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DEVELOPMENT OF HIGH FIBRE AND ANTIOXIDANT-RICH FRUIT BARS FROM BLACK CARROT POMACE

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ABSTRACT

Extraction of black carrot juice results in generation of pomace as waste which contains good amount of phytochemicals. The purpose of this study was to utilize this waste for the formulation of bar. Bars were prepared by varying sugar (40-60%), citric acid (1-1.5%) and carboxymethyl cellulose (CMC) (0.15-0.25%). Based on preliminary sensory trials, bar prepared with addition of 50% sugar, 1.50% citric acid and 0.25% CMC followed by drying at 60ºC for 5 h was selected for product preparation and storage studies. The bars contained 10.55% crude fibre, ascorbic acid (6.21mg/100g), total phenols (1454.0 mg GAE/100g), carotenoids (3.15 mg/100g), and anthocyanins (72.30 mg CE/100g) with 60.32% DPPH free radical scavenging activity. Storage studies (at 28-37 ºC/RH 35-87%) revealed that crude fiber and anthocyanins changed non-significantly while other bioactive components such as ascorbic acid, carotenoids, total phenols, and antioxidant activity significantly ($p<0.05$) decreased while maintaining good overall acceptability. Prepared bars can be stored safely for six months at ambient temperature with nutrient constituents intact.

Keywords: Bars, Fibre, Pomace, Phytochemicals, Storage studies

Black carrots are highly nutritious containing a high amount of phenols, anthocyanins, zinc, sodium, potassium, calcium, copper, iron, magnesium, calcium, manganese, and flavonols like beta carotene, and have antioxidant activity four times higher than the red carrots (Sharma *et al.,* 2012). Black carrots are a rich source of natural food colorings having great nutraceutical value. Anthocyanins are the pigments present in black carrot extracts which provide them with their color and also have a number of health benefits, which include the ability to treat neurological dysfunctions, treating cancer; they are remarkable blood cleansers and have the potential to treat anaemia deficiency, constipation and stomach disorders (Micheal *et al.,* 2007; Mills *et al.,* 2008). Black carrots are sown from August to September and are available for a short span from December to February. Although purple and black carrots are underutilized in India as vegetables, they are mostly used for the preparation of fermented beverages which act as good appetizers (Tuker *et al.,* 2004).

During the processing of fruits and vegetables, 40-60% goes waste in the form of peel, seeds, and pomace along with many nutrients and phytonutrients such as vitamin C and B, antioxidants, carotenoids, flavonoids, fiber, folate, anthocyanins and polyphenols present in the juice residue (Sagar *et al.,* 2018). The residues are also rich in polyphenols, which can become active pharmaceutical ingredients. No work has been reported for the utilization of black carrot pomace in the preparation of high fiber, and antioxidant-rich bars. Fruit bar is a concentrated fruit product that has superior nutritive and energy values. In addition, compared to fresh fruits, fruit bars tend to possess extended shelf life. In addition, fruit bars can be an exceptional instant food that can deliver the required dietary fiber and other bioactive compounds required to meet the daily requirements in humans (Parn *et al.,* 2015).

Keeping in view the above rationale, the present study was planned to utilize black carrot juice residue (pomace) for the formulation of high fiber and antioxidant-rich bars and to ascertain the shelf stability of prepared bars.

MATERIALS AND METHODS

Procurement of raw material

Freshly harvested black carrots var. *Punjab Black Beauty* were procured from the Department of Vegetable Science, Punjab Agricultural University, Ludhiana, India for the preparation of bars. Analytical grade chemicals were purchased from SRL Chemicals Pvt. Ltd., India.

Preparation of fruit bars

Extraction of juice

Carrots were washed in an automatic washer equipped with scrubbing to remove extraneous matter

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from the outer surface, followed by extraction of juice. The juice waste (pomace) was collected and packed in high-density polythene pouches (2 Kg capacity) and stored at refrigerated (4±1 ºC) temperature till further used.

