Indian Journal of Agricultural Sciences **88** (2): 228–33, February 2018/Article

Influence of drying techniques on retention of anthocyanin and their antioxidant activities in chrysanthemum (*Chrysanthemum* × *morifolium*) flowers

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Received: 20 January 2017; Accepted: 24 October 2017

ABSTRACT

The influence of different drying methods, viz. shade drying, hot air oven drying and microwave oven drying on total anthocyanin content, total phenolic content and their antioxidant activities (measured by CUPRAC, FRAP and DPPH assay) of dried ray florets of five anthocyanin rich chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.) varieties was studied in the present investigation. Microwave oven drying was the best method *w.r.t.* retaining its total anthocyanin content, total phenolic content and antioxidant activities. Microwave oven drying method had retained highest total anthocyanins (208.26 mg/100g), total phenolic content (36.17mg GAE/g DW) and antioxidant activities (CUPRAC= 280.74 µmol trolox/g dry weight, FRAP= 145.26 µmol trolox/g dry weight and DPPH= 31.44%) in variety Red Gold. In microwave oven drying method, total anthocyanin content of dried ray florets revealed significantly and positively correlation with total phenolic content (0.922) and CUPRAC (0.892), FRAP (0.976), DPPH (0.904) assay of antioxidant activities. Total phenolic content also exhibited positive and significant correlation with CUPRAC (0.995), FRAP (0.982), DPPH (0.998) assay of antioxidant activities. The antioxidant activity assays of microwave dried ray florets also exhibited positive and significant correlation among themselves.

Key words: Anthocyanin, Antioxidant activities, Correlation, Drying, Phenolic content

Chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.) is one of the most economically important flower crops cultivated all over the world. It belongs to family Asteraceae and native to Asia and north eastern Europe. Its flowers have been consumed as herbal medicines, beverages and vegetables (Anderson *et al.* 1998). Chrysanthemum flowers are famed for their beautiful colours including red, pink, orange, magenta, scarlet colours, etc. are contributed by anthocyanins particularly cyanidin. Anthocyanins are phenolic plant metabolites which are water-soluble pigments, versatile in nature and serve as key antioxidants. These pigments have a high free radical scavenging capacity and play an essential role in the prevention of cardiovascular disease, obesity, cancer, diabetes and other diseases (Prior and Wu 2006). Now-a-days, the private sector has been more active in development of bioactive compounds both in India and across the world. Moreover, synthetic pigments used as food colourants are dominating over the market which is more detrimental to human health. On the other hand, natural pigments are environment-friendly and safe for human beings. Chrysanthemum is considered as known source for anthocyanin extraction due to presence of numerous flowers in a single plant with wide range of flower colour (Gantait *et al.* 2010). Antioxidant activity of the plants depends upon the pigment composition and content of the phenolic and other bioactive compounds (Stalikas 2007).

Dehydration is an important process for extending the storage life of flowers so as to retain their physical and biochemical properties. It also ensures the inhibition of enzymatic degradation and microbial growth (Ahrne *et al.* 2007, Pereira *et al.* 2007). Drying not only affects the water content of the product but also alters other physical, biological and chemical properties, such as enzymatic activity, microbial spoilage, etc. Dehydration of flowers also leads to easy extraction of pigments. Shade drying is the most commonly used method for dehydrating flowers and is used in production of powder for use in food and feed industries. However, this method of drying has disadvantages like contamination especially in the rainy season, longer drying times and inability to handle the large quantities to achieve quality standards (Maskan 2000). Hot air (HA) oven drying is another commonly used commercial method for drying flowers, vegetables and fruits, but the long drying time cause degradation of product quality. Microwave drying

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is one of the new methods which is able to retain product quality in terms of shelf-life and pigment retention. The time between harvesting and consumption might be long and during this period biochemical changes could happen that affect the nutraceutical value in chrysanthemum. Moreover, there are very few published reports on the effect of efficient dehydration techniques on retention of total anthocyanins and their antioxidant activity of flowers. Therefore, different dehydration techniques are needed to be optimized for higher retention of anthocyanins and their antioxidant activities in chrysanthemum flowers.

