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Standardization of storage conditions of marigold (*Tagetes* sp.) petal extract for retention of carotenoid pigments and their antioxidant activities

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ABSTRACT

Present investigation was carried out to find the effect of different storage temperatures and durations on retention of total carotenoids, antioxidant activities and other secondary metabolites of marigold petal extract in varieties Pusa Arpita, Pusa Basanti Gainda, and Pusa Narangi Gainda. It was observed that the highest retention of carotenoids, lutein, β-carotene, phenolic and flavonoid contents and antioxidant activities were recorded at storage temperature of -20°C followed by 4°C and lowest retention was observed at ambient storage temperature. It was revealed from the studies that the carotenoids, total phenolics, total flavonoids and antioxidant activities were decreased during storage. Among varieties, Pusa Narangi Gainda retained more carotenoids (1 925.26 mg/100g DW) and antioxidant activities (FRAP- 647.83 µ mol $FeSO_4$/g; DPPH-59.51%), whereas, Pusa Arpita retained more lutein (228.87 µg/g) and β- carotene content (15.49µg/g) stored at -20°C. The storage of vacuum dried petals at -20°C temperatures was found suitable for higher retention of bioactive compounds.

Key words: Antioxidant activity, β-carotene, Carotenoid, Flavonoid, Lutein, Marigold, Phenol

Marigold (*Tagetes* sp.) flower petals are considered as an important source of carotenoid pigments, especially the yellow carotenoids (β-carotenes), xanthophylls (lutein, zeaxanthin) (Ahluwalia *et al.* 2014) and polyphenols (Siriamornpun *et al.* 2012). These pigments are recognized as safe chemicals for nutraceutical purpose because of their concentrated colour, their role as precursor for vitamin A synthesis and antioxidant activity in human beings. Carotenoids reduces the risk of chronic diseases, auto-oxidation of cellular lipids and age related macular degeneration, enhance immune function and provides protection against oxidant induced cell damage. Carotenoids are inherited instable pigments. Since these pigments are highly unsaturated molecules, hence, subjected to isomerization causing colour loss and oxidation. In enzyme extraction, carotenoid pigments still bind to proteins and keep their natural state 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 2004). Carotenoid degradation during storage not only affects colour but also flavour and nutritive value. There are very few studies on carotenoid behaviour during storage (Premavalli and Arya 1991, Yen and Chen 1995) but reported in crops such as orange, carrot and sweet potato (Cinar 2004).

Plants synthesized secondary metabolites such as phenolic compounds during the period of development and in response to infection, wounding and ultra violet radiations. There are approx. 8 000 naturally occurring compounds which belong to phenolics group. Plant phenolics include simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, tannins, lignans and lignins, etc. (Stalikas 2007). Antioxidant activity of the plants depends upon the composition and content of the phenolic and other bioactive compounds. Ayala *et al.* (2004) reported that changes occur in antioxidant activities of the pigments present in horticultural crops during storage. Storage at different temperature is also an important factor for retention of carotenoid pigments and their antioxidant properties in fruits, vegetables and flowers (Lee and Kader 2000).There are very few published reports on the effect of efficient dehydration and storage techniques on retention of carotenoid content and antioxidant activities of flowers. The time between harvesting and consumption might be long and during this period, biochemical changes could happen that affect the nutraceutical value in marigold. Therefore, there is need to standardize storage at different temperature and for different durations to recover maximum of carotenoids and other bioactive compounds and high retention of antioxidant activities of pigments in marigold flowers.

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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 are given in Table 1 and Fig. 1, respectively. These were grown and maintained at research farm of the Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi during 2013-15. Fresh marigold flowers were harvested at full bloom stage for drying of petals in vacuum oven (LVO-2030, Daihan Labtech Co. Ltd.). The petals were spread uniformly in the trays of vacuum oven, at pressure of 0.08k Pascal at 60 °C till the constant weight was obtained. The inner chamber of oven is electropolished, had two rack holders and equipped with vacuum system ranging from 0 to 0.1 K Pascal, temperature control and pressure and temperature gauge. All the valves and connections of vacuum oven were made of corrosion resistant steel. 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 carotenoid extracts were subjected to different storage temperatures such as ambient temperature, 4° and -20°C and different durations of 0 day, 20, 40 and 60 days.

The total carotenoids were extracted and estimated using method given by Ranganna (1995) with minor modifications. Sample preparation for Lutein and β carotene was done using a modification of procedure described by Barba *et al.* (2006). Analysis of lutein and β-carotene was carried out using HPLC.

The phenolic compounds in vacuum dried petals of marigold were extracted using a modification of the procedure described by Uzelac *et al*. (2005). Total phenolic content (TPC) was estimated according to procedure given by Singleton and Rossi (1965). The colorimetric method described by Abu Bakar *et al.* (2009) was used to determine total flavonoid content (TFC).

The sample was extracted using the procedure as in case of phenolic compounds. Total antioxidants were estimated using FRAP (Ferric Reducing Antioxidant Potential) method as described by Benzie and Strain (1996). The DPPH free radical scavenging activity assay method is based on the reduction of DPPH, a stable free radical. The antioxidant activity of the extracts was determined using DPPH assay described by Braca *et al.* (2001).

The data was statistically analyzed in completely randomized design (CRD) using Statistical analysis system (SAS) software. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% confidence ($P<0.05$).

RESULTS AND DISCUSSION

The results obtained from the storage of vacuum dried marigold flower petal extract exhibited the decreasing trend of total carotenoids, lutein content and β-carotene during storage. At -20°C temperature, extract had highest

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

Pusa Narangi Gainda Pusa Basanti Gainda Pusa Arpita

Fig. 1 Genotypes used for determination of total carotenoids, lutein, carotene, phenolic and flavonoid content and antioxidant activities of extract of vacuum dried petals of marigold (*Tagetes* sp.)

retention of all carotenoid pigments studied, followed by 4°C temperature. At ambient temperature, the rate of depletion of pigments was found to be faster, thus, retaining less carotenoid pigments during storage of dried petal extract.

Retention of total carotenoids

Total carotenoids decreased from 1108.76 on 0 day to 461.64 mg/100g DW after 60 days of storage of extract of vacuum dried petals of French marigold variety, Pusa Arpita at ambient temperature (Table 2). When petal extract stored at 4°C temperature, the total carotenoids decreased from 1108.76 on 0 day to 945.57 mg/100g DW after 60 days of storage. Total carotenoids decreased to 1059.74 mg/100g DW after 20 days, 1036.37 mg/100g DW after 40 days and 1002.33 mg/100g DW, after 60 days of storage at -20°C temperature. It is depicted from Fig 2 that after 60 days only 41.63% of total carotenoids had retained in petal extract stored at ambient temperature, whereas 85.28% total carotenoids retained in the extract which was stored at 4°C temperature. Highest retention during storage was observed at -20°C temperature, which had 90.40% of total carotenoids after 60 days of storage.The data in Table 2 shows that in African marigold variety, Pusa Narangi Gainda, total carotenoids decreased from 2765.76 on 0 day to 1558.58 mg/100g DW after 60 days of storage at ambient temperature. When marigold petal extract stored at 4°C temperature, the total carotenoids decreased from 2765.76 on 0 day to 1908.84 mg/100g DW after 60 days of storage. Total carotenoids decreased to 2261.99 mg/100g DW after 20 days, 2106.11 mg/100g DW after 40 days and 1925.26 mg/100g DW, after 60 days of storage at -20°C temperature. It is depicted from Fig 3 that after 60 days only

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

Variety	Storage temperature (°C)	Duration (days)	Total carotenoids (mg/100g)		Lutein (µg/g)		β-carotene (µg/g)	
			Mean	S D	Mean	S D	Mean	S D
Pusa Arpita	Ambient	0	1108.76	87.03	252.51	9.43	17.00	0.24
		20	759.17	144.68	173.35	33.04	11.46	1.04
		40	514.71	71.04	117.53	16.22	8.36	0.85
		60	461.64	42.76	105.41	9.76	7.14	0.94
	4	0	1108.76	87.03	252.51	9.43	17.00	1.35
		20	1030.07	180.14	235.20	41.13	15.55	0.90
		40	1009.43	100.37	230.49	22.92	15.82	1.03
		60	945.57	59.84	215.91	13.66	14.53	1.10
	-20°C	0	1108.76	87.03	252.51	9.43	17.00	1.35
		20	1059.74	146.81	241.98	33.53	16.52	1.01
		40	1036.37	35.06	239.32	19.87	15.74	2.03
		60	1002.33	16.76	228.87	3.83	15.49	1.22
Pusa Narangi Gainda	Ambient	0	2765.76	56.39	295.15	10.38	10.86	0.92
		20	1705.40	67.94	182.51	7.27	4.36	0.41
		40	1642.01	60.80	175.72	6.51	3.97	0.37
		60	1558.58	25.31	166.79	2.71	3.46	0.16
	4	0	2765.76	56.39	295.15	10.38	10.86	0.92
		20	2184.57	80.88	233.79	8.66	7.26	0.49
		40	2024.33	25.59	216.64	2.74	6.29	0.16
		60	1908.84	31.80	204.28	3.40	5.59	0.19
	-20	0	2765.76	56.39	295.15	10.38	10.86	0.92
		20	2261.99	134.57	242.08	14.40	7.74	0.82
		40	2106.11	21.93	225.39	2.35	6.79	0.13
		60	1925.26	16.97	206.03	1.82	5.69	0.11

Contd.

Table 2. (*Concluded*)

Variety	Storage temperature (°C)	Duration (days)	Total carotenoids (mg/100g)		Lutein (µg/g)		β-carotene (µg/g)	
			Mean	S D	Mean	S D	Mean	S D
Pusa Basanti Gainda	Ambient	0	144.90	8.06	79.21	4.40	4.65	0.98
		20	107.80	4.11	59.30	2.26	3.47	0.13
		40	99.85	8.16	54.93	4.49	3.22	0.26
		60	86.23	6.15	47.44	3.39	2.78	0.20
	4	0	144.90	8.06	79.21	4.40	4.65	0.98
		20	124.33	4.00	68.39	2.20	4.01	0.13
		40	118.60	6.90	65.24	3.80	3.82	0.23
		60	107.60	3.80	59.19	2.09	3.47	0.13
	-20	0	144.90	8.06	79.21	4.40	4.65	0.98
		20	130.67	3.61	71.88	1.98	4.21	0.12
		40	123.63	5.65	68.01	3.11	3.99	0.18
		60	115.97	3.71	63.79	2.04	3.74	0.12
CD ($P \leq 0.05$)	Variety (A)		31.88**		6.33**		0.38**	
	Temperature (B)		31.881**		6.33**		0.38**	
	Duration (C)		36.81**		7.30**		0.44**	
	Variety × Temp. (A×B)		55.22**		10.96**		0.65**	
	Variety × Duration (A×C)		63.76**		12.65**		0.75**	
	Temperature ×Duration (B×C)		63.76**		12.65**		0.75**	
	Variety × Temperature × Duration (A×B×C)		110.44**		21.91**		1.31**	

** Significant at 1% , *Significant at 5%, NS=Non Significant.

56.35% of total carotenoids had retained in extract stored at ambient temperature, whereas 69.02% total carotenoids retained in the extract which was stored at 4°C temperature. Highest retention during storage was observed at -20°C temperature, which had 69.61% of total carotenoids after 60 days of storage. In African marigold (*Tagetes erecta* L.) variety, Pusa Basanti Gainda, total carotenoids decreased from 144.90 on 0 day to 86.23 mg/100g DW after 60 days of storage at ambient temperature. When marigold petal extract stored at 4°C temperature, the total carotenoids decreased from 144.90 on 0 day to 107.60 mg/100g DW after 60 days of storage. Total carotenoids decreased to 130.67 mg/100g DW after 20 days, 123.63 mg/100g DW after 40 days and 115.97 mg/100g DW, after 60 days of storage at -20°C temperatures. It is depicted from Fig. 4 that after 60 days only 59.51% of total carotenoids had retained

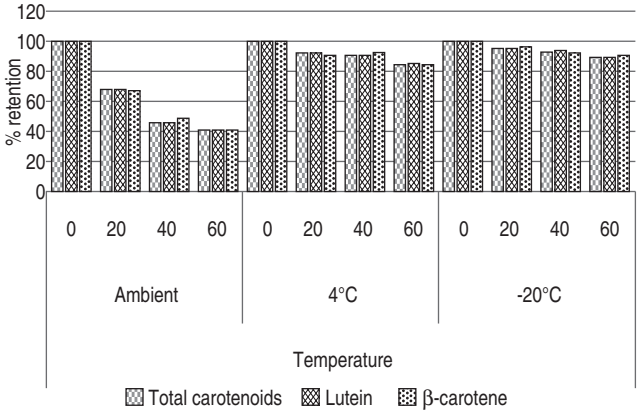

Fig 2 Carotenoids, lutein and β-carotene, retention in *Targetes Patula* var Pusa Arpita.

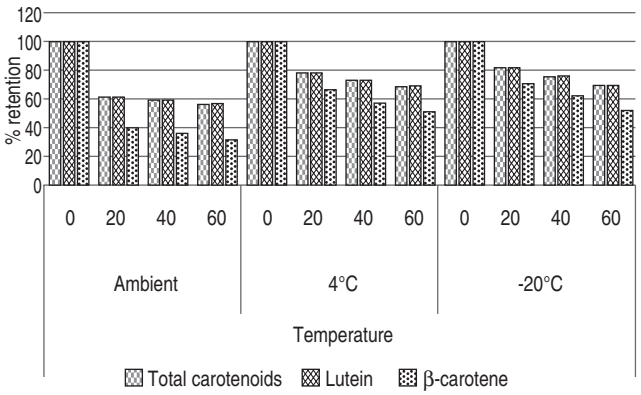

Fig 3 Carotenoids, lutein and β-carotene, retention in *Tagetes erecta* var. Pusa Narangi Gainda.

in extract stored at ambient temperature, whereas, 74.26% total carotenoids retained in the dried marigold petal extract which was stored at 4°C temperature. Highest retention during storage was observed at -20°C temperature, which had 80.03% of total carotenoids after 60 days of storage (Table 2). The loss of carotenoid pigments during storage is probably due to low stability of carotenoid compounds. Blessington *et al.* (2007) also reported the significant loss in carotenoid pigments. 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 (Dutta 2005). Similarly, Bechoff (2010) reported that there was a loss of carotenoids (68.2 %) in sweet potato chips during storage of 4 months. The results were also in confirmation with Blessington *et al*. (2007) who reported a decrease in carotenoids in potato during storage. The interaction between varieties × temperature, varieties × duration, temperature × duration varieties × temperature × duration for total carotenoids is significant at 1% level of significance.

Retention of lutein content

The data presented in the Table 2 shows that in French marigold variety, Pusa Arpita, lutein content decreased from 252.51 on 0 day to 105.41 µg/g DW after 60 days of storage at ambient temperature. When marigold petal extract stored at 4°C temperature, the lutein content decreased from 252.51 on 0 day to 215.91 µg/g DW after 60 days of storage. Lutein content decreased to 241.98 µg/g DW after 20 days, 239.32 µg/g DW after 40 days and 228.87 µg/g DW, after 60 days of storage at -20°C temperature. It is depicted from Fig. 2 that after 60 days only 41.74% of lutein content had retained in extract stored at ambient temperature, whereas 85.51% lutein content retained in the dried marigold petal extract which were stored at 4°C temperature. Highest retention during storage was observed at -20°C temperature, which had 90.64% of lutein content after 60 days of storage. The data in the Table 2 shows that in African marigold variety, Pusa Narangi Gainda, lutein content decreased from 295.15 on 0 day to 166.79 µg/g DW after 60 days of storage at ambient temperature. When marigold petals stored at 4°C temperature, the lutein content decreased from 295.15 on 0 day to 204.28 µg/g DW, after 60 days of storage. Lutein content decreased to 242.08 µg/g DW after 20 days, 225.39 µg/g DW 40 days and 206.03 µg/g DW, after 60 days of storage at -20°C temperature. It is depicted from Fig. 3 that after 60 days only 56.51% of lutein content had retained in marigold petal extract stored at ambient temperature, whereas 69.21% lutein content retained in the extract which was stored at 4°C temperature. Highest retention during storage was observed at -20°C temperature, which had 69.81% of lutein content after 60 days of storage. In African marigold variety, Pusa Basanti Gainda, lutein content decreased from 79.21 on 0 day to 47.44 µg/g DW after 60 days of storage of extract at ambient temperature. When marigold petal extract stored at 4°C temperature, the lutein content decreased from 79.21 on 0 day to 59.19 µg/g DW after 60 days of storage. Lutein content decreased to 71.88 µg/g DW after 20 days, 68.01 µg/g DW after 40 days and 63.79 µg/g DW, after 60 days of storage at -20°C temperature. It is depicted from Fig. 4 that after 60 days only 59.89% of lutein content had retained in marigold petal extract stored at ambient temperature, whereas 74.73% lutein content retained in the extract which was stored at 4°C temperature. Highest retention during storage was observed at -20°C temperature, which had 80.53% of lutein content after 60 days of storage.The interaction between varieties × temperature, varieties × duration, temperature × duration and varieties × temperature × duration for lutein content is significant at 1% level of significance.