Standardization of recipe for fruit bars

Five different formulations were prepared by varying sugar (40-60%), citric acid (1-1.50%), and carboxymethyl cellulose (CMC) (0.15-0.25%). The detailed formulations are given below:

F1: Sugar (40%), citric acid (1%)

F2: Sugar (50%), citric acid (1.25%)

F3: Sugar (60%), citric acid (1.50%)

F4: Sugar (50%), citric acid (1.50%), CMC (0.15%)

F5: Sugar (60%), citric acid (1.50%), CMC (0.25%)

Method of preparation

The collected black carrot pomace was ground to a fine pulp using a domestic grinder (Momenta NV 750, Havels, India). The fine pulp was then cooked at 80 ºC with selected concentrations of sugar, citric acid, and CMC gum till TSS reached. The resultant mixture was then poured onto a polyethylene sheet smeared with oil, followed by drying at 60 ºC for 5 hours in a hot-air cabinet drier (Model- 1254, Pharma Tech International, Kolkata, India; with airflow rate of 2 m/s). The dried product was then allowed to cool, cut into rectangular shape bars, and packed in polyethylene pouches of 100 gauge thickness. Based on preliminary sensory trials, formulation F4 was selected for product development and storage studies at room temperature (28-37˚C/ RH 35-87%) temperature for storage studies. The flow sheet of the process is given in Fig. 1.

Collection of black carrot pomace
↓
Cooking of juice waste (at 80°C for 15 minutes)
↓
Addition of sugar (50%), CMC gum (0.25%), and citric acid (1.50%)
↓
Pouring on oil smeared polythene sheet
↓
Drying in tray drier (60°C for 5h)
↓
Cutting into suitable pieces
↓
Packaging
↓
Storage at ambient temperature (28-37 °C)

Fig. 1. Flow chart of the preparation of bars from black carrot pomace

Quality analysis of black carrot juice waste and bar

Physicochemical analysis

The total solids were determined by drying 5 g of fresh tissue in hot air oven at 80±2 °C till constant weight was obtained and were calculated on fresh weight basis % (AOAC, 2020). The total soluble solids (TSS) and pH were determined using hand refractometer (Erma, Japan) and pH meter (Mettler Toledo, India), respectively. Total sugars and reducing sugars were determined by the phenol sulphuric acid method and Nelson Somogyi method as outlined by Kaur *et al.,* (2022a). The titratable acidity was determined by using the standard titration method (Ranganna, 2017) and results were expressed in % acidity (citric acid). Crude fibre was estimated by the method of Ranganna (2017) using fibertec (FOSS instrument, Sweden).

Phytochemical analysis

Bioactive compounds such as ascorbic acid, total monomeric anthocyanins, total phenols and DPPH free radical scavenging activity were studied.

Ascorbic acid was determined following standard titrimetric method using 2, 6- Dichlorophenol indophenol dye solution that gets reduced to colorless by ascorbic acid (Aggarwal *et al.*, 2022).

Total phenols were measured with Folin-Ciocalteau reagent spectrophotometrically, as per standard procedure (Aggarwal *et al.,* 2022). Briefly, 0.5 ml of extract was mixed with 0.5 ml of Folin-Ciocalteu reagent, followed by addition of 1.5 ml of sodium carbonate (20% w/v) solution and 7.5 ml of double-distilled water. The absorbance was read at 760 nm after incubating the samples for 2 h at room temperature. The results were expressed as mg gallic acid equivalents (GAE)/100 g.

Total monomeric anthocyanins were estimated using pH differential method as reported by Kaur *et al.,* (2022b). One ml aliquot was transferred to 10 ml volumetric flasks and the volume was made up with both the buffers i.e. pH 1.0 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium acetate) separately, followed by keeping the flasks in dark for 2 h at 25 °C and absorbance was read at 520 and 700 nm, using a UV/Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The total monomeric anthocyanins were calculated as mg cyanidin-3-o-glucoside equivalent (CE)/100 g using the following equation:

$$TMAC = \frac{A \times Mw \times DF \times 1000}{\in \times L}$$

Where, A= $(A_{520}\text{-}A_{700})_{pH1.0} - (A_{520}\text{-}A_{700})_{pH4.5}$; M_w = 449.2 g/mol (molecular weight of cyanidin-3-glucoside), DF is the dilution factor, ε = 26,900 L/cm mol (molar absorptivity of cyanidin-3-glucoside), and L= 1 cm (standard optical path length); 1000 is the factor to convert grams to milligrams.

The antioxidant activity of the samples was estimated spectrophotometrically by DPPH free radical scavenging activity (Kaur *et al.,* 2022b). The results were expressed % inhibition of DPPH radicals.

