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Influence of storage conditions of marigold flowers on retention of carotenoids and antioxidant activities

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ABSTRACT

The present investigations were carried out to find the effect of different storage temperatures and durations on retention of total carotenoids and antioxidant activities of dried marigold flower petals in varieties Pusa Arpita of *Tagetes patula* L., Pusa Basanti Gainda and Pusa Narangi Gainda of *Tagetes erecta* L. The results revealed that vacuum dried petals of varieties, namely Pusa Arpita, Pusa Narangi Gainda and Pusa Basanti Gainda recorded the highest content of carotenoids (952.78, 1923.94 and 119.75 mg/100 g DW), lutein (220.48, 205.26 and 65.87 µg/g DW), β carotene (14.61, 11.68 and 3.86 µg/g DW), total phenolic content (46.50, 45.48 and 63.34 mg GAE/g DW), total flavonoid content (30.85, 29.32 and 31.28 mg GAE/ g DW) and antioxidant activities {FRAP (430.59, 595.29 and 509.57 µmol $FeSO_4$/g DW) and DPPH (49.28, 59.73 and 51.30%)} after 60 days of storage temperature at -20°C, respectively followed by 4°C and lowest content was observed in dried marigold flower petals stored at ambient temperature. It was also revealed from the studies that carotenoids, total phenolic content, total flavonoids and antioxidant activities were found decreased during storage at all the temperatures. The retention of carotenoids and their antioxidant activities was found to be high in vacuum dried petals stored at -20°C. Among varieties, Pusa Narangi Gainda of African marigold retained better carotenoids and antioxidant activities after 60 days of storage at -20°C.

Key words: *Tagetes* sp., β-carotene, flavonoid, lutein, phenols.

INTRODUCTION

Flower crops are also one of the potential sources of pigments, however, due to lack of awareness it remained as an unexploited area. Among flowers, marigold is one of the economically important loose flower crops which are further used for their diuretic, antiseptic, depurative, insect repellent activities. Since marigold petals are a rich source of carotenoids especially the yellow carotenoids (β-carotenes), xanthophylls (lutein, zeaxanthin) and polyphenols, it is used as food colourant and animal feed. Since these pigments are inherited unstable, highly unsaturated molecules, hence, subjected to isomerisation causing colour loss and oxidation. Carotenoid pigments still bind to proteins and keep their natural state by providing stability to pigment colour and structure during storage. However, the retention of carotenoids during storage is an important aspect to get acceptable end product (Cinar, 7). Carotenoid degradation during storage not only affects colour but also flavour and nutritive value.

Antioxidant activity of the plants also depends upon the composition and content of the phenolic and flavonoid content present in the plants. Storage at different temperatures is also an important factor for retention of carotenoid pigments and their antioxidant activities in fruits, vegetables and flowers (Lee and Kader, 11). Effect of efficient storage techniques on retention of carotenoid content and antioxidant activities of marigold flowers was supported by very few published reports. The time between harvesting and consumption might be long and during this period, biochemical changes could happen that affect the nutraceutical value in marigold, hence, there is need to standardize storage of dry petals at different temperatures and for different durations to recover maximum content of carotenoids and other bioactive compounds and high retention of antioxidant activities of pigments in marigold flowers.

MATERIALS AND METHODS

The plant material utilized for conducting the experiment consisted of two varieties of African marigold (*Tagetes erecta* L.), namely, Pusa Narangi Gainda and Pusa Basanti Gainda and one variety of French Marigold (*Tagetes patula* L.), namely, Pusa Arpita. The features of varieties used in the present investigation are given in Table 1. These were grown and maintained at research farm of the Division of Floriculture and Landscaping and estimation was done in laboratory of the Division of Agricultural Chemicals, ICAR-IARI, New Delhi during 2013-15.

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Table 1. Salient features of marigold genotypes.

Genotype	Flower type	Flower form	Flower size	Flower colour	Species	Flowering time	Source
Pusa Arpita	Semi double	Petalous	Medium	Orange	*Tagetes patula* L.	Mid Dec.,- Mid Feb.,	ICAR-IARI
Pusa Narangi Gainda	Semi double	Petalous	Medium	Orange	*Tagetes erecta* L.	Mid Feb.,- Mid April	ICAR-IARI
Pusa Basanti Ganida	Semi double	Petalous	Medium	Yellow	*Tagetes erecta* L.	Mid Feb.,- Mid March	ICAR-IARI

Fresh marigold flowers were harvested at full bloom stage for drying of petals in vacuum drying oven. The petals were spread uniformly in the trays of vacuum oven, at pressure of 0.08 kP at 60°C till the constant weight was obtained. The dried petals were subjected to different storage temperatures such as ambient temperature, 4° and -20°C for different durations of 0, 20, 40 and 60 days. The dried petals were further used for extract preparation (petroleum ether extract for carotenoids, ethanolic extract for antioxidant, and lutein extract for lutein and β-carotene estimation).

