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Studies on drying methods for evaluation of biochemical properties in African marigold (*Tagetes erecta* L.)

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Abstract
The influence of different drying methods on biochemical properties of African marigold (*Tagetes erecta* L.) cultivar "Seracole", was assessed in this experiment. The petals of marigold flowers were dried by five different methods viz. sun drying, shade drying, oven drying, cabinet drying and infrared drying. The biochemical constituents were analyzed on total carotenoids (mg/100g sample), ferric reducing antioxidant power (FRAP) (µ mol trolox/g), vitamin C (mg/100g sample) and dry matter (%). Infrared dried petals exhibit the highest yield of total carotenoids (T_{10}; 333.33 mg/100g sample) and ascorbic acid (T_9; 75.89 mg/100g sample) and the lowest value found in sun drying (T_0; 246.23 mg/100g sample & T_0; 61.89 mg/100g sample respectively). The highest content of FRAP assay and dry matter content were found in cabinet drying (T_8; 532.70 µ mol trolox/g & T_6; 17.44% respectively).

Keywords: *Tagetes erecta*, total carotenoids, frap, vitamin C, dry matter

Introduction
African marigold (*Tagetes erecta* L.), one of the most important flowers, belongs to the family Asteraceae. It grows widely in warm, temperate and Mediterranean region. In India, flowers are of a great demand in domestic market as loose flower or garland making. Moreover, marigold is especially used for beautification in landscape plans due to its variable height and colour of flowers. Marigold leaves and flowers are equally important from medicinal point of view. A number of marigold species are reported to possess therapeutic usage in various ailments, such as skin complaints, wounds and burns, conjunctivitis and poor eyesight, menstrual irregularities, varicose veins, hemorrhoids, duodenal ulcers, etc. (Wichtl and Bisset, 1994; Ćetković *et al.*, 2004) [22, 7]. Over the past few years there has been an increasing interest in natural antioxidants and their role in human health and nutrition. The fact that the oxidation process plays host to several degenerative diseases and can contribute significantly to the risk of human aging and cancer has focused the interest in this subject (Jacob *et al.*, 1994) [11]. Besides fulfilling the basic physiological function, they are the best sources of dietary antioxidants which have health promoting effects. The flowers contain a desirable amount of carotenoids and essential oils and are used for antioxidant, antibacterial and antifungal properties, as a spice and to colour human foods (Li *et al*., 2007; Breithaupt *et al.*, 2002; Dutta *et al*., 2007) [13, 6, 8]. Carotenoid specially Lutein has beneficial effects on eye health (Roberts *et al*., 2009) [17], cardiac health (Alves-Rodrigues and Shao, 2004) [1], skin health (Astner *et al*., 2007; Roberts *et al.*, 2009) [3, 17] and other disease conditions like cancer (Slattery *et al*., 2000) [21]. Vitamin C is required for the production of collagen in the connective tissue, teeth and bones. FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method. Dietary antioxidants are substances in foods that significantly decrease the adverse effect of these free radicals. Thus high intake of fruits and vegetables are known to boost endogenous antioxidant defences in the body and mitigate oxidative damages. Considering all these points, present investigation was carried out to study the performance of different drying on nutraceutical properties in African marigold (*Tagetes erecta* L.) cultivar 'Seracole' under West Bengal conditions. This study is directed towards determining the best drying methods for commercial applications, with respect to carotene yield and antioxidant activity.

Materials and Methods

The experiment was conducted at Dept. of Floriculture & Landscape Architecture, BCKV, Mohanpur, Nadia, West Bengal, India. African marigold (*Tagetes erecta* L.) flower specially "Seracole" variety was collected from Mondouri farm, BCKV and the laboratory analysis were carried out at the laboratory of Dept. of Floriculture & Landscape Architecture, BCKV.

