions no anthocyanin content was recorded after 40th day of storage in plant material. Decreasing trend was recorded in Table 2 and every storage interval was significantly different from each other except the values recorded under refrigerated conditions for plant material on 0 and 10th day of storage and under ambient conditions for plant extract on 0 and 10th day of storage. Pigments present in the plant material and extract stored under refrigerated conditions lasted longer than the pigments present in the plant material and extract stored under ambient storage conditions. The amount of the total anthocyanin content decreased with increase in days of storage and was not detected after some days of storage. The drastic decrease of anthocyanins was because of rapid degradation. Anthocyanin molecules are unstable and very sensitive to technological processing particularly when heat is involved. Waskar and Khurdiya (1987) have reported degradation of anthocyanin in Phalsanector, concentrate, squash and crush during entire period of storage. Forni et al. (1993) reported anthocyanin loss in thermal processing and storage in osmodehydrated and pasteurized cherries. Similar findings have been reported by Siddiq et al. (1994) in plum juice, Uygan and Acar (1995) in cherry nectar, Iversen (1999) in black currant nectar, Gimenez et al. (2001) in strawberry jam, Kmiecik et al. (2001), in bilberry and strawberry jam, Kim and Pandilla (2004) in cherry, plum and raspberry. Anthocyanin degradation during refrigerated storage is believed to be due to native enzymes, particularly polyphenoloxidase.

Total carotenoid content (mg/100 g)

In Pot Marigold (*Calendula officinalis* L. var. Gitana Orange) total carotenoid content ranged between 170.00 to 288.33 mg/100g under ambient storage conditions while as under refrigerated storage conditions it ranged between 176.83 to 288.33



mg/100g (Table 1; Fig. 1a & b). In the extract of the species the pigments ranged between 183.00 to 288.33 mg/100g under ambient storage conditions and 190.67 to 288.33 mg/100g under refrigerated storage conditions. Highest (288.33 mg/100g) pigment content was recorded during day 0 of storage and lowest (170.00 and 176.83 mg/100g) on 90th day of storage in plant material under ambient and refrigerated storage conditions respectively. However, in extract lowest pigment content to the tune of 183.00 mg/100 g was recorded under ambient and 190.67 mg/100g under refrigerated storage conditions on 90th day of storage. Decreasing trend was recorded in Table 1 and every storage interval was significantly different from each other. In Pot Marigold (Calendula officinalis L. var. Gitana Yellow) total carotenoid content ranged between 121.00 to 205.50 mg/100g under ambient storage conditions while as under refrigerated storage conditions it ranged between 131.00 to 205.50 mg/100g. In the extract of the species the pigments ranged between 141.33 to 205.50 mg/100g under ambient storage conditions and 148.50 to 205.50 mg/100g under refrigerated storage conditions. Highest (205.50 mg/100g) pigment content was recorded during on day 0 and lowest on 90th day of storage in plant material under ambient storage conditions (121.00 mg/100g) and refrigerated storage conditions (131.00 mg/100g), while as in extract lowest pigment to the tune of 141.33 mg/100g and 148.50 mg/100g was recorded under ambient and refrigerated storage conditions respectively on 90th day of storage (Table 2, Fig. 2a & b). Decreasing trend was recorded in Table 2 and every storage interval is significantly different from each other. Carotenoids present in the plant material and extract stored under refrigerated conditions lasted longer than those present in the plant material and extract stored under ambient conditions. Decrease in total carotenoid content was observed with increase in days of storage and in some test species. The total carotenoid content was not detected after some days of storage. The decrease in content during storage may be attributed to degradation of carotenoids. The loss of carotenoids may be due to increased temperature during storage. The above findings regarding the decrease in total carotenoid content during storage are in conformity with that of Rao (1970) in mango juice, Sadhu and Bose (1976) in mango cultivars and Krishnaveni et al. (2001) in jackfruit RTS beverages. Similar results have been observed by Szymczak and Plocharski (2000); Peggy and James (2003); Raynal *et al.* (1989) and Maria *et al.* (2002). The decreasing trend of carotenoids was also observed by Srivastava *et al.* (1985) in blending of mango varieties for juice preparation during storage, Sahani *et al.* (1994) in carotene profile of mango and Deka *et al.* (2005) in spiced mango, pineapple beverages and Punchok (2005) in apricot and seabuckthorn squash.

Color parameters (L^* , a^* , b^* , C^* and h°)

The L* value of Pot Marigold (Calendula officinalis L. var. Gitana Orange) ranged between 64.33 to 72.21. The L* value showed the decreasing trend with increase in storage time in both ambient as well as refrigerated conditions, showing darker color with storage. The a* value ranged between 65.32 to 68.93. Similar a* value of 65 was reported by Kishimoto et al. (2007) in orange flowered cultivars of Calendula officinalis L. The a* value showed the increasing trend with storage in both storage conditions reflecting the reddening of the plant material. The b* value ranged between 59.94 to 67.29. The b* value showed the decreasing trend with increase in storage time in both storage conditions. The Ho ranged between 41.01 to 45.85° and also showed decreasing trend. These values of H^o are in agreement to the values reported by Kishimoto et al. (2007) who reported the H° between the range of 38-80° for orange flowered cultivars of Calendula officinalis L. Further the C* ranged between 91.35 to 93.78 showing the decreasing trend reflecting the transformation from more vivid sample to dull one in both the storage conditions. ΔE indicating the total color difference between two samples ranged between 96.03 to 97.05. The L* value of Pot Marigold (Calendula officinalis L. var. Gitana Yellow) ranged between 63.79 to 81.52. The L* value showed the decreasing trend with increase in storage time in both ambient as well as refrigerated conditions, showing darker color with storage. These values were lower when compared with the values (83.2-85.3) reported by Kishimoto et al. (2007) in yellow flowered varieties of Calendula officinalis L. The a* value ranged between 35.29 to 47.43. The a* value showed the decreasing trend with increase in time of storage in both storage conditions due to the degradation of anthocyanins. The b* value ranged between 48.95 to 77.09. The b* value showed the decreasing trend with increase in time of storage in both storage conditions. The Ho ranged between 54.21 to 58.40° and also showed decreasing trend reflecting the color change from dark yellow to light yellow. Further the C* ranged between 60.34 to 90.51 showing the decreasing trend, it might be due to the transformation of the more vivid colored plant material to dull one under both storage conditions, however enough literature regarding this could not be found. ΔE indicating the total color difference between two samples ranged between 67.40 to 91.83, it showed the decreasing trend.

Trend of hue angle (H°) changes

The hue angle (H°) of the selected plant species during 0 and 90 days of storage under ambient and refrigerated are shown in Table 3 and 4. The less changes in hue angle (H°) were recorded under refrigerated conditions as compared to the ambient storage conditions in selected plant species. Thus, among the two storage conditions, the refrigerated condition is the best condition for maintaining the hue angle (H°).

Table 1: Changes in anthocyanin content (mg/100 g) and total carotenoid content (mg/100 g) at different storageintervals of plant material and extract of Pot Marigold (*Calendula officinalis* var. Gitana Orange) under ambient (12-
28 °C) and refrigerated (4 °C) storage conditions

Storage	Pot Marigold (Calendula officinalis var. Gitana Orange)										
intervals	Aı	nthocyanin co	ntent (mg/10	0 g)	Total carotenoid content (mg/100 g)						
(days)	Plant 1	naterial	Ex	tract	Plant	material	Extract				
	Ambient	Refrigerated	Ambient	Refrigerated	Ambient	Refrigerated	Ambient	Refrigerated			
D ₀	16.29 (4.16)	16.29 (4.16)	16.29 (4.16)	16.29 (4.16)	288.33	288.33	288.33	288.33			
D ₁₀	10.86 (3.44)	12.04 (3.61)	13.06 (3.75)	13.91 (3.86)	265.50	271.33	277.50	280.83			
D ₂₀	7.97 (2.99)	9.16 (3.19)	11.03 (3.47)	12.55 (3.68)	252.67	263.00	272.50	275.50			
D ₃₀	3.73 (2.16)	5.93 (2.62)	9.16 (3.18)	11.03 (3.47)	245.50	251.83	261.83	267.83			
D_{40}	1.86 (1.68)	2.71 (1.92)	7.12 (2.85)	7.12 (2.85)	234.00	244.83	251.50	257.17			
D ₅₀	0.34 (1.14)	1.18 (1.47)	4.92 (2.43)	5.43 (2.53)	219.50	227.50	233.33	242.17			
D ₆₀	0.00 (1.00)	0.67 (1.28)	2.88 (1.96)	4.24 (2.28)	207.67	213.67	219.83	228.17			
D ₇₀	-	0.34 (1.14)	0.00 (1.00)	2.20 (1.78)	195.17	200.67	206.83	216.83			
D ₈₀	_	0.00 (1.00)	-	1.01 (1.40)	181.50	188.00	194.50	207.50			
D_{90}	-	-	_	0.00 (1.00)	170.00	176.83	183.00	190.67			
C.D(p≤0.05)	0.220	0.204	0.141	0.183	4.938	4.633	4.753	4.228			

*Figures in parentheses are square root transformed means



Fig. 1a & b: Changes in anthocyanin content (mg/100 g) and total carotenoid content (mg/100 g) at different storage intervals of plant material and extract of Pot Marigold (*Calendula officinalis* var. Gitana Orange) under ambient (12-28 °C) and refrigerated (4 °C) storage conditions



Table 2: Changes in anthocyanin content (mg/100 g) and total carotenoid content (mg/100 g) at different storage intervals of plant material and extract of Pot Marigold (*Calendula officinalis* var. Gitana Yellow) under ambient (12-28 °C) and refrigerated (4 °C) storage conditions

	Pot Marigold (Calendula officinalis var. Gitana Yellow)											
Storage intervals (days)	Aı	nthocyanin co	ntent (mg/10	0 g)	Total carotenoid content (mg/100 g)							
	Plant 1	material	Ex	tract	Plant	material	Extract					
-	Ambient	Refrigerated	Ambient	Refrigerated	Ambient	Refrigerated	Ambient	Refrigerated				
D ₀	10.01 (3.32)	10.01 (3.32)	10.01 (3.32)	10.01 (3.32)	205.50	205.50	205.50	205.50				
D ₁₀	6.44 (2.72)	8.82 (3.13)	9.50 (3.24)	9.76 (3.28)	187.83	192.17	197.00	200.50				
D ₂₀	4.92 (2.43)	6.10 (2.66)	7.97 (2.99)	9.16 (3.18)	181.67	186.17	190.50	197.67				
D ₃₀	1.69 (1.62)	4.07 (2.24)	6.78 (2.79)	8.14 (3.02)	174.83	179.67	186.17	192.67				
D_{40}	0.00 (1.00)	1.69 (1.62)	5.26 (2.50)	6.61 (2.76)	168.17	171.50	179.83	188.33				
D ₅₀	-	0.00 (1.00)	3.73 (2.17)	5.26 (2.50)	158.17	162.17	171.00	178.50				
D ₆₀	-	-	2.37 (1.82)	4.24 (2.28)	147.50	153.67	163.00	172.33				
D ₇₀	-	-	0.00 (1.00)	3.49 (1.90)	137.67	145.00	154.83	165.33				
D ₈₀	-	-	-	1.60 (1.53)	133.17	138.83	149.67	156.83				
D ₉₀	_	_	-	1.11 (1.28)	121.00	131.00	141.33	148.50				
C.D(p≤0.05)	0.197	0.207	0.146	0.165	4.872	3.617	3.271	3.071				

*Figures in parentheses are square root transformed means



Fig. 2a & b: Changes in anthocyanin content (mg/100 g) and total carotenoid content (mg/100 g) at different storage intervals of plant material and extract of Pot Marigold (*Calendula officinalis* var. Gitana Yellow) under ambient (12-28 °C) and refrigerated (4 °C) storage conditions