The total carotenoids were extracted and estimated using method given by Ranganna (13) with minor modifications. Sample preparation for Lutein and β-carotene was done using a modification of procedure described by Barba *et al.* (2). Analysis of lutein and β-carotene was carried out using high performance liquid chromatography. Sample was prepared for estimation of phenolic compounds and assessment of antioxidant activity was done using a method described by Uzelac *et al*. (17) with some modifications. Total phenolic content (TPC) was estimated according to procedure given by Singleton and Rossi (14). The colorimetric method described by Abu Bakar *et al.* (1) was used to determine total flavonoid content (TFC). Total antioxidants were estimated using FRAP method as described by Benzie and Strain (3). The antioxidant activity of the extracts was determined using DPPH assay described by Braca *et al.* (5). The data was statistically analyzed in completely randomized design (CRD) using statistical analysis system (SAS) software. All determinations were done in triplicate and were averaged.

RESULTS AND DISCUSSION

The data presented in the Table 2 revealed that there was significantly decreasing trend for carotenoid pigments in vacuum-dried marigold flowers as the storage duration is increased. The rate of depletion of carotenoid pigments and antioxidant activities in petals stored at -20°C temperature was very slow, followed by petals stored at 4°C. Total carotenoids, lutein and β-carotene in petals of French marigold (*Tagetes patula* L.) variety, Pusa Arpita was decreased from 1108.76 mg/100 g DW, 252.51 87 µg/g DW and 17.00 µg/g DW on 0 day to 952.78 mg/100 g DW, 220.48 µg/g DW and 14.61 µg/g DW after 60 day of storage at -20°C temperature, respectively. Highest retention of total carotenoids (85.93%), lutein (87.31%) and β-carotene (85.94%) during storage was observed at -20°C temperature, whereas, only 74.49% of total carotenoids, 73.80% of lutein and 68.70% of β-carotene was retained at ambient temperature after 60 days of storage of dried petals (Fig. 1). In African marigold (*Tagetes erecta* L.) variety, Pusa Narangi Gainda, total carotenoids, lutein and β-carotene content decreased from 2765.76 mg/100 g DW, 295.15 µg/g DW and16.44 µg/g DW on 0 day to 1923.94 mg/100 g DW, 205.26 µg/g DW and 16.44 µg/g DW after 60 days of storage at -20°C, respectively. It is depicted from Fig. 3 that highest retention of carotenoids (69.56%), lutein (69.54%) and β-carotene (71.04%) was observed at -20°C, however, only 61.69% of total carotenoids, 61.68% of lutein and 63.01% of β-carotene was retained after 60 days of storage of dried petals at ambient temperature (Fig. 2). The data revealed that in African marigold, Pusa Basanti Gainda, total carotenoids, lutein and β-carotene were decreased from 144.90

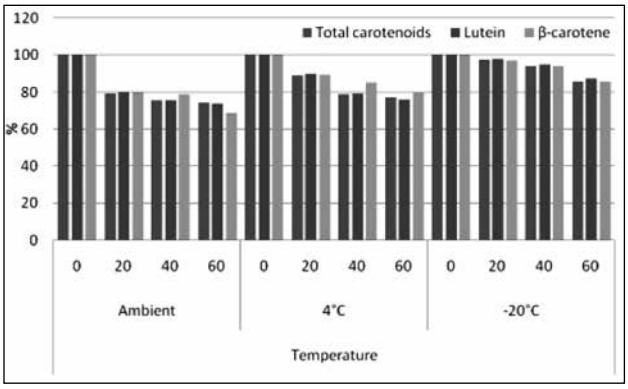

Fig. 1. Carotenoids retention in vacuum dried flowers of French marigold variety Pusa Arpita during storage.

Table 2. Effect of storage temperature and duration on retention of high carotenoid pigments in vacuum dried flowers of marigold.

Variety	Storage temp. (°C)	Duration (days)	Total carotenoids (mg/100 g)		Lutein (µg/g)		β-carotene (µg/g)	
			Mean	SD	Mean	SD	Mean	SD
Pusa Arpita	Ambient	0	1108.76	87.03	252.51	9.43	17.00	0.24
		20	882.85	14.53	202.57	10.37	13.55	0.89
		40	837.33	20.79	191.06	3.44	13.45	1.71
		60	826.00	18.31	186.36	5.28	11.68	0.95
	4	0	1108.76	87.03	252.51	9.43	17.00	0.24
		20	989.35	22.37	226.95	6.03	15.22	0.97
		40	876.33	20.44	200.95	8.61	14.49	1.09
		60	859.24	27.99	191.86	7.46	13.68	1.95
	-20	0	1108.76	87.03	252.51	9.43	17.00	0.24
		20	1081.21	51.70	247.08	5.36	16.51	1.98
		40	1044.58	22.39	240.54	19.01	16.01	1.07
		60	952.78	9.38	220.48	4.01	14.61	1.40
Pusa Narangi Gainda	Ambient	0	2765.76	56.39	295.15	10.38	16.44	1.99
		20	2175.47	108.26	232.10	11.55	13.21	0.66
		40	1830.11	88.72	195.26	9.47	11.11	0.54
		60	1706.34	18.71	182.05	2.00	10.36	0.12
	4	0	2765.76	56.39	295.15	10.38	16.44	1.99
		20	2278.08	31.37	243.05	3.35	13.83	0.19
		40	2026.12	49.64	216.17	5.30	12.30	0.30
		60	1918.33	25.29	204.67	2.70	11.65	0.15
	-20	0	2765.76	56.39	295.15	10.38	16.44	1.99
		20	2292.47	16.71	244.58	1.78	13.92	0.10
		40	2068.31	48.42	220.67	5.17	12.56	0.30
		60	1923.94	25.47	205.26	2.72	11.68	0.16
Pusa Basanti Gainda	Ambient	0	144.90	8.06	79.21	4.40	4.65	0.98
		20	113.60	9.24	62.49	5.08	3.66	0.30
		40	101.56	6.87	55.87	3.78	3.27	0.22
		60	91.10	5.16	50.11	2.83	2.94	0.17
	4	0	144.90	8.06	79.21	4.40	4.65	0.98
		20	126.43	5.90	69.55	3.25	4.08	0.19
		40	120.33	5.86	66.19	3.22	3.88	0.19
		60	114.70	7.75	63.09	4.27	3.69	0.25
	-20	0	144.90	8.06	79.21	4.40	4.65	0.98
		20	131.77	11.70	72.48	6.44	4.25	0.38
		40	125.67	6.90	69.12	3.80	4.05	0.23
		60	119.75	5.73	65.87	3.15	3.86	0.19
$CD_{0.05}$	Variety (A)		20.15**		3.35**		0.46**	
	Temperature (B)		20.15**		3.35**		0.46**	
	Duration (C)		23.27**		3.87**		0.53**	
	Variety × Temp. (A×B)		34.90**		5.81**		0.79^{NS}	
	Variety × Duration (A×C)		40.30**		6.70**		0.91**	
	Temperature × Duration (B×C)		40.30**		6.70**		0.91^{NS}	
	Variety × Temp. × Duration (A×B×C)		69.80*		11.61*		1.58^{NS}	

**,*Significant at 1 and 5% levels

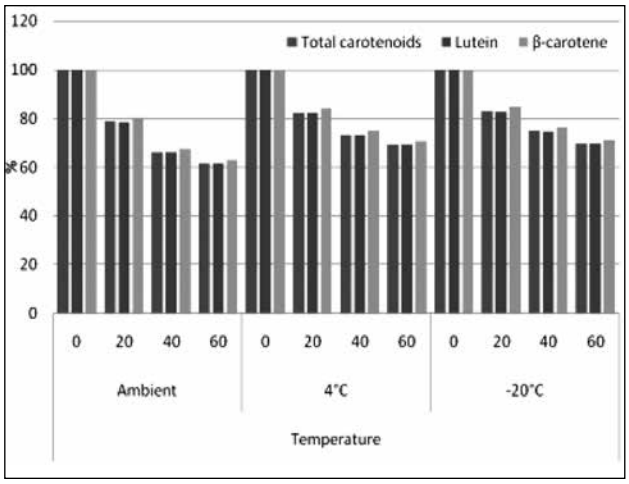

Fig. 2. Carotenoids retention in vacuum dried flowers of African marigold variety Pusa Narangi Gainda during storage.

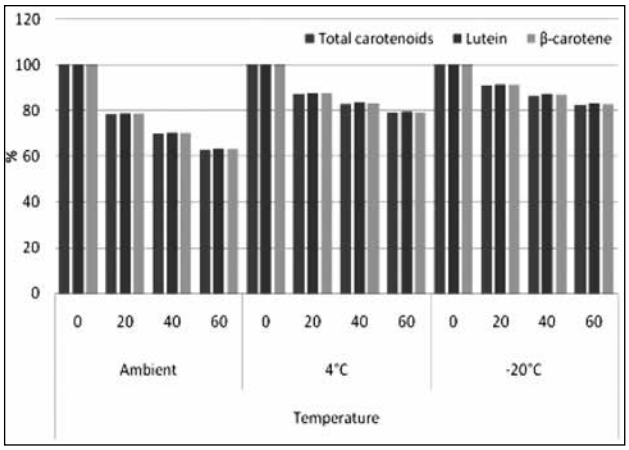

Fig. 3. Carotenoids retention in vacuum dried flowers of African marigold variety Pusa Basanti Gainda during storage.

mg/100 g DW, 79.21 µg/g DW and 4.65 µg/g DW on 0 day to 119.75 mg/100g DW, 65.87 µg/g DW and 3.86 µg/g DW after 60 days of storage at -20°C temperature. Highest retention of total carotenoids (82.64%), lutein (83.16%) and β-carotene (83.01%) was observed at -20°C temperature after 60 days of storage of dried petals (Fig. 3). The interaction between variety × temperature, variety × duration, temperature × duration for total carotenoids and lutein content was significant at 1% level of significance. However, interaction between variety × temperature × duration was significant at 5% level of significance. For β-carotene, interaction between variety × temperature, temperature × duration and variety × temperature × duration was non-significant, whereas, interaction between variety × duration was significant at 1% level of significance. Carotenoids are sensitive to heat, light and oxygen and the major cause of carotenoid destruction during processing and storage is enzymatic or non enzymatic oxidation in carrots (Dutta, 9). The results were also in confirmation with Blessington *et al*. (4) in potato and Siriamornpun *et al.* (15) in marigold.

The data presented in the Table 3 depicts that the phenolic content, flavonoid content and antioxidant activities (FRAP and DPPH) in marigold petals tend to decrease during storage. The storage temperature of -20°C had high retention of phenolic content, flavonoid content and antioxidant activities and the rate of depletion was much faster under ambient temperature. In French marigold variety, Pusa Arpita, total phenolic content (84.24 mg GAE/g DW) and total flavonoids content (47.74 mg GAE/g DW) on 0 day, were decreased to 46.50 mg GAE/g DW and 30.85 mg GAE/g DW after 60 days of storage at -20°C, respectively. Highest retention of total phenolic content (55.20%) and total flavonoid content (49.67%) during storage was observed at -20°C temperature, whereas, only 39.10% of phenolic content and 39.00% of total flavonoid content was retained at ambient temperature after 60 days of storage of dried petals (Fig. 4). The data shows that in African marigold (*Tagetes erecta* L.) variety, Pusa Narangi Gainda, total phenolic content (83.89 mg GAE/g DW) and total flavonoid content (46.61 mg GAE/g DW) on 0 day decreased to 45.48 mg GAE/g DW and 29.32 mg GAE/g DW after 60 days of storage at -20°C temperature, respectively. Highest retention of total phenolic content (54.21%) and total flavonoid content (62.90 %) during storage was observed at -20°C temperature, whereas, only 37.68% of phenolic content and 39.30% of total flavonoids content was retained at ambient temperature after 60 day of

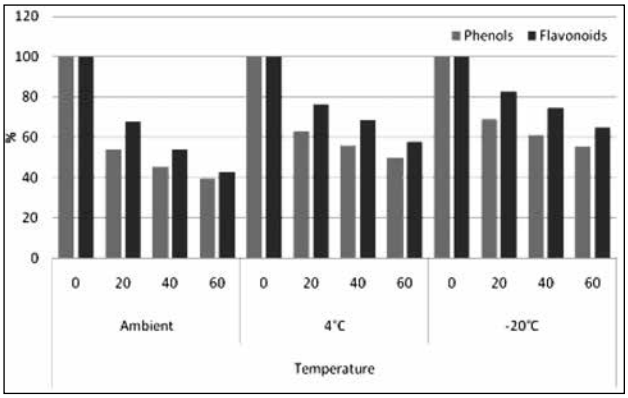

Fig. 4. Retention of Phenolic compounds in vacuum dried flowers of French marigold variety Pusa Arpita during storage.