Table 1: Treatments of sun drying, shade drying, oven drying, cabinet drying and infrared drying

Treatments	Types of drying	Conditions of drying
T_0	Sun drying	February (30 ˚C + 70% RH + 10 days)
T_1	Shade drying	February (22 ˚C + 65% RH + 15 days)
T_2	Shade drying	December (19 ˚C + 65% RH + 15 days)
T_3	Oven drying	60 ˚C + 4 hours + 30% RH
T_4	Oven drying	60 ˚C + 6 hours + 30% RH
T_5	Oven drying	60 ˚C + 8 hours + 30% RH
T_6	Cabinet drying	55 ˚C + 2 hours + 70% RH
T_7	Cabinet drying	55 ˚C + 4 hours + 70% RH
T_8	Cabinet drying	55 ˚C + 6hours + 70% RH
T_9	Infrared drying	50 ˚C + 1 hour + 70% RH
T_{10}	Infrared drying	50 ˚C + 2 hours + 70% RH
T_{11}	Infrared drying	50 ˚C + 3 hours + 70% RH

Total Carotenoids was estimated by extracting pigment from 5 g sample by using pestle mortar and homogenizer (POLYTRON® PT 1600 E) where 30 ml acetone was taken as solvent. Extraction was repeated until the entire pigments were extracted and the residue became colourless. Then it was filtered by Whatman no. 1 filter papers. The extract was then purified using separating funnel (previously leached with petroleum ether) where the total extract was poured along with 20 ml petroleum ether and a few drops of water containing 5 percent anhydrous sodium sulphate. Whenever the two layers were distinguished properly, the lower layer was drained out and upper extract was collected into a beaker fitted with Whatman filter paper on it along with cotton plug containing 5% sodium sulphate. Extraction of acetone phase was repeated with small volume of petroleum ether until no more colours extracted. The extract then diluted 10 times adding petroleum ether and the absorbance measured in UV-VIS spectrophotometer (VARIAN CARY®, USA) at 452 nm. Petroleum ether was taken as blank and the value calculated using following formula (Rangana, 1986) [16].

$$\text{Total Carotenoids (mg/100g sample)} = \frac{3.857 \times \text{OD at 452 nm} \times \text{volume made up} \times \text{dilution} \times 100}{1000 \times \text{weight of sample}}$$

The estimation of Ferric reducing antioxidant power (FRAP) assay was performed according to Benzie and Strain (1996) [5] with slight modification. In this procedure, Frap reagent was prepared by mixing acetate buffer (pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) and ferric chloride at the ratio of 10:1:1 and allowed to keep it in a dark coloured bottle. Then ethanol extract was prepared by homogenizing 5 g sample in 30 ml ethanol with pestle mortar and homogenizer (POLYTRON® PT 1600 E). Then it was centrifuged (SIGMA 3K30, UK) at 10,000 rpm for 15 min. at 4^0C and the supernatant was stored at -20 ˚C. 100 µl sample was then mixed with 2.9 ml FRAP reagent and kept in dark for 30 min. Absorbance was recorded in UV-VIS spectrophotometer (VARIAN CARY®, USA) at 593 nm and the value was calculated using following formula.

$$\text{µ mol trolox/g} = \frac{\text{OD at 593 nm} \times \text{volume made up} \times \text{dilution} \times 100}{1.2 \times \text{weight of sample}}$$

Estimation of dry matter and Vitamin C (%) were done by AOAC International Methods.

The experimental data collected from five different types of drying methods were subjected to the statistical analysis appropriate to completely randomized designs (CRD). The critical difference between the entries was at 5% level of significance.

Results and Discussion

A significant and wide variation was recorded for biochemicals in dried marigold petal extract of twelve treatments (Table 1). The distribution of carotenoids in plants appears to be shaped by the changes in physiological, biochemical and genetic factors (Goldman *et al.*, 1999) [10]. Carotenoids content of the dried sample (*Tagetes erecta* L.) increased significantly with decrease in moisture content as compared to fresh sample irrespective of drying method and variety. The results for total carotenoids content in different extracts were presented in Table 2. From the obtained results for marigold dried flowers, T_{10} (333.33 mg/100g sample) recorded the highest total carotenoids which was followed by T_8 (331.33 mg/100g sample) and T_0 recorded the lowest (246.23 mg/100g sample). The results showed near about similar with the findings of Singh *et al.*, (2008) [20] where carotenoids content recorded the highest (355.04 mg/100 g) from cabinet dried petals of marigold cv. Pusa Narangi Gainda.

FRAP is considered to be rapid and a semi-quantitative assay. In FRAP assay, antioxidant capacity is evaluated based on the ability of the sample extracts to reduce ferric tripyridyltriazine complexes to ferrous tripyridyltriazine. The biochemical estimations resulted the maximum FRAP concentration in T_8 (532.70 µ mol trolox/g) which was at par T_7 (529.40 µ mol trolox/g) and followed by T_6 (524 µ mol trolox/g). The value was recorded the lowest in T_0 (447.83 µ mol trolox/g) followed by T_2 (453 µ mol trolox/g). This finding is almost similar to Padalia *et al.*, (2014)[14] who reported that the FRAP values of *Tagetes erecta* L. in FIR-HA drying had the greatest reducing power (972.7 µmol $FeSO_4$/g DW), followed by fresh (821.0 µmol $FeSO_4$/g DW), FD (811.0 µmol $FeSO_4$/g) and then HA (730.7 µmol $FeSO_4$/g DW) while Kaisoon *et al.*, (2011) [12] reported that *T. erecta* L. had the ferric reducing antioxidant power (FRAP) value 60 mmol $FeSO_4$/g 100 DW.

The results of estimation of vitamin C through various drying methods are presented in Table 2. Obtained results showed that T_9 was the richest source of ascorbic acid content (75.89 mg/100g sample) which was at par T_{10} (73.95 mg/100g sample) and followed by T_6 (70.48 mg/100g sample) while T_0 (61.89 mg/100g sample) showed the lowest amount of

ascorbic acid followed by T_2 (67.26 mg/100g sample). Singh *et al.,* (2013) [18] reported that the ascorbic acid contents in flower of *Hibiscus sabdariffa* L. was estimated 164.32 mg/100 mg while Barros *et al.,* (2010) [4] reported that in *Malva sylvestris* L., the highest amount of ascorbic acid was found in flowers (1.11 mg/g of extract), while leaves presented the lowest levels (0.17 mg/g).

The distributed estimation revealed that the maximum dry matter content was recorded in T_6 (17.44%) followed by T_3 (17.20%) and the lowest dry matter content was exhibited by the T_1 (11.46%) followed by T_2 (12.10%). Singh (2014) [19] conducted a study on the drying behaviour of French marigold flowers (variety Jafri) while it was drying in mechanical drier. The drying curves for moisture content of drying sample (db) vs drying time (min) and drying rate (dM/dt) vs drying time (min) at different air velocities were drawn. The experimental flowers had initial moisture content of 84 ± 1% (wb) and the samples were dried up to 19 to 20 per cent moisture content (wb). There was a non-linear decrease of moisture with drying time for all the air velocities as well as temperatures. The effect of temperature on the drying time was more significant as compared to drying air velocities. The drying time required for reaching a moisture content of 19 to 20 per cent at 45 °C was more than three times the time required at 55 °C.

The results revealed that the infrared drying and cabinet drying gave the best results from the preservation of quality with respect to chemical and functional constituents' point of view.

Table 2: Total Carotenoids, FRAP, Vitamin C and Dry matter content in different types of drying methods

Treatments	Total Carotenoids (mg/100g sample)	FRAP (µ moltrolox/g)	Vitamin C (mg/100g sample)	Dry matter (%)
T_0	246.23	447.83	61.89	16.9
T_1	257.56	453.83	68.20	11.5
T_2	250.93	453.00	67.26	12.1
T_3	317.26	503.33	68.82	17.2
T_4	321.63	503.67	68.50	16.8
T_5	325.66	515.50	67.59	16.5
T_6	324.26	524.00	70.48	17.4
T_7	329.16	529.40	70.09	16.0
T_8	331.33	532.70	67.62	15.5
T_9	325.6	519.83	75.89	14.6
T_{10}	333.33	519.67	73.95	14.1
T_{11}	328.1	522.00	69.16	13.8
SEm (±)	1.011	1.381	1.161	0.012
CD (5%)	2.975	4.062	3.416	0.036

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