	Pot Marigold (Calendula officinalis L. var. Gitana Orange)												
Storage intervals (days)	Color coordinates							Hue angle		Chroma		Total color	
	L*		a*		b*		(H°)		(C*)		change (ΔE)		
	Α	R	Α	R	A	R	Α	R	Α	R	A	R	
D ₀	72.21	72.21	65.32	65.32	67.29	67.29	45.85	45.85	93.78	93.78	96.70	96.70	
D ₁₀	69.95	71.17	65.73	65.51	66.01	66.93	45.11	45.61	93.15	93.65	96.59	96.80	
D ₂₀	69.31	70.22	66.49	65.70	65.45	66.63	44.55	45.40	93.30	93.57	96.88	96.93	
D ₃₀	68.14	69.58	66.81	65.98	64.79	65.71	44.12	44.88	93.07	93.12	96.95	96.64	
D_{40}	67.70	68.72	67.23	66.27	63.85	65.18	43.52	44.52	92.72	92.95	96.73	96.69	
D_{50}	66.80	68.42	67.72	66.69	63.28	64.52	43.06	44.05	92.68	92.79	96.93	96.61	
D ₆₀	66.26	68.07	68.16	67.01	62.77	63.69	42.64	43.54	92.66	92.45	97.05	96.37	
D ₇₀	65.47	67.50	68.38	67.24	61.82	63.00	42.12	43.14	92.18	92.14	96.81	96.22	
D ₈₀	64.83	66.89	68.69	67.53	60.94	62.12	41.58	42.61	91.83	91.76	96.65	96.01	
D ₉₀	64.33	66.35	68.93	67.82	59.94	61.60	41.01	42.25	91.35	91.62	96.34	96.03	
C.D(p≤0.05)	0.142	0.143	0.089	0.186	0.134	0.115							

Table 3: Chromaticity of the Pot Marigold (*Calendula officinalis* L. var. Gitana Orange) under ambient (12-28 °C)and refrigerated (4 °C) conditions

A= Ambient; R = Refrigerated

Table 4: Chromaticity of the Pot Marigold (Calendula officinalis L. var Gitana Yellow) under ambient (12-28 °C) and
refrigerated (4 °C) conditions

Storage intervals (days)	Pot Marigold (Calendula officinalis L. var. Gitana Yellow)												
	Color coordinates							Hue angle				Total color	
	L*		a*		b*		(H°)				change (∆E)		
	Α	R	A	R	A	R	Α	R	Α	R	Α	R	
D ₀	81.52	81.52	47.43	47.43	77.09	77.09	58.40	58.40	90.51	90.51	91.83	91.83	
D ₁₀	78.68	80.47	44.90	46.59	71.64	74.03	57.92	57.82	84.55	87.47	86.31	88.95	
D ₂₀	76.73	78.92	44.13	45.77	69.74	72.15	57.68	57.61	82.53	85.44	84.66	87.17	
D ₃₀	75.07	77.62	43.54	45.07	65.94	68.05	56.56	56.48	79.02	81.62	81.56	83.62	
D ₄₀	74.07	75.93	42.51	43.62	63.49	65.27	56.20	56.25	76.41	78.50	79.24	80.88	
D ₅₀	72.78	74.97	42.35	43.63	61.16	63.48	55.30	55.50	74.39	77.03	77.60	79.65	
D ₆₀	71.33	73.62	39.90	42.04	57.78	60.63	55.37	55.26	70.22	73.78	73.97	76.80	
D ₇₀	68.68	71.43	37.95	41.26	55.47	58.95	55.62	55.01	67.21	71.95	71.91	75.61	
D ₈₀	66.17	70.96	37.04	38.94	52.97	56.53	55.04	55.44	64.64	68.64	70.38	72.57	
D ₉₀	63.79	69.40	35.29	38.54	48.95	54.54	54.21	54.75	60.34	66.78	67.40	71.28	
C.D(p≤0.05)	0.159	0.126	0.146	0.130	0.119	3.992							

A= Ambient; R = Refrigerated





Analysis of anthocyanins and carotenoids in two varieties of Calendula officinalis L. flowers



Conclusion

From the present study it could be concluded that both varieties of Pot Marigold have good levels of total carotenoids, thus petals of both varieties can be used as a raw material for extraction of yellow to orange natural dyes. However further research must be done in identifying the new areas of demand and application.

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