Sensory quality

The sensory evaluation of prepared bar was carried out for sensory attributes i.e. appearance, mouth-feel, flavor, and overall acceptability using 9- point hedonic scale by a panel of 20 semi-trained panelists from the Department of Food Science and Technology, Punjab Agricultural University, Ludhiana, India. The score of 9 indicates liked extremely to 1 for disliked extremely.

Storage studies

Black carrot pomace bar was packed in HDPE pouches (100 g) and stored at room temperature (28-37˚C/RH 35-87%) for six months. Storage stability was assessed by determining the changes in physico- and phytochemical constituents, antioxidant activity, and sensory quality parameters at regular intervals of 1 month.

Statistical analysis

All the experiments were carried out in triplicates and the results were reported as mean ± standard deviation. One way analysis of variance was performed using the SPSS version 20.0 (Statistical Package for Social Sciences) followed by Tukey's *post hoc* test at 5% level of significance.

RESULTS AND DISCUSSION

Quality analysis of raw black carrot, extracted juice and juice waste

The data regarding different physico- and phytochemical constituents of raw black carrot, extracted juice and pomace is presented in Table 1. A significant ($p<0.05$) difference was observed in total solids of the black carrot and pomace with 14.66% total solids in black carrot and 16.26% in pomace(Table 1). The pH of the pomace significantly ($p<0.05$) decreased from 6.84 to 6.54 after the extraction of juice, however, a non-significant variation was observed in pH of fresh black carrot and its juice (Table 1). Juice was found to have the highest acidity of 0.29% followed by 0.24% in raw carrot and 0.11% in juice waste. Sagar *et al.,* (2004) reported acidity of fresh carrots in the range of 0.08-1.0%. The pH values of raw carrot, juice waste and carrot juice were observed as 6.84, 6.88 and 6.54 respectively. Total sugars and reducing sugars were found to be highest in raw carrots (6.95%, 3.36%) followed by extracted juice (6.75, 3.41) and juice waste (6.02, 2.91%) respectively. Sagar *et al.,* (2004) reported higher total sugars (11.46-13.25%) in different varieties of carrot while Sethi and Anand (1982) reported lower total sugars (5.23%) for fresh blanched carrots. Juice waste was found to have higher crude fibre (1.51%) than raw carrots which contained 1.02% crude fibre (Table 1). Goplan *et al.,* (1982) and Sandhu *et al.,* (2005) reported 1.20% and 1.01% crude fibre, respectively in fresh carrots.

Ascorbic acid content was found to be highest in black carrot juice (8.0 mg/100g) followed by raw carrot (7.34 mg/100g) and pomace (7.30 mg/100g) with non-significant ($p>0.05$) difference (Table 1). Teaotia *et al.,* (1972) reported ascorbic acid content of carrot varieties as 6.21-6.18% respectively. Total phenols in raw black carrot were found to be maximum (496 mg GAE/100g) followed by juice and pomace with 438.05 mg GAE/100 g and 411 mg GAE/100 g total phenols, respectively (Table 1). Alasalvar *et al.,* (2001) reported 74.60 mg GAE/100 g (FW) total phenolics.

Juice contained the maximum monomeric anthocyanins (159.2 mg CE/100g) followed by raw carrots (126.4 mg CE/100g) and pomace (121.79 mg CE/100g) (Table 1). Sezen *et al.,* (2014) observed 486.0 mg/100g (DW) monomeric anthocyanins in raw black carrot. Total anthocyanin content of black carrot can be influenced by differences in the degree of root coloring, cultivars and growing conditions (Kammerer *et al.,* 2004; Montilla *et al.,* 2011). The DPPH free radical scavenging activity of raw carrot, pomace, and juice was recorded as 58.40, 56.84 and 60.57%, respectively, with carrot juice showed the maximum antioxidant activity (Table 1). The antioxidant capacity of black carrot was studied by Sezen *et al.,* (2014) using two different in *vitro* methods, DPPH free radical scavenging activity and Cupric Ion Reducing Antioxidant Capacity with mean values were 3887.0 and 12362.0 mg trolox equivalent/ 100 g dry weight.