MATERIALS AND METHODS

The plant material utilized for conducting the experiment consisted of five anthocyanin rich genotypes of chrysanthemum namely Red Gold, Red Spoon, Red Stone, Jaya and Lalpari. The main features and photograph of genotypes used in the present investigation 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 2014-16. The ray florets harvested at full bloom stage were subjected to different drying methods and then dried ray florets were used for the determination of anthocyanin content, phenolic content and antioxidant activities.

The three drying methods such as shade drying, hot air oven drying and microwave oven drying were used for dehydration of ray florets. In shade drying, samples were uniformly spread over blotting sheets and kept under room temperature (25 ± 2°C) until the flower petals were fully dried and had constant weight. In hot air oven drying, the ray florets were separated, cleaned and weighed thoroughly with water. Then, these were transferred to the trays of hot air oven and spread uniformly in a single layer. The hot air oven used for drying had the capacity of three trays and equipped with an exhaust outlet. The temperature was maintained at 60°C and air velocity of 0.12-.16 m/sec. The position of trays was interchanged regularly and ray florets were dried till constant weight was attained. In microwave oven drying, the chrysanthemum ray florets were spread uniformly in the microwave oven for duration of 90 seconds.

Table 1 Salient features of chrysanthemum genotypes used in drying

Genotype	Flower type	Flower size	Flower colour	Flowering time	Source
Red Gold	Double	Medium	Dark red	Mid Nov-Mid Jan	ICAR-IARI
Lalpari	Semi double	Medium	Dark red	Mid Nov-Mid Jan	ICAR-IARI
Red Stone	Semi double	Medium	Red	Mid Nov-Mid Jan	ICAR-IARI
Red Spoon	Semi double	Medium	Red	Mid Nov-Mid Jan	ICAR-IARI
Jaya	Semi double	Medium	Dark Red	Mid Nov-Mid Jan	ICAR-IARI

The total anthocyanins were extracted and estimated using method given by Wrolstad (2005). The phenolic compounds in dried petals of chrysanthemum 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 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), DPPH assay described by Braca *et al.* (2001) and CUPRAC method standardized by Apak *et al.* (2004).

The data were 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

Retention of total anthocyanin content in dried ray florets

Data presented in the Table 2 revealed that the ray florets dried in microwave oven had significantly higher retention of total anthocyanin content than hot air oven and shade drying. Microwave oven drying method had retained highest total anthocyanins in variety Red Gold (208.26 mg/100g) followed by varieties namely Lal Pari (107.47 mg/100g), Red Stone (91.68 mg/100g), Red Spoon (81.97 mg/100g) and Jaya (65.38 mg/100g) on dry basis. Followed by microwave drying, shade drying exhibited best results in retaining high anthocyanin. The ray florets of

Fig 1 Chrysanthemum genotypes used for drying and estimation of total anthocyanin content, total phenolic content and antioxidant activities.

Table 2 Estimation of total anthocyanins, total phenolic content and their antioxidant activities in dried ray florets of promising genotypes of chrysanthemum

Drying technique	Variety	Total anthocyanin content (mg/100g)	Total phenolic content (mg GAE/g)	Antioxidant activities		
				CUPRAC (µmol trolox/g)	FRAP (µmol trolox/g)	DPPH (%)
Shade drying	Red Gold	200.03	24.47	271.21	140.45	27.33
	Lalpari	101.77	23.34	250.34	123.81	25.16
	Red Stone	87.12	14.36	228.74	116.97	21.05
	Red Spoon	75.38	11.20	219.16	116.83	19.91
	Jaya	60.97	6.42	200.35	112.99	17.25
Microwave oven drying	Red Gold	208.26	36.17	280.74	145.26	31.44
	Lalpari	107.47	28.30	259.65	130.61	28.36
	Red Stone	91.68	21.16	237.87	125.23	25.13
	Red Spoon	81.97	18.31	221.54	121.83	23.49
	Jaya	65.38	13.11	207.74	116.91	21.33
Hot air oven drying	Red Gold	195.37	20.36	260.37	135.09	21.65
	Lalpari	96.126	16.26	240.19	117.42	20.04
	Red Stone	83.26	10.68	217.03	115.86	16.49
	Red Spoon	71.22	8.37	207.27	111.06	14.29
	Jaya	56.17	4.25	194.22	107.81	13.42
LSD (P=0.05)	Drying method (A)	0.44	3.33	0.02	0.70	0.02
	Variety (B)	0.57	4.30	0.02	0.915	0.03
	A × B	1.45	7.44	0.08	1.58	0.05

chrysanthemum dried by shade drying method had higher anthocyanin content than the hot air oven drying method, whereas, less than microwave drying technique. Ray florets of chrysanthemum variety Red Gold dried under shade exhibited total anthocyanin content of 200.03 mg/100g followed by varieties namely Lalpari (101.77 mg/100g), Red Stone (87.12), Red Spoon (75.38 mg/100g) and Jaya (60.97 mg/100g) on dry weight basis. However, the petals dried in hot air oven showed less anthocyanin content as compared to other drying methods. The hot air oven dried petals had highest anthocyanin content of 195.37 mg/100g in variety Red Gold variety followed by 96.126 mg/100g in var. Lalpari, 83.26 mg/100g in var. Red Stone, 71.22 mg/100g in var. Red Spoon and 56.17 mg/100g in var. Jaya. The interaction between drying methods and varieties for total anthocyanin content is significant at 5% level of significance. Our results were also in association with the study of Rabeta *et al.* (2013) as they determined the effect of different drying methods on the total anthocyanin content and found that microwave oven drying is the best method for retaining high total anthocyanin content. Our study was in a line with the investigation of Mitra *et al.* (2013) in Saskatoon berries where the highest retention of anthocyanin was exhibited in microwave-vacuum dried berries, thin layer hot air dried berries and vacuum dried berries.

Retention of total phenolic content in dried ray florets

The highest total phenolic content was retained in microwave oven dried ray florets of variety Red Gold (36.17 mg GAE/g DW) followed by shade drying of petals of variety Red Gold (24.47 mg GAE/g DW) and hot air oven dried petals of variety Red Gold (20.36 mg GAE/g DW). In microwave oven drying, ray florets of variety Red Gold shown highest phenolic content followed by varieties namely Lalpari (28.30 mg GAE/g DW), Red Stone (21.16 mg GAE/g DW), Red Spoon (18.31 mg GAE/g DW) and Jaya (13.11 mg GAE/g DW). Shade drying retained a content of 24.47 mg GAE/g DW) in ray florets of variety Red Gold, 23.34 mg GAE/g DW in var. Lalpari, 14.36 mg GAE/g DW in var. Red Stone, 11.20 mg GAE/g DW in var. Red Spoon and 6.42 mg GAE/g DW in var. Jaya. However, lowest total phenolic content was retained in hot air oven dried petals. The total phenolic content in Red Gold is reduced to 20.36 mg GAE/g DW. Similar findings were obtained by Ramamoorthy and Bono (2007) in extracts of *Morinda citrifolia* fruit as they reported highest total phenolic content in vacuum dried samples than samples dried by other drying methods. Khattak (2014) reported phenolic content in sun dried sample of marigold flowers as compard to other drying methods. Hajimehdipoor *et al.* (2012) reported the highest total phenolic content in microwave-dried *Anethum graveolense.*