Table 3. Effect of storage temperature and duration on retention of antioxidant activities, phenolic and flavonoid content in vacuum dried flowers of marigold.

Variety	Storage temperature (°C)	Duration (days)	Total Phenolic content (mg GAE/g)		Total Flavonid content (mg RE/g)		FRAP (µmol $FeSO_4$/g)		DPPH (%)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pusa Arpita	Ambient	0	84.24	1.99	47.74	1.31	655.75	35.28	76.63	2.70
		20	45.36	6.54	32.37	2.11	538.89	30.26	52.31	1.66
		40	38.16	2.04	25.72	2.23	406.99	45.62	37.62	0.89
		60	32.94	1.48	20.35	2.21	306.98	32.93	29.53	0.85
	4	0	84.24	1.99	47.74	1.31	655.75	35.28	76.63	2.70
		20	52.78	3.24	36.33	3.10	562.28	32.30	58.15	1.87
		40	46.83	1.71	32.59	3.14	440.48	13.12	51.35	2.52
		60	41.85	1.12	27.55	1.91	370.82	65.05	45.69	0.89
	-20	0	84.24	1.99	47.74	1.31	655.75	35.28	76.63	2.70
		20	58.03	1.43	39.44	1.80	594.61	35.38	62.34	1.95
		40	51.28	2.58	35.44	2.90	530.23	20.93	54.28	2.94
		60	46.50	2.15	30.85	1.12	430.59	22.82	49.28	1.94
Pusa Nara-ngi Gainda	Ambient	0	83.89	2.75	46.61	2.00	838.83	27.46	71.99	3.69
		20	43.66	1.04	28.49	1.04	602.44	13.23	54.88	0.63
		40	37.66	0.80	23.41	2.00	461.05	9.54	41.81	1.69
		60	31.61	0.75	18.32	1.23	309.50	13.71	30.39	0.94
	4	0	83.89	2.75	46.61	2.00	838.83	27.46	71.99	3.69
		20	53.33	0.85	36.04	1.63	709.51	21.73	64.98	0.82
		40	46.10	1.55	29.56	1.51	639.20	2.85	58.98	1.79
		60	41.43	0.66	25.63	1.16	524.82	8.19	54.53	0.98
	-20	0	83.89	2.75	46.61	2.00	838.83	27.46	71.99	3.69
		20	57.45	1.68	38.00	0.38	734.92	10.81	67.16	0.77
		40	50.27	1.98	33.97	1.32	677.27	4.59	63.26	0.87
		60	45.48	1.04	29.32	1.56	595.29	11.31	59.73	0.44
Pusa Basanti Gainda	Ambient	0	93.00	2.05	46.61	2.00	716.05	98.44	73.15	0.90
		20	62.45	4.35	29.85	2.45	552.56	89.26	53.49	3.70
		40	51.83	6.36	24.48	2.24	434.03	38.17	41.04	1.38
		60	44.33	5.25	19.47	0.99	347.30	25.58	31.95	1.62
	4	0	93.00	2.05	46.61	2.00	716.05	98.43	73.15	0.90
		20	70.98	2.62	34.47	2.67	623.00	41.09	59.92	1.34
		40	64.06	3.92	30.50	1.11	503.86	27.84	54.16	0.99
		60	58.55	1.61	28.45	2.02	434.48	26.00	48.30	0.93
	-20	0	93.00	2.05	46.61	2.00	716.05	98.43	73.15	0.90
		20	74.09	2.34	38.31	3.75	641.94	40.28	64.45	2.27
		40	68.27	4.06	34.70	1.50	564.27	47.20	57.22	1.08
		60	63.34	7.03	31.28	2.17	509.57	45.44	51.30	1.27
$CD_{0.05}$	Variety (A)		1.40**		0.93**		2.03**		0.92**	
	Temperature (B)		1.40**		0.93**		2.03**		0.92**	
	Duration (C)		1.62**		1.08**		2.34**		1.06**	
	Variety × Temp. (A×B)		2.43NS		1.62 NS		35.16**		1.59**	
	Variety × Duration (A×C)		2.81**		1.87 NS		40.60 NS		1.83**	
	Temperature × Duration (B×C)		2.81**		1.87**		40.60**		1.83**	
	Variety × Temp. × Duration (A×B×C)		4.86 NS		3.23 NS		70.32 NS		3.17 NS	

**,*Significant at 1 and 5% levels

storage of dried petals (Fig. 5). In African marigold (*Tagetes erecta* L.) variety, Pusa Basanti Gainda, total phenolic and flavonoid content decreased from 93.00 mg GAE/g DW and 46.61 mg GAE/g DW on 0 day to 63.34 mg GAE/g DW and 63.34 mg GAE/g DW, respectively, after 60 days of storage at ambient temperature. Highest retention of total phenolic content (68.10%) and total flavonoid content (67.11%) was observed after 60 days of storage at -20°C temperature whereas, only 47.66% of total phenolic content and 41.77% of total flavonoid content had retained in marigold petals stored at ambient temperature (Fig. 6). The interaction between variety × duration, temperature × duration for total phenolic content is significant at 1% level of significance. However, interaction between variety × temperature, variety × temperature × duration are non-significant.

Data with respect to FRAP and DPPH activities showed that the antioxidant activities decreased as the storage temperature and duration increased. It was observed that the FRAP and DPPH values decreased from 655.75 µmol $FeSO_4$/g DW and 76.63% to 430.59 µmol $FeSO_4$/g DW and 49.28% retaining 65.66 and 64.30% reducing power in flowers of variety Pusa Arpita after 60 days of storage at -20°C temperature, respectively (Table 3; and Fig. 7). In vacuum dried flowers of variety Pusa Narangi Gainda, the FRAP and DPPH values decreased from 838.83 µmol $FeSO_4$/g DW and 71.99% to 595.29 µmol $FeSO_4$/g DW and 59.73% retaining 70.96 and 82.96% reducing power in flowers after 60 days of storage at -20°C temperature, respectively (Table 3, Fig. 8). The Table 3 and Fig. 9 exhibited that the FRAP and DPPH values decreased from 716.05 µmol $FeSO_4$/g DW and 73.15% to 509.57 µmol $FeSO_4$/g DW and 51.30% retaining 71.16% and 70.12% reducing power in flowers of variety Pusa Arpita after 60 day of storage at -20°C, respectively. The interaction between variety × temperature and temperature × duration for ferric

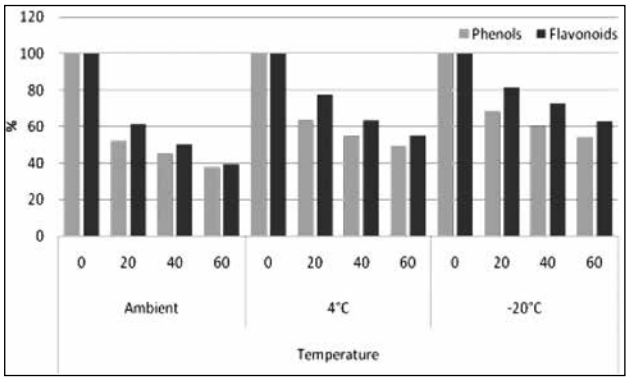

Fig. 5. Retention of Phenolic compounds in vacuum dried flowers of African marigold variety Pusa Narangi Gainda during storage.

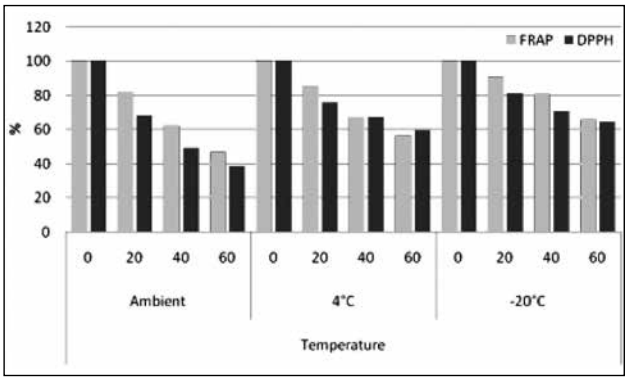

Fig. 7. Retention of antioxidant activities in vacuum dried flowers of French marigold variety Pusa Arpita during storage.

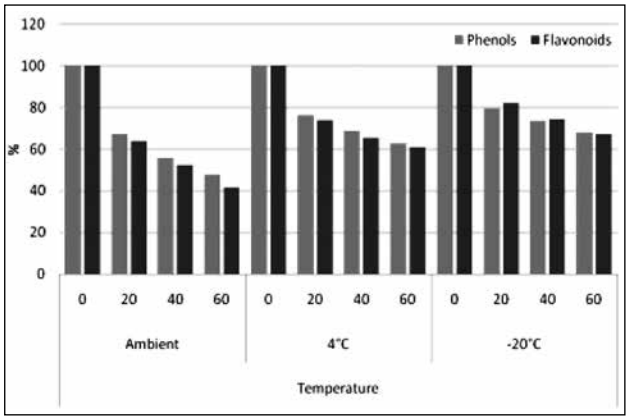

Fig. 6. Retention of Phenolic compounds in vacuum dried flowers of African marigold variety Pusa Basanti Gainda during storage.

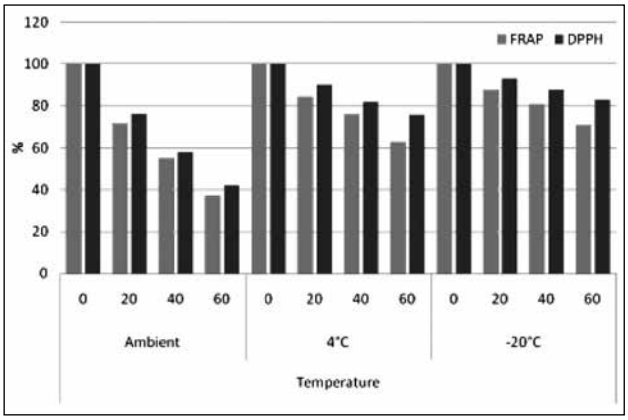

Fig. 8. Retention of antioxidant activities in vacuum dried flowers of African marigold variety Pusa Narangi Gainda during storage

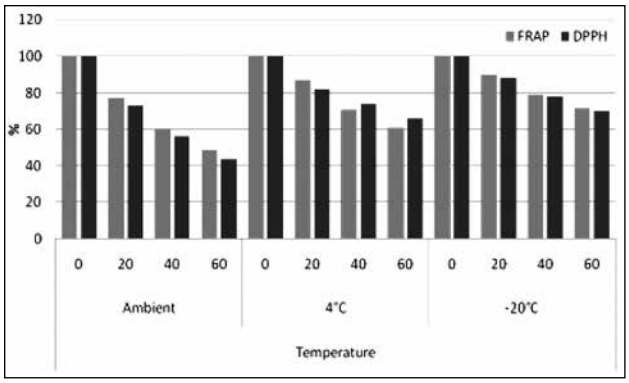

Fig. 9. Retention of antioxidant activities in vacuum dried flowers of African marigold variety Pusa Basanti Gainda during storage.

reducing antioxidant power and DPPH; variety × duration for DPPH were significant at 1% level of significance. However, interaction between variety × duration for FRAP values and variety × temperature × duration for both FRAP and DPPH values were non-significant. Klimczak *et al.* (10) reported the decrease in antioxidant activity of orange juices by 18, 45 and 84% after 6 months of storage at 18, 28 and 38°C, respectively. Similarly, De Ancos *et al.* (8) reported that the freezing process at -20°C during storage produced a decrease of antiradical efficiency in the four cultivars of raspberry ranging between 4 and 26%. Our results are in confirmation with the results of Cao *et al*. (6) who reported that low temperature storage maintained higher content of total phenolics and higher levels of DPPH radical scavenging activity and reducing power in Loquat fruit. Patthamakanokporn *et al.* (12) reported that the antioxidant activity and total phenolic compounds in the homogenised guava decreased significantly during storage at -20°C for 2 week and continued to decrease during 3 month of storage. Similar results were obtained by Siriamornpun *et al.* (15) in marigold and Tavarini *et al.* (16) in kiwifruit cultivar Hayward.

It was concluded from the investigations that among varieties, Pusa Narangi Gainda of African marigold retained more carotenoids, lutein and β-carotene content and antioxidant activities. The storage of vacuum dried petals at -20°C temperatures was found suitable for higher retention of bioactive compounds.

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