Retention of β-carotene

β-carotene decreased from 17.00 on 0 day to 7.14 mg/100g DW after 60 days of storage of vacuum dried petal extract of French marigold variety, Pusa Arpita at ambient temperature (Table 2). When extract was stored at 4°C temperature, the β-carotene decreased from 16.33 on 0 day to 14.53 µg/g DW after 60 days of storage. β-carotene decreased to 16.52 µg/g DW after 20 days, 15.74/g DW after 40 days and 15.49 µg/g DW, after 60 days of storage at -20°C temperature. Highest retention during storage was observed at -20°C temperature, which had 91.12% of β-carotene after 60 days of storage. After 60 days, 85.47% β-carotene retained in the dried marigold petal extract which was stored at 4°C, whereas only 42.00% of β-carotene had retained in marigold petals stored at ambient temperature (Fig 2). In African marigold variety, Pusa Narangi Gainda, β-carotene was decreased from 10.86 on 0 day to 3.46 µg/g DW after 60 days of storage at ambient temperature. When marigold petal extract stored at 4°C temperature, the β-carotene decreased from 10.86 on 0 day to 5.59 µg/g DW after 60 days of storage. β-carotene decreased to 7.74 µg/g DW after 20 days, 6.79 µg/g DW 40 days and 5.69 µg/g DW, after 60 days of storage at -20°C temperature. Highest retention during storage was observed at -20°C temperature, which had 52.39% of β-carotene after 60 days of storage. It is depicted from Fig. 3 that after 60 days 51.47% β-carotene retained in the petal extract which was stored at 4°C, whereas only 31.86% of β-carotene had retained in marigold petal extract stored at ambient temperature. The data in the Table 2 shows that in African marigold variety, Pusa Basanti Gainda, β-carotene decreased from 4.65 on 0 day to 2.78 µg/g DW after 60 days of storage of extract at ambient temperature. When marigold petal extract stored at 4°C temperature, the β-carotene decreased from 4.65 on 0 day to 3.47 µg/g DW after 60 days of storage. β-carotene decreased to 4.21 µg/g DW after 20 days, 3.99µg/g DW after 40 days and 3.74 µg/g DW, after 60 days of storage at -20°C temperature. Highest retention during storage was observed at -20°C temperature, which had 80.43% of β-carotene after 60 days of storage of extract. It is depicted from Fig. 4 that after 60 days 74.62% β-carotene retained in the dried marigold petal extract which was stored at 4°C, whereas only 59.78% of β-carotene had retained in extract stored

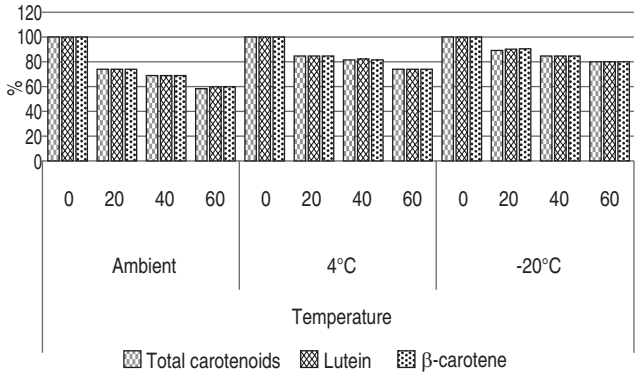

Fig 4 Carotenoids, lutein and β-carotene retention in *Tagetes erecta* var. Pusa Basanti Gainda.

at ambient temperature. Similar work was carried out by Subadra *et al.* (1997) who worked on dehydrated drumstick leaves (*Moringa oleifera*) and found that the retention of β carotene immediately after dehydration was 59% and at the end of one month of storage was 51% and at the end of 90 days of storage was 47%.The interaction between varieties × temperature, varieties × duration, temperature × duration and varieties × temperature × duration for β-carotene is significant at 1% level of significance.

Total phenolic and flavonoid content

The data in Table 3 indicates that the total phenolic and flavonoid content in carotenoid extract tends to decrease during storage. The rate of depletion of total phenolic and flavonoid content was observed much faster under ambient temperature conditions. The storage of extract at temperature of -20°C was the best treatment that reported highest retention of the total phenolic and flavonoid content during storage.