Quality analysis of fruit bars

Fresh bars were observed to contain 22.81% moisture content. The effect of storage on moisture content of bars was found to be significant ($p<0.05$). There was a gradual and slow decrease in moisture content of bars throughout the storage period from an initial value of 22.81% to 20.99% after 6 months of storage (Table 2). A similar decreasing trend in moisture content of guava leather was observed by Safdar *et al.,* (2014) during storage at ambient temperature. There was an increase in TSS of bars with increase in storage time with initial value 65.67to 67.39ºB after 6 months of storage (Table 2). The increase in TSS during the storage period might be due to conversion of insoluble polysaccharides into soluble sugars (Sandhu *et al.,*

Table 1. Quality characteristics of raw black carrot, juice and pomace

Parameters	Black carrot	Juice	Pomace
Total solids (%)	14.66±0.20[a]	-	16.26±0.10[b]
TSS (°B)	7.00±0.02[a]	7.5±0.05[a]	7.00±0.07[a]
Acidity (% citric acid)	0.24±0.02[a]	0.29±0.10[c]	0.113±0.06[b]
pH	6.84±0.03[a]	6.54±0.06[b]	6.88±0.05[a]
Total sugars (g/100 g)	6.95±0.25[a]	6.75±0.10[b]	6.02±0.09[c]
Reducing sugars (g/100 g)	3.36±0.10[a]	3.41±0.8[a]	2.91±0.7[b]
Crude fiber (%)	1.02±0.03[b]	-	1.512±0.08[a]
Ascorbic acid (mg/100 g)	7.34±0.72[a]	8.00±0.56[b]	7.30±0.61[a]
Total phenolics (mg GAE/100 g)	496±1.5[a]	438.05±0.25[b]	411.00±0.10[c]
Total carotenoids (mg/100 g)	1.84±0.02[a]	1.71±0.07[b]	1.69±0.05[c]
Anthocyanins (mg CE/100 g)	126.4±0.50[b]	159.2±0.38[a]	121.79±0.43[c]
DPPH free radical scavenging activity (%)	58.4±0.65[b]	60.57±0.47[a]	56.86±0.36[c]

Values are Mean±SD, *n=3*; Values with different superscript letters are significantly (p<0.05) different among columns; GAE: Gallic acid equivalent; CE: Cyanidin-3-glucoside

2001). Another reason for this increasing trend could be the moisture loss resulting in the concentration effect on the product during storage period. Similar trend was reported by Rani and Bhatia (1985) in pear candy and Sharma *et al.,*(1998) in apple candy. An increasing trend for acidity and declining trend for pH was observed during the storage of fruit bar; with initial acidity (0.69%) and pH (5.05) values significantly ($p \leq 0.05$) changed to 0.79% and 4.80 after 6 months of storage (Table 2). The increase in acidity might be due to rise in the concentration of weakly ionized acids and their salts during storage. Apart from this, formation of acids by degradation of polysaccharides and oxidation of reducing sugars or by breakdown of pectic substances and uronic acid might be contributed to increase in titratable acidity (Mann*et al.,* 2019). Aruna *et al.,* (1999) reported similar results during storage of papaya fruit bar. Variation in pH is directly related to variation in acidity of fruit bars, hence, a decrease in pH was observed during storage in the present study.

For total sugars and reducing sugars, a significant ($p<0.05$) increasing trend was observed during the storage period. The initial total sugars and reducing sugars were 61.84 and 33.66 g/100g, respectively

Table 2. Effect of storage on quality characteristics of black carrot pomace bars

Parameter	Storage periods (months)			
	0	2	4	6
Physicochemical				
Moisture (%)	22.81±0.23[a]	22.44±0.20[b]	21.95±0.30[c]	20.99±0.35[d]
TSS (°B)	65.67±0.42[c]	64.05±0.32[d]	66.77±0.28[b]	67.39±0.20[a]
Titratable acidity (% citric acid)	0.69±0.05[d]	0.72±0.08[c]	0.74±0.04[b]	0.79±0.05[a]
pH	5.05±0.02[a]	5.02±0.05[a]	5.01±0.02[a]	4.80±0.03[a]
Total sugars (g/100g)	61.84±0.61[c]	61.92±0.58[c]	63.74±0.50[b]	65.61±0.42[a]
Reducing sugars (g/100g)	33.66±0.88[c]	33.96±0.82[c]	34.98±0.70[b]	36.25±0.70[a]
Crude fiber (%)	10.55±1.0[a]	10.56±0.9[a]	10.56±0.9[a]	10.58±1.1[a]
Sensory				
Appearance	7.70±0.04[a]	7.50±0.04[a]	7.20±0.03[b]	7.10±0.03[c]
Flavor	7.00±0.02[a]	7.00±0.02[a]	6.70±0.02[b]	7.00±0.04[a]
Mouth feel	8.09±0.03[a]	7.80±0.05[b]	7.50±0.04[d]	7.23±0.04[c]
Overall acceptability	9.00±0.02[a]	9.00±0.04[a]	8.80±0.02[b]	8.59±0.02[c]