Recovery of antioxidant activities in dried ray florets

CUPRAC (Cupric Reducing Antioxidant Capacity) assay: The CUPRAC values among genotypes of chrysanthemum were highest in microwave oven dried petals compared to shade drying and hot air oven drying (Table

2). The highest reducing power was observed in microwave oven dried petals of Red Gold (280.74 µmol trolox/g fresh weight) followed by CUPRAC value of 271.21 µmol trolox/g of petals dried in shade and 260.37 µmol trolox/g of petals dried in hot air oven. Petals dried by hot air oven exhibited lowest reducing power. In microwave drying method, ray florets of variety Red Gold shown highest phenolic content followed by varieties namely Lalpari (259.65 µmol trolox/g), Red Stone (237.87 µmol trolox/g), Red Spoon (221.54 µmol trolox/g) and Jaya (207.74 µmol trolox/g).

FRAP (Ferric Reducing Antioxidant Potential) assay: The FRAP values of the dried chrysanthemum flower of promising five genotypes are shown in Table 2. The highest FRAP values was observed in dried ray florets of variety Red Gold in microwave oven drying (145.26 µmol trolox/g dry weight) followed by shade drying (140.45 µmol trolox/g) and hot air oven drying (135.09 µmol trolox/g dry weight). In microwave drying method, ray florets of variety Red Gold shown highest FRAP values followed by varieties namely Lalpari (130.61 µmol trolox/g), Red Stone (125.23 µmol trolox/g), Red Spoon (121.83 µmol trolox/g) and Jaya (116.91 µmol trolox/g).

DPPH free radical scavenging activity: DPPH scavenging activity of dried ray florets of five promising chrysanthemum genotypes is presented in Table 2. For the DPPH assay, free radical scavenging activity was expressed as % inhibition. Microwave oven dried petals showed remarkably high DPPH activity as compared to shade drying and hot air oven drying. DPPH values of microwave oven dried petals ranged from 31.44% (Red Gold) to 23.49% (Jaya) followed by shade drying and hot air oven drying. DPPH values of shade dried petals ranged from 27.33 % (Red Gold) to 17.25% (Jaya) and hot air oven dried petals the DPPH values ranged from 21.65% (Red Gold) to 13.42% (Jaya).

In our studies, highest retention of antioxidant activities was reported in microwave oven drying as compared to the shade drying and hot air oven drying. Similar findings were reported by Duy *et al.* (2012) and observed significant losses in the antioxidant capacity as measured by DPPH scavenging activity of oven dried samples of different vegetables. Similarly, Annamalai *et al.* (2011) observed higher DPPH radical scavenging activity in microwave dried samples of *Cardiospermum halicacabum* than samples obtained by sun drying. The lowest antioxidant activities was observed in sun dried sample of marigold flowers by Khattak *et al.* (2014). Hajimehdipoor *et al.* (2012) reported highest antioxidant activity in microwave-dried *Anethum graveolense*.

Correlation between anthocyanin content and antioxidant activities of ray florets dried under different drying methods

In the present study, the data presented in Tables 3, 4 and 5 showed a strong correlation between total anthocyanin content, total phenolic content and antioxidant activities in ray florets of chrysanthemum dried in microwave oven, hot air oven and under shade. However, strong and significantly positive correlation was observed in microwave drying

Table 3 Linear correlation coefficient (r) between total anthocyanin content, total phenolic content and antioxidant activities (CUPRAC, FRAP and DPPH) in ray florets of chrysanthemum dried under shade

	Total anthocyanin content	Total phenolic content	CUPRAC	FRAP	DPPH
Total anthocyanin content	1.000	0.635**	0.903**	0.980**	0.661**
Total phenolic content		1.000	0.755**	0.725**	0.994**
CUPRAC			1.000	0.925**	0.778**
FRAP				1.000	0.751**
DPPH					1.000

**Correlation is significant at the 0.01 level (2-tailed).

Table 4 Linear correlation coefficient (r) between total anthocyanin content, total phenolic content and antioxidant activities (CUPRAC, FRAP and DPPH) in ray florets of chrysanthemum dried using microwave oven drying technique.

	Total anthocyanin content	Total phenolic content	CUPRAC	FRAP	DPPH
Total anthocyanin content	1.000	0.922**	0.892**	0.976**	0.904**
Total phenolic content		1.000	0.995**	0.982**	0.998**
CUPRAC			1.000	0.968**	0.998**
FRAP				1.000	0.974**
DPPH					1.000

Table 5 Linear correlation coefficient (r) between total anthocyanin content, total phenolic content and antioxidant activities (CUPRAC, FRAP and DPPH) in ray florets of chrysanthemum dried using hot air oven drying technique

	Total anthocyanin content	Total phenolic content	CUPRAC	FRAP	DPPH
Total anthocyanin content	1.000	0.856**	0.975**	0.987**	0.910**
Total phenolic content		1.000	0.936**	0.894**	0.992**
CUPRAC			1.000	0.994**	0.965**
FRAP				1.000	0.934**
DPPH					1.000

method. It is also clear from scatter plot diagrams (Fig 2) that as anthocyanin content increases, phenolic content and antioxidant activity also increases in all drying methods. Moreover, the increase in total phenolic content is also positively correlated with increase in antioxidant activities in all drying methods.

In shade drying methods, total anthocyanin content of dried ray florets of chrysanthemum showed significantly and positively correlation with total phenolic content (0.635) and CUPRAC (0.903), FRAP (0.980), DPPH (0.661) assay of antioxidant activities. Total phenolic content also exhibited positive and significant correlation with CUPRAC (0.755), FRAP (0.725), DPPH (0.994) assay of antioxidant activities. The different assay of antioxidant activities also exhibited positive and significant correlation among themselves (Table 3). Scatter plot diagram (Fig 2) also showed significant relationship ($r^2 = 0.876$) between total anthocyanin content and total phenolic content, total anthocyanin content with CUPRAC ($r^2 = 0.816$), FRAP ($r^2 = 0.979$), DPPH ($r^2 = 0.759$) assay, total phenolic content with CUPRAC ($r^2 = 0.827$), FRAP ($r^2 = 0.514$), DPPH ($r^2 = 0.878$) assay of antioxidant activities.

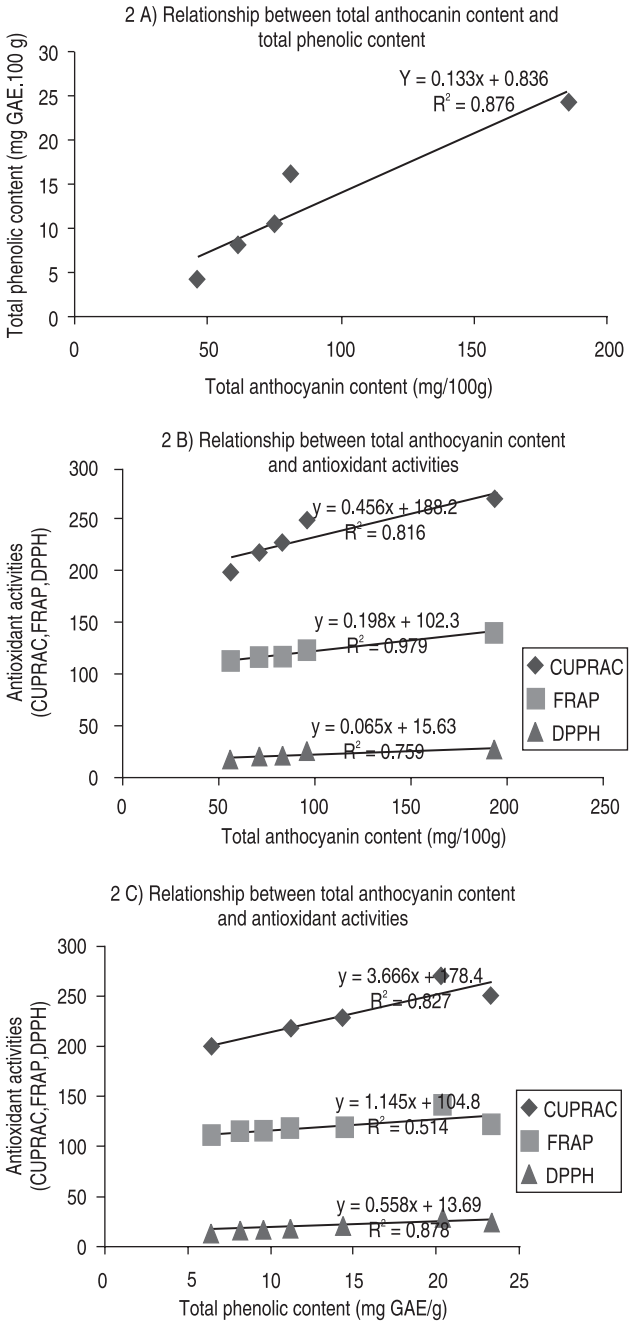

Fig 2 Scatter plot diagram of total anthocyanin content, total phenolic content and antioxidant activities in ray florets of chrysanthemum dried under shade. 2A) Total anthocyanin content *v/s* total phenolic content 2B) Total anthocyanin content *v/s* antioxidant activities 2C) Total phenolic content *v/s* antioxidant activities.

In microwave oven drying method, total anthocyanin content of dried ray florets revealed significantly and positively correlation with total phenolic content (0.922) and CUPRAC (0.892), FRAP (0.976), DPPH (0.904) assay of antioxidant activities. Total phenolic content also exhibited positive and significant correlation with CUPRAC (0.995), FRAP (0.982), DPPH (0.998) assay of antioxidant activities. The antioxidant activity assays of microwave dried ray florets also exhibited positive and significant correlation among themselves (Table 4). Scatter plot diagram also showed significant relationship ($r^2 = 0.849$) between total anthocyanin content and total phenolic content, total anthocyanin content with CUPRAC ($r^2 = 0.796$), FRAP ($r^2 = 0.953$), DPPH ($r^2 = 0.818$) assay, total phenolic content with CUPRAC ($r^2 = 0.989$), FRAP ($r^2 = 0.966$), DPPH ($r^2 = 0.997$) assay of antioxidant activities.

In hot air oven drying method, total anthocyanin content of dried ray florets also exhibited significant and positive correlation with total phenolic content (0.856) and CUPRAC (0.975), FRAP (0.987), DPPH (0.910) assay of antioxidant activities. Total phenolic content also showed positive and significant correlation with CUPRAC (0.936), FRAP (0.894), DPPH (0.992) assay of antioxidant activities. The antioxidant activity assays of hot air oven dried ray florets also exhibited positive and significant correlation among themselves (Table 5). Significant relationship ($r^2 = 0.875$) between total anthocyanin content and total phenolic content, total anthocyanin content with CUPRAC ($r^2 = 0.799$), FRAP ($r^2 = 0.984$), DPPH ($r^2 = 0.698$) assay, total phenolic content with CUPRAC ($r^2 = 0.988$), FRAP ($r^2 = 0.931$), DPPH ($r^2 = 0.937$) assay of antioxidant activities was also showed in scatter plot diagrams (Fig. 4). Our study was in a line with the investigation of Adina *et al.* (2013) in *Adenium obesum,* Mitra *et al.* (2013) in Saskatoon berries and Akshaya (2015) in marigold. Their results also showed that the antioxidant activities are highly correlated with the total anthocyanin content. Chrysanthemum flowers with deep colour and rich in anthocyanin are the best choices as sources of antioxidants and retention of antioxidant activity during drying process.

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