Total phenolic content

The total phenolic content was presented in the Table 3 and in marigold extract of the variety Pusa Arpita when stored at ambient temperature for duration of total 60 days, there was decrease in the total phenolic content from 84.24 on 0 day to 27.65 mg GAE/g DW after 60 days. The per cent retention in total phenolic content after 60 days of storage at ambient temperature was reported as 32.82% (Fig 5). However, at storage temperature of 4°C, the phenolic content was reduced to 36.74 mg GAE/g DW after 60 days of storage. The per cent retention in total phenolic content after 60 days of storage at 4°C temperature was reported as 43.61% (Fig 5). However the best results for maximum retention of total phenolic content was observed at -20°C temperature. At -20°C, the phenolic content was found to decrease to 43.45 mg GAE/g DW after 60 days of storage. The per cent retention in total phenolic content after 60 days of storage at -20°C temperature was reported as 51.58% (Fig. 5). In the extract of African marigold variety Pusa Narangi Gainda stored at ambient temperature for duration of total 60 days, there was decrease in the total phenolic content from 83.89 on 0 day to 23.63 mg GAE/g DW after 60 days of storage. The per cent retention in total phenolic content after 60 days of storage at ambient temperature was reported as 28.17% (Fig 6). However, at storage temperature of 4°C, the phenolic content was reduced to 38.65 mg GAE/g DW after 60 days of storage. The per cent retention in total phenolic content after 60 days of storage at 4°C temperature was reported as 46.07% (Fig. 6). However the best results for maximum retention of total phenolic content was observed at -20°C temperature. At -20°C, the phenolic content was found to decrease to 45.13 mg GAE/g DW after 60 days of storage. The per cent retention in total phenolic content after 60 days of storage at -20°C °C temperature was reported as 53.80% (Fig 6).The data depicted in Table 3 exhibited the decrease in the total phenolic content of the extract of African marigold variety Pusa Basanti Gainda stored at ambient temperature from 93.00 on 0 day to 41.34 mg GAE/g DW after 60 days of storage. The per cent retention in total phenolic content after 60 days of storage at ambient temperature was reported as 47.66% (Fig.7). However, at storage temperature of 4°C, the phenolic content was reduced to 60.25 mg GAE/g DW after 60 days of storage. The per cent retention in total phenolic

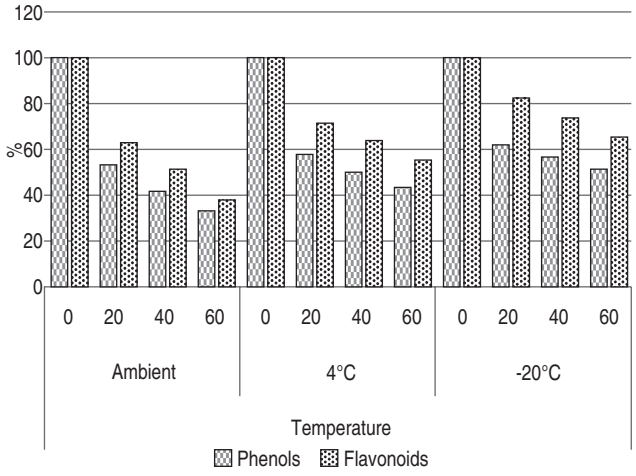

Fig 5 Retention of phenolic compounds in *Tagetes patula* var. Pusa Arpita

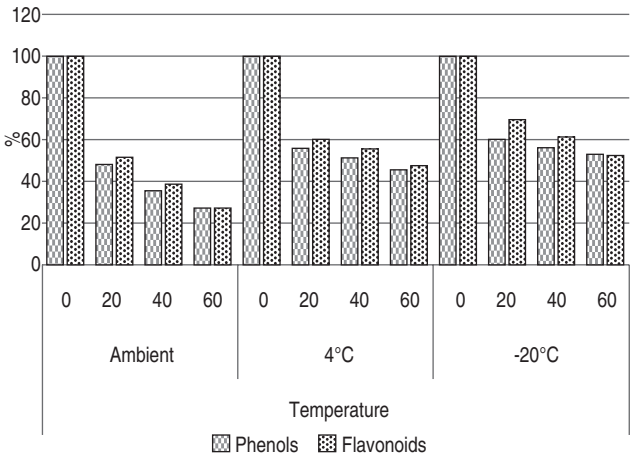

Fig 6 Retention of phenolic compounds in *Tagetes erecta*var. Pusa Narangi Gainda

content after 60 days of storage at 4°C temperature was reported as 62.95% (Fig. 7). However the best results for maximum retention of total phenolic content was observed at -20°C temperature where phenolic content was found to decrease to 66.38 mg GAE/g DW after 60 days of storage. The per cent retention in total phenolic content after 60 days of storage at -20°C temperature was reported as 68.10% (Fig. 7). Zheng and Wang (2001) observed that antioxidant activity of phenols is due to their redox properties which absorbs and neutralizes free radicals, quenching singlet and triplet oxygen and decomposing peroxidises. The interaction between varieties × duration, temperature × duration for

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

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	S D	Mean	S D	Mean	S D	Mean	S D
Pusa Arpita	Ambient	0	84.24	1.99	47.74	1.31	655.75	35.28	76.63	2.70
		20	44.36	1.69	30.06	2.33	563.08	72.11	47.90	2.84
		40	35.13	3.12	24.41	2.09	375.00	130.32	37.38	13.80
		60	27.65	0.68	18.34	1.96	314.70	118.64	28.69	3.39
	4	0	84.24	1.99	47.74	1.31	655.75	35.28	76.63	2.70
		20	49.15	3.37	34.30	1.05	521.41	95.26	54.02	1.66
		40	42.30	2.58	30.49	2.01	433.13	10.06	49.48	3.11
		60	36.74	3.54	26.39	2.05	372.74	15.45	46.17	4.83
	-20	0	84.24	1.99	47.74	1.31	655.75	35.28	76.63	2.70
		20	52.43	2.46	39.37	0.81	572.81	19.96	62.25	2.57
		40	47.80	1.87	35.33	1.99	455.84	23.77	55.40	1.97
		60	43.45	1.12	31.43	3.06	397.87	12.93	50.22	2.98
Pusa Narangi Gainda	Ambient	0	83.89	2.75	46.61	2.00	838.84	27.45	71.99	3.69
		20	40.44	3.16	24.32	0.99	593.77	10.94	51.63	0.69
		40	29.95	0.27	18.11	0.72	440.12	7.99	39.92	1.71
		60	23.63	0.85	13.12	1.08	281.06	8.67	27.27	0.88
	4	0	83.89	2.75	46.61	2.00	838.83	27.46	71.99	3.69
		20	47.46	0.92	28.05	1.76	688.95	16.53	63.84	0.36
		40	43.64	1.35	26.34	1.93	640.54	2.18	59.41	1.15
		60	38.65	0.49	22.48	3.15	605.44	13.19	54.55	1.13
	-20	0	83.89	2.75	46.61	2.00	838.83	27.46	71.99	3.69
		20	51.05	2.69	32.45	4.00	722.40	24.62	66.76	2.03
		40	47.61	1.16	28.48	1.74	682.32	21.68	64.07	0.69
		60	45.13	0.72	24.50	3.91	647.83	6.71	59.51	0.64
Pusa Basanti Gainda	Ambient	0	93.00	2.05	46.61	2.00	716.05	98.43	73.15	0.90
		20	60.85	10.11	26.66	2.94	506.06	51.21	49.20	1.00
		40	51.15	1.73	20.64	6.24	412.39	40.75	38.35	1.23
		60	41.34	4.30	12.61	3.58	290.42	28.55	27.31	1.08
	4	0	93.00	2.05	46.61	2.00	716.06	98.42	73.15	0.90
		20	75.68	2.85	32.14	4.02	588.82	29.67	61.44	0.82
		40	67.54	2.90	27.59	2.95	522.23	58.81	53.37	0.96
		60	60.25	3.05	24.51	1.78	453.21	27.25	46.49	1.19
	-20	0	93.00	2.05	46.61	2.00	716.06	98.43	73.15	0.90
		20	76.38	5.37	37.76	3.50	628.60	33.31	63.44	0.80
		40	72.51	3.26	33.57	4.98	562.71	40.42	56.28	1.02
		60	66.38	1.76	29.66	3.96	514.43	26.04	51.49	0.99

Contd.

Table 3. (*Concluded*)

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	S D	Mean	S D	Mean	S D	Mean	S D
CD (P≤0.05)	Variety (A)		1.39**		1.26**		24.43**		1.47**	
	Temperature (B)		1.39**		1.26**		24.43**		1.47**	
	Duration (C)		1.60**		1.46**		28.21**		1.70**	
	Variety × Temp. (A×B)		2.41**		2.19 NS		42.31**		2.54*	
	Variety × Duration (A×C)		2.79**		2.53**		48.85 NS		2.94**	
	Temperature ×Duration (B×C)		2.79**		2.53**		48.85**		2.94**	
	Variety× Temperature ×Duration (A×B×C)		4.82 NS		4.38 NS		84.62 NS		5.09 NS	

** Significant at 1%, *Significant at 5%, NS=Non Significant.

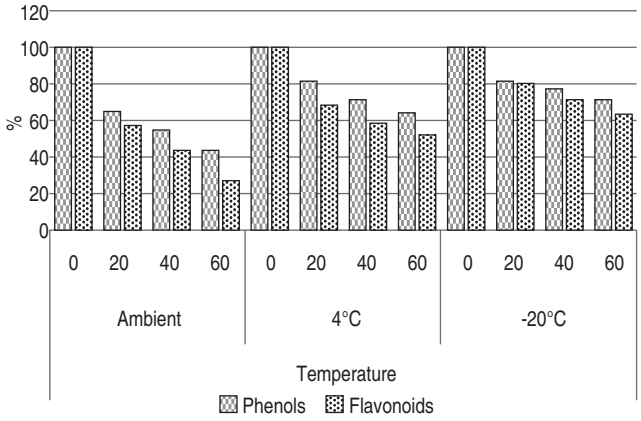

Fig 7 Retention of phenolic compounds in Tagetes *erecta* var. Pusa Basanti Gainda

total phenolic content at 1% level of significance was found to be significant however, interaction between varieties × temperature, varieties × temperature× duration for total phenolic content was found to be non significant**.**

Total flavonoid content

The total flavonoid content was presented in the Table 3. In the extract of dry petals of African marigold variety Pusa Arpita stored at ambient temperature for duration of 60 days, there was observed a decrease in the total flavonoid content from 47.74 on 0 day to 18.34 mg RE/g DW after 60 days of storage. The per cent retention in total flavonoid content after 60 days of storage at ambient temperature was reported as 38.42% (Fig 5). However, at storage temperature of 4°C, the flavonoid content was reduced to 26.39 mg RE/g DW after 60 days of storage. The per cent retention in total flavonoid content after 60 days of storage at 4°C temperature was reported as 55.28% (Fig 5). However the best results for maximum retention of total flavonoid content was observed at -20°C temperature where the flavonoid content was found to decrease to 31.43 mg RE/g DW after 60 days of storage. The per cent retention in total flavonoid content after 60 days of storage at -20°C temperature was reported as 65.84% (Fig 5).The total flavonoid content was decreased from 46.61on 0 day to 13.12 mg RE/g DW after 60 days of storage of dry petal extract of the variety Pusa Narangi Gainda at ambient temperature. The per cent retention in total flavonoid content after 60 days of storage at ambient temperature was reported as 28.15% (Fig 6). However, at storage temperature of 4°C, the flavonoid content was reduced to 22.48 mg RE/g DW after 60 days of storage. The per cent retention in total flavonoid content after 60 days of storage at 4°C temperature was reported as 48.23% (Fig 6). However, the best results for maximum retention of total flavonoid content was observed at -20°C temperature where flavonoid content was found to decrease to 24.50 mg RE/g DW after 60 days of storage. The per cent retention in total flavonoid content after 60 days of storage at -20°C temperature was reported as 52.56% (Fig 6).In marigold extract of the variety Pusa Basanti Gainda stored at ambient temperature, for duration of 60 days, there was decrease in the total flavonoid content from 46.61 on 0 day to 12.61 mg RE/g DW. The per cent retention in total flavonoid content after 60 days of storage at ambient temperature was reported as 27.05% (Fig 7). However, at storage temperature of 4°C, the flavonoid content was reduced to 24.51 mg RE/g DW after 60 days of storage. The per cent retention in total flavonoid content after 60 days of storage at 4°C temperature was reported as 52.59% (Fig 7). However the best results for maximum retention of total flavonoid content was observed at -20°C temperature. At -20°C, the flavonoid content was found to decrease to 29.66 mg RE/g DW after 60 days of storage. The per cent retention in total flavonoid content after 60 days of storage at -20°C temperature was reported as 63.63% (Fig 7).The interaction between temperature × duration for total flavonoid content at 1% level of significance was found to be significant however, interaction between varieties × temperature, varieties × duration, varieties × temperature× duration for total flavonoid content was found to be non significant**.**

Antioxidant activities

Ferric reducing antioxidant power (FRAP)

The FRAP value of the carotenoid extract was also presented in the Table 3. In petal extract of the French marigold variety Pusa Arpita stored at ambient temperature for duration of 60 days, there was decrease in FRAP value from 655.75 on 0 day to 314.70 µmol $FeSO_4$/g DW after 60 days of storage. The per cent retention in reducing power after 60 days of storage at ambient temperature was reported as 47.99% (Fig.8). However, at storage temperature of 4°C, the FRAP was reduced to 372.74 µmol $FeSO_4$/g DW after 60 days of storage. The per cent retention in reducing power after 60 days of storage at 4°C temperature was reported as 56.84% (Fig 8). However the best results for maximum retention of reducing power was observed at -20°C temperature. At -20°C, the FRAP value was found to decrease to 397.87 µmol $FeSO_4$/g DW after 60 days of storage. The per cent retention in reducing power after 60 days of storage at -20°C temperature was reported as 60.67% (Fig 8).The FRAP value of the carotenoid extract was presented in the Table 3. The data depicted that, marigold, for duration of 60 days, There was decrease in FRAP value from 838.83 on 0 day to 281.06 µmol $FeSO_4$/g DW after 60 days of storage of petal extract of the variety Pusa Narangi Gainda stored at ambient temperature. The per cent retention in reducing power after 60 days of storage at ambient temperature was reported as 33.51% (Fig 9). However, at storage temperature of 4°C, the FRAP was reduced to 605.44 µmol $FeSO_4$/g DW after 60 days of storage. The per cent retention in reducing power after 60 days of storage at 4°C temperature was reported as 72.18% (Fig 9). However the best results for maximum retention of reducing power was observed at -20°C temperature. At -20°C, the FRAP value was found to decrease to 647.83 µmol $FeSO_4$/g DW after 60 days of storage. The per cent retention in reducing power after 60 days of storage at -20°C temperature was reported as 77.23% (Fig 9). The FRAP value of the carotenoid extract was presented in the Table 3 and it was observed a decrease in FRAP value

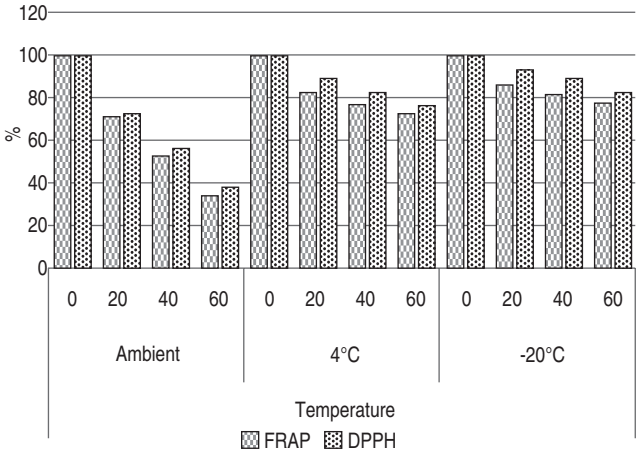

Fig 9 Retention of antioxidant activities in *Tagetes erecta* var. Pusa Narangi Gainda

from 716.05 on 0 day to 290.42 µmol $FeSO_4$/g DW after 60 days of storage marigold extract of the variety Pusa Basanti Gainda stored at ambient temperature. The per cent retention in reducing power after 60 days of storage at ambient temperature was reported as 40.56% (Fig 10). However, at storage temperature of 4°C, the FRAP was reduced to 453.21 µmol $FeSO_4$/g DW after 60 days of storage. The per cent retention in reducing power after 60 days of storage at 4°C temperature was reported as 63.29% (Fig 10). However the best results for maximum retention of reducing power was observed at -20°C temperature. At -20°C, the FRAP value was found to decrease to 514.43 µmol $FeSO_4$/g DW after 60 days of storage. The per cent retention in reducing power after 60 days of storage at -20°C temperature was reported as 71.84% (Fig 10). Klimczak *et al.* (2007). They 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.* (2000) 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%.The interaction between varieties × temperature

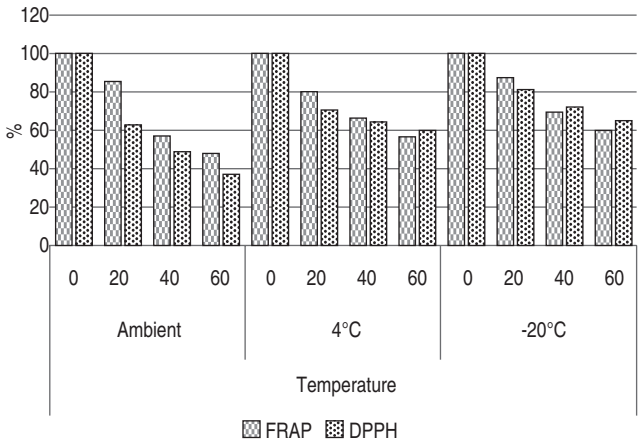

Fig 8 Retention of antioxidant activities in *Tagetes patula* var. Pusa Arpita

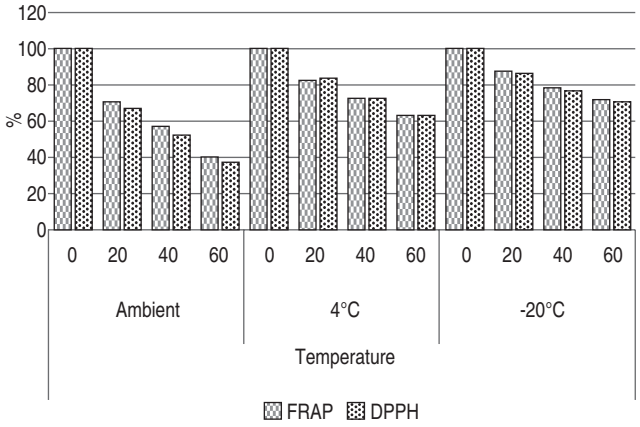

Fig 10 Retention of antioxidant activities Tagetes *erecta* var. Pusa Basanti Gainda

, temperature × duration for Ferric reducing antioxidant power at 1% level of significance was found to be significant however, interaction between varieties × duration, varieties × temperature× duration for Ferric reducing antioxidant power was found to be non significant**.**

DPPH radical scavenging activity

The DPPH radical-scavenging activity of the dry petal extract of the variety Pusa Arpita stored at ambient temperature was decreased from 76.63% on 0 day to 28.69% after 60 days of duration. The per cent retention in radical scavenging activity after 60 days of storage at ambient temperature was reported as 37.44% (Fig 8). However, at storage temperature of 4°C, the radical scavenging activity was reduced to 46.17% after 60 days of storage. The per cent retention in radical scavenging activity after 60 days of storage at 4°C temperature was reported as 60.25% (Fig 8). However the best results for maximum retention of radical scavenging activity was observed at -20°C temperature where radical scavenging activity was found to decrease to 50.22% after 60 days of storage. The per cent retention in radical scavenging activity after 60 days of storage at -20°C temperature was reported as 65.54% (Fig 8). The DPPH radical-scavenging activity of the dry petal extract of the variety Pusa Narangi Gainda stored at ambient temperature was decreased from 71.99% on 0 day to 27.27% after 60 days of duration. The per cent retention in radical scavenging activity after 60 days of storage at ambient temperature was reported as 37.88% (Fig 9). However, at storage temperature of 4°C, the radical scavenging activity was reduced to 54.55% after 60 days of storage. The per cent retention in radical scavenging activity after 60 days of storage at 4°C temperature was reported as 75.55% (Fig 9). However, the maximum retention of radical scavenging activity was observed at -20°C temperature. At -20°C, the radical scavenging activity was found to decrease to 59.91% after 60 days of storage. The per cent retention in radical scavenging activity after 60 days of storage at -20°C temperature was reported as 82.66% (Fig 9). The DPPH radical-scavenging activity of the dry petal extract of the variety Pusa Basanti Gainda stored at ambient temperature was decreased from 73.15% on 0 day to 27.31% after 60 days of duration. The per cent retention in radical scavenging activity after 60 days of storage at ambient temperature was reported as 37.33% (Fig 10). However, at storage temperature of 4°C, the radical scavenging activity was reduced to 46.49% after 60 days of storage. The per cent retention in radical scavenging activity after 60 days of storage at 4°C temperature was reported as 63.55% (Fig 10). However, maximum retention of radical scavenging activity was observed at -20°C temperature. At -20°C, the radical scavenging activity was found to decrease to 51.49% after 60 days of storage. The per cent retention in radical scavenging activity after 60 days of storage at -20°C temperature was reported as 70.39% (Fig 10). Thus it is proven that -20°C followed by 4°Ccan retain much higher antioxidants than storing at room temperature. Our results are in confirmation with the results of Cao *et al*. (2006). They 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.* (2008) reported that the antioxidant activity and total phenolic compounds in the homogenised guava decreased significantly during storage at −20°C for 2 weeks and continued to decrease during 3 months of storage. Similar results were obtained by Tavarini *et al.* (2008). They found a general decrease in antioxidant capacity, after two months of cool storage at 0°C in kiwifruit cultivar Hayward. The interaction between varieties × duration, temperature × duration for DPPH radical-scavenging activity at 1% level of significance was found to be significant however, interaction between varieties × temperature, varieties × temperature× duration for DPPH radical-scavenging activity was found to be non significant**.**

Among varieties, petal extract of Pusa Narangi Gainda of African marigold retained more carotenoids and antioxidant activities, whereas, petal extract of Pusa Arpita of French marigold retained more lutein and β- carotene content. The storage of vacuum dried petals at -20°C temperatures was found suitable for higher retention of bioactive compounds.

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