Values are Mean±SD, Values within a row with different superscript letters are significantly (p<0.05) different.

which increased significantly to 65.61 and 36.25 g/100g, respectively after 6 months of storage (Table 2). This might be due to gradual inversion of non-reducing sugars and also due to breakdown of insoluble polysaccharides and other carbonate polymers to soluble sugars (Sharma *et al.,* 2013).

Ascorbic acid, most important non-phenolic antioxidant; is known to be a highly unstable vitamin. During storage, the ascorbic acid content of fruit bar was found to decrease significantly ($p<0.05$). The initial ascorbic acid content of bar was 6.21 mg/100 g, which decreased consistently to 4.47 mg/100g, after 6 months of storage (Fig. 2). This might be due to oxidation of the thermoliabile ascorbic acid into dehydroascorbic acid upon storage (Selvamuthukumaran and Khanum, 2014). The ascorbic acid loss accounted to 28.0% after 6 months of storage period. Rao and Roy (1980) observed higher losses (around 47.32%) in during storage of mango leather for three months. Other factors which influence the degradation of ascorbic acid are oxygen, light, metal ion catalyst and pH (Mann *et al.,* 2019).

Anthocyanins; the potent antioxidant, is responsible for the attractive red, blue and purple color of the fruits, vegetables, and flowers. The initial total anthocyanin content of fruit bar was 72.30 mg CE/100 g which slightly decreased to 72.18 mg CE/100g after 6 months of storage and the decrease was found to be non-significant ($p>0.05$) (Fig. 3). However, Mann *et al.,* (2019) reported 13.70% loss in total anthocyanin content of strawberry jam stored for 6 months at room temperature.

The phenolic compounds contribute immensely towards better human health due to their multiple biological effects such as antioxidant capacity, antimutagenic and anticarcinogenic activity (Selvamuthukumaran and Khanum, 2014, Kaur *et al.,* 2019b). The total phenols of fruit bar was estimated to be 1454 mg GAE /100g initially which significantly ($p<0.05$) decreased to 1399 mg GAE/100g, after 6 months of storage (Fig. 2). This might be due to the sensitivity of the phenolic component to oxidation at above storage conditions. According to Pinelli *et al.,(*2015), the processing and storage conditions lead to co-pigmentation and self-association of molecules, reducing the number of hydroxyl groups available to react with Folin-Ciocalteau colorimetric assay, apparently reducing the concentration of total phenolic content. Mann *et al.,* (2019) observed 14.61% losses of total phenolics in strawberry jam after six months of storage at 25 ºC. In our study, the loss in total phenol content accounted for 3.78% under room temperature after six months of storage.

Carotenoids are fat soluble compounds which are used in food fortification because of their possible activity as provitamin A and their biological functions to health benefit, such as strengthening the immune system, reducing the risk of degenerative diseases, antioxidant properties and antiobesity/ hypolipidemic activities (Mezzomo and Ferreira 2016).During storage, a significant ($p<0.05$) decrease in total carotenoid content was observed in fruit bar (Fig. 2). Initially, total carotenoids were 3.15 mg/100g which slightly but significantly ($p<0.05$) decreased to 3.01 mg/100g after 6 months of storage at room temperature (Fig. 2a). According to Kidmose *et al.,* (2004) decrease in total carotenoids might be due to oxidation of highly unsaturated carotenoid structure. However, degradation in carotenoids could also be due to sensitivity of pigments to heat, light and air. The change in total carotenoids content of black carrot pomace bar during

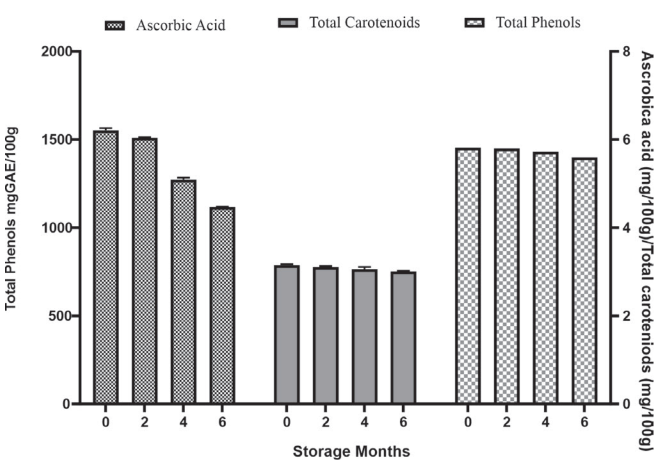

Fig. 2. Effect of storage on a) ascorbic acid b) total carotenoids c) total phenols of the black carrot pomace bar; GAE: Gallic acid equivalent

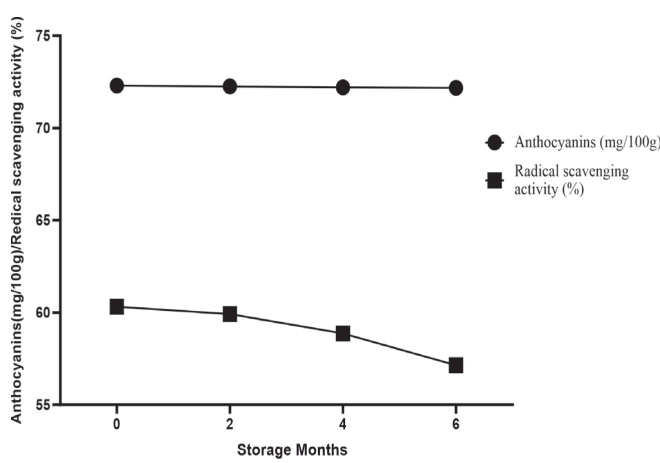

Fig. 3. Effect of storage on a) anthocyanins (mg CE/100 g) b) DPPH free radical scavenging activity(%) of black carrot pomace bar; CE: Cyanidin-3-glucoside

the entire storage period was approximately 4.44%. The results of loss in carotenoids during storage were in conformity with report on papaya toffee and papaya leather by Attri *et al.,* (2014).

Antioxidants in fruits and vegetables have been recognized as an important component in reducing the risk of several chronic diseases including cancer, diabetes, coronary heart diseases; hypertension etc. Exposure to oxygen, light and heat may reduce the antioxidative properties and health beneficial effects of food (Wicklund*et al.,* 2005). A significant ($p<0.05$) decrease in DPPH free radical scavenging activity of black carrot pomace bar was observed up to 6 months of storage (Fig. 3). The initial radical scavenging activity of the bar was 60.32% which significantly ($p<0.05$) decreased to57.15% after six months of storage (Fig. 3). The decrease in the antioxidant activity might be linked to decrease in phytochemicals (ascorbic acid, anthocyanins, and total phenols) during storage. Kanwal *et al.,* (2017) observed 5.50% loss of total antioxidant activity in guava jam after 60 days storage.

Sensory quality

The effect of storage on the sensory quality attributes overall acceptability of black carrot pomace is represented in Table 2. Although sensory attributes significantly ($p<0.05$) decreased with the advancement of storage, however, the bar remained highly acceptable up to six months of storage.

It may be concluded fibre and phytochemical-rich bar can be prepared from black carrot pomace. Bars prepared with 50% sugar, 1.50% citric acid and 0.25% CMC followed by drying at 60ºC for 5 hrs retained the physico-chemical and bioactive components including total phenolics, carotenoids, anthocyanins and total anti-oxidant activity. The fruit bar was found to be highly acceptable up to six months of storage at ambient temperature with its color and nutrients intact.

Authors' contribution

Conceptualization of research work and designing of experiment: (PA, AS); Execution of the field/ lab experiments and data collection: (AS); Analysis of the data and interpretation: (SK, NK, AS); Preparation of the manuscript: (SK, NK, PA, AS)

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