Characterization of major betalain pigments -gomphrenin, betanin and isobetanin from *Basella rubra* L. fruit and evaluation of efficacy as a natural colourant in product (ice cream) development

S. Sravan Kumar · P. Manoj · N. P. Shetty ·
Maya Prakash · P. Giridhar

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Abstract *Basella rubra* L. (Basellaceae) commonly known as Malabar spinach is a leafy vegetable which accumulates pigments in its fruits. To find out the feasibility of utilizing pigment rich extracts of its fruit as natural food colourant, fruits at different stages were analysed for pigment profiling, carbohydrate content, physical dimensions and weight. Total betalains content increased rapidly from early (green) through intermediate (half-done red-violet) to matured stage (red-violet). Maximum pigment content was observed in ripened fruits (143.76 mg/100 g fresh weight). The major betalain pigment characterized was gomphrenin I in ripened fruits (26.06 mg), followed by intermediate fruits (2.15 mg) and least in early fruits (0.23 mg) in 100 g of fresh deseeded fruits. Total carbohydrates content and the chroma values (redness) were also increased during ontogeny of *B. rubra* fruits. The textural characters of developing fruits showed the smoothness of green fruits with lower rupture force (0.16 N/s) than ripe ones (0.38 N/s). The pigment-rich fruit extract was used as natural colourant in ice-cream, to evaluate its effect on physicochemical properties and acceptability of the product. After six months of storage at -20°C , 86.63 % colour was retained in ice-cream. The ice-cream had good overall sensorial quality and was liked by consumers indicating that addition of *B. rubra* fruit extract did not alter the sensory quality of the product. The colour values also indicate that there was no significant decrease of this pigment-rich extracts of fruits for its incorporation in food products.

Keywords *Basella rubra* · Betalains · Gomphrenin I · Chlorophyll · Tinctorial strength

Introduction

Basella rubra L. (Basellaceae), normally called as Malabar spinach is a succulent, smooth, annual, climbing and herbaceous vine found in tropical and sub-tropical areas (Khare 2004) and one of the commonly consumed leafy vegetable. The leaves of this pot shrub is an excellent source of calcium and iron (Anusuya et al. 2012). In India the leaves are used to treat mouth ulcers, headache, gonorrhoea and balanitis (Nadkarni 1996). Sporadic pharmacological reports on *B. rubra* showed hypoglycemic effects (Nirmala et al. 2009). *B. rubra* plant has pale pink flowers (Oladele and Aborisade 2009). The fruits are fleshy, stalk less, ovoid or nearly spherical. Green to red violet fruits are observed during ontogeny (Wealth of India 2000). Three to four months old plant produce approximately 2 kg of fresh fruits. Lin et al. (2010) identified the red violet pigments present in *B. alba* and *B. rubra* fruits.

The major pigments, such as chlorophyll, carotenoids, flavonoids (anthocyanins and betalains) are responsible for colouration of leaves and fruits (Khan et al. 2011). The pigment content of fruits changes during ontogeny to adapt to the environmental condition, stress and damage (Biswall 1995; Lichtenthaler 1996). Betalains and anthocyanins are the most important water soluble pigments in nature but never been found in the same plant (Stafford 1998). Betalains are nitrogen containing plant pigment which replaces anthocyanins in flowers and fruits of plants of family Caryophyllales (Clement and Mabry 1996). The other sources of betalains are beet root, *Gomphrena globosa* flowers, amaranth plant,

S. S. Kumar · P. Manoj · N. P. Shetty · P. Giridhar (✉)
Plant Cell Biotechnology Departments, Central Food Technological
Research Institute, Mysore 570020, India
e-mail: parvatamg@yahoo.com

M. Prakash
Sensory Science Department, CSIR-CFTRI, Mysore 570020, India

Rivina humilis fruits and cactus fruits (Lin et al. 2010; Khan et al 2012; Khan and Giridhar 2014a). Betalains may be orange to yellow betaxanthins and red to violet betacyanins, which can be classified into four types based on their chemical structure such as betanin type, amaranthin type, gomphrenin type and bouganvillein type (Cai et al. 2005). During fruit development of cherry, increase in fruit size, total soluble solids content and pigment content was reported (Ishida et al. 1997). Components, such as pigment, sugars, proteins, phenols and organic acids of fruits and vegetables play important roles in maintaining quality and nutritive value. Moreover, the concentration of these constituents in fruits has been of interest because of their influence on organoleptic properties. Although *B. rubra* twine is a familiar leafy vegetable, but its fruits, richly endowed with betalain pigments are underutilized. Hence, it is worthy to document the pigment accumulation pattern in different developmental stages of *B. rubra* so that if we have to use this fruit as betalains enriched food additive, proper harvesting time to yield maximum pigment as well as nutrients can be set up.

Moreover, consumers prefer natural over artificial colours especially while using them in processed foods. This prompted demand for convenient, natural and foods fortified with natural ingredients. In view of this, the aim was to investigate the pigment profile in *B. rubra* fruits at developmental stages, and concomitant changes in total carbohydrates which are important for processing and organoleptic properties, along with the use of this betalain rich concentrate as natural colourant in ice cream.

Materials and methods

Source of fruits

Basella rubra L. fruits were collected from 3 months old twines of greenhouse maintained plants at CFTRI, Mysore campus located geographically between 12° 18' 26' North latitude and 76° 38' 59' East longitude in the months of December 2012 through March 2013. During the period, the maximum and minimum temperatures were 28 °C and 14 °C, respectively and rainfall was 5–10 cm. Based on the visual observations such as colour and position, fruits were divided into three groups, such as early (green, 2 wk after flowering), intermediate (half-done red violet, 7 wk after flowering) and matured stage (red violet, 9 wk after flowering). Plants were taxonomically identified, and Herbarium sheet of the plant was deposited at Herbarium Collection Centre, University of Mysore (Reference No. 02/08/05/13). The fruits were randomly distributed into three distinct colours or stages viz. green (early stage), half-done red violet (intermediate stage), red violet (matured or ripened stage).

Physical dimensions and weight of fruits

Fresh weight and physical dimensions of *B. rubra* fruits in different ontogenial stages were measured immediately after collecting from greenhouse using an analytical balance (Mettler AE 163, Marshall Scientific, USA) according to the method of Khan et al. (2011).

Measurement of tinctorial strength and chemical analysis of betalains

Fresh deseeded fruits were weighed (1.0:10 w/v) and homogenized in distilled water, methanol, ethanol, methanol:water (60:40) and ethanol:water (60:40) using mortar and pestle and centrifuged at 12,000 rpm for 10 min at 4 °C. The prepared extracts were diluted in deionized water to adjust a constant absorbance value of 1.0 to 1.1. The resulting dilution factors ranged from 306 in ethanol extract and 455 in water extract. From these values, the tinctorial strength of all the extracts was calculated. The total soluble solid (°Brix) in extracts was recorded using digital refractometer at 20 °C (Model: RX-500, Atago Co. Ltd., Tokyo, Japan). Similarly the pH of all the extracts were measured (Cyberscan 500, Eutech instrument, Singapore). All measurements were made in triplicates. The colour of above extracts were monitored (Kunnuka and Pranee 2011) using colour measurement instrument by measuring L, a* and b* values where, L values indicates lightness, a* (−/+) indicates green to red and b* (−/+) values indicates blue to yellow using colour measurement instrument (Konica Minolta CM5, Germany).

Colour measurement

The colour of aqueous extracts of fruits at different developmental stages (1.0:10 w/v) was monitored using the above described method (Kunnuka and Pranee 2011). L, C* (Chroma) and h* (Hue angle) values are calculated from the L, a* and b* values. The C* (Chroma) values and h* value (Hue angle) were calculated as the square root of (a²+b²) and tan^{−1} (b/a), respectively.

Spectrophotometric analysis of pigments

Fruits of respective ontogeny stages were manually squeezed to remove seed. Deseeded fruits were weighed (1.0:10 w/v) and homogenized in distilled water using mortar and pestle and centrifuged at 12,000 rpm for 10 min at 4 °C. The collected supernatant was further analyzed for its absorbance maxima of pigments using spectrum from 400 to 600 nm, pigment quantity and characterization by photometrically according to Castellanos–Santiago and Yahia (2008) and

HPLC method (Lin et al. 2010). All the measurements were made in triplicates.

$$BC(\text{mg/g}) = [A(\text{DF})(\text{MW})V_d/\varepsilon L W_d]$$

Where A is the absorption value at the absorption maxima of 535 and 477 nm for betacyanins and betaxanthins, respectively, DF is the dilution factor, V_d is the solution volume (ml), W_d is the deseeded fruit weight (g), and L is the path length (1 cm) of the cuvette. The molecular weight (MW) and the molar extinction coefficient (ε) of betanin [MW=550 g/mol; ε =60,000 L/ (mol cm) in water were applied to quantify the betacyanins. Quantitative equivalents of the major betaxanthins (Bx) were determined by applying the mean molar extinction coefficient ε =48,000 L/ (mol cm) in H₂O].

HPLC analysis of *B. rubra* fruit red violet pigment during ontogeny

HPLC analysis was conducted using an HPLC pump equipped with a C₁₈ column (Sunfire, Waters Corporation, Milford, MA, USA) of 250×4.6 mm i.d and the UV detector was set at 477 nm and 535 nm. Twenty micro liters of extract were injected, and mobile phase consists of (A) 0.1 % trifluoro acetic acid in water and (B) 100 % methanol. The binary gradient solvent program was set at 0 min, 85 % A and 15 % B; 10 min, 70 % A and 30 % B; 15 min, 20 % A and 80 % B; 17 min, 10 % A and 90 % B; 25 min, 9 % A and 91 % B; 26 min, 0 % A and 100 % B; and 28 min, 85 % A and 15 % B, with a flow rate of 1.0 ml/min (Lin et al. 2010).

Determination of chlorophyll content

The fruit pulp of different ontogeny stages (1.0:10 w/v fresh weight) was separated and extracted with 80 % acetone (5×2 ml with grinding for 2 min each), centrifuged (8,000×g, 15 min, 4 °C), filtered through qualitative Whatman no.1 filter paper (particle retention-11 μm) and concentrated in a rotavapor (Buchi, Model 490, Switzerland) under reduced pressure (150 mbar) at 30 °C. The absorbance was measured at 663 nm and 645 nm with a double beam spectrophotometer (UV-160 A, Shimadzu Corporation, Kyoto, Japan). The concentration of chlorophyll a (chl-a), chlorophyll b (chl-b) and

Fig. 1 Changes in fresh weight (g/100 fresh fruits) of *B. rubra* fruits during ontogeny stages. Values are mean±SD. Significance was tested by Duncan Multiple Range Test at p 0.05, and values with different superscript were found not significant difference from each other

total chlorophyll (chl-t) were calculated using the equation as follow (Arnon 1949).

$$\text{Chl a } (\mu\text{g/ml}) = 12.72 A_{663} - 2.59 A_{645}$$

$$\text{Chl b } (\mu\text{g/ml}) = 22.9 A_{645} - 4.67 A_{663}$$

$$\text{Chl t } (\mu\text{g/ml}) = 20.31 A_{645} + 8.05 A_{663}$$

Determination of total carbohydrate content

Deseeded fruit sample (100 mg) was weighed into boiling tube. The sample was hydrolyzed by boiling with 5 ml of 2.5 N HCl for 3 h, cooled to room temperature and neutralized with sodium carbonate. The volume was made up to 10 ml and centrifuged. The supernatant was collected, and 0.05 and 0.1 ml aliquots were taken for analysis. Standards were prepared with 0.1 mg/ml of glucose. The volume was made to 1 ml with distilled water in all the tubes including the sample tubes. 4 ml of anthrone reagent was added and heated for 8 min in boiling water bath and cooled rapidly. The green colour developed was read at 630 nm. By using a standard graph, the amount of carbohydrate present in the sample was calculated (Sadasivam and Manickam 1992).

Table 1 Physical characteristic of matured *B. rubra* fruits

Sample	Circumference (cm)		Fresh weight of 100 fruits (g)	Fresh weight of 100 seeds after peeling pulp (g)	Fresh weight of pulp for 100 fruits (g)
	Whole fruit	Seed			
<i>B. rubra</i> fruit	2.41±0.16	0.9±0.08	18.64±0.24	5.69±0.22	10.40±0.48

Values are mean±S.D (n=20)

Table 2 Tinctorial strength and chemical analysis of betalains of matured *B. rubra* fruits

Extracted solvent	TSS	RI	pH	TDF	A at max	Tinctorial strength
water	0.73±0.04	1.33±0.00	5.69±0.09	455.00±7.07	1.04±0.02	473.36±1.33
methanol	0.42±0.04	1.33±0.00	7.24±0.02	325.24±15.12	1.05±0.04	340.04±13.28
ethanol	19.95±0.09	1.36±0.00	6.43±0.06	306.25±8.84	1.06±0.01	325.65±6.12
methanol:water (60:40)	6.30±0.02	1.34±0.00	6.76±0.12	376.15±6.73	1.09±0.01	408.57±18.23
ethanol:water (60:40)	19.32±0.02	1.36±0.00	6.55±0.01	406.67±9.43	1.08±0.03	439.93±20.26

TSS total soluble solids, RI refractive index, Tinctorial strength=Absorbance (A) at $\lambda_{\max}$ * total dilution factor (TDF). Values are mean±S.D (n=3)

Texture measurement of ontogenial fruits

The texture measurement of different ontogeny stages of fruits was analyzed by using universal texture measuring system (UTM Lloyd, LR-5 k, Lloyd Instrument Ltd., Fareham, U.K).

A cylindrical pointed penetration probe with a diameter of 2 mm was allowed to penetrate into ontogeny stages of fruit at constant speed to a depth of 3 mm without touching the seed. The force of penetration into ontogenial fruit was expressed as Newtons /s (Breene 1975).

Fig 2 Colour intensity (Hunter) of *B. rubra* fruits (BRF) extracted in different solvents (a) and different stages of ontogeny (b). Values are mean±SD (n=3). Significance was tested by Duncan Multiple Range Test at p 0.05, and values with same superscript were found not significant difference from each other. Separate significance was conducted for L, chroma and hue

Preparation of ice cream using pigment rich extracts of *B. rubra* fruits and sensory analysis

The ice cream (10 L) was prepared using ingredients, such as milk powder (16 kg); sugar (28 kg), and butter (15 kg). All these were mixed in water and boiled for 1 h at 100 °C. The ice cream without coloured extract (control) and treated sample (with extract) containing concentrated fruit juice of 100 ml) was added and subjected to freezing for 12 h. The volume of juice was fixed for good colour with previous experimentation on the appearance and overall quality, as suggested by the trained panelist. The ice cream samples were subjected to sensory analysis by 15–25 trained panelists, selected among scientists, staff members and students familiar with sensory analysis techniques and experience in evaluating food formulations. Evaluations were conducted under white fluorescent light in sensory booths maintained at 25 ± 2 °C and relative humidity of 50 ± 5 %. A suitable score card was developed using “free-choice profiling” method selecting suitable terminology. Ice cream (control and treated) samples were provided in two coded plates to visually examine and taste under consumer acceptance study using hedonic test. The 7- point hedonic scale was used for evaluation which ranged from “Like Very Much” (LVM) to “Dislike Very Much” (DVM) with “Neither Like Nor Dislike” (NLND) as the midpoint. “Overall Quality” was judged for the above mentioned ice cream samples.

Fig. 3 UV spectrum showing the absorbance maxima of ontogenial fruits (1), HPLC chromatograms monitored at 535 nm showing major pigments (betanin (1), isobetanin (2) and gomphrenin I (3)) of *B. rubra* fruits during ontogenesis i.e., **a**. green immature fruit, **b**. partially mature fruit and **c**. ripened fruit

Table 3 Pigment content (mg/100 g f. wt.) of different ontogenial stages of *B. rubra* fruits

Fruit stage	Betacyanins	Betaxanthins	Total Betalains
Early	6.89±0.21	15.41±0.92	22.32±1.56
Intermediate	55.90±3.19	31.81±1.64	87.71±5.37
Matured	124.18±5.41	19.16±1.35	143.76±7.04

Values are mean±S.D (n=3)

Colour stability of ice cream using colour measurement instrument

The colour of ice cream was monitored (Kunnuka and Pranees 2011) by measuring (L, a* and b*) values to know the betalain stability during preservation where, L values indicates lightness, a* (–/+) indicates green to red and b* (–/+) values indicates blue to yellow using colour measurement instrument (Konica Minolta CM5, Germany).

Statistical analysis

All values presented are mean±S.D. of three analyses. Data were subjected to one-way ANOVA followed by post hoc Duncan's Multiple Range Test (DMRT) using SPSS 17 (SPSS Inc., Chicago, IL, USA) for determining significant differences. A difference was considered significant at p 0.05.

Table 4 Chlorophyll content in different stages of fruits

Sample	Early fruit	Intermediate fruit	Matured fruit
Chl a	23.07±0.49	8.94±0.13	ND
Chl b	24.51±0.71	12.17±0.16	ND
Chl t	47.57±0.21	21.10±0.29	ND

Results are expressed in mg/100 g fresh weight of deseeded fruits. Values are mean±SD; $n=3$. Chl, Chlorophyll; a

Results and discussion

Physical dimensions and weight of ontogenial fruits

Physical dimensions of *B. rubra* red violet fruits were given in Table 1. The whole fruit ranged from 2.40 to 2.51 cm in circumference, whereas the circumference of the seeds ranged from 0.9 to 1.0 cm. The weight of 100 fruits was approximately 18.64 g, whereas 100 seeds weighed around 5.69 g. The fresh weight of pulp for 100 red violet fruits was 10.4 g. The weight of ontogenial fruits was shown in Fig. 1.

Measurement of tinctorial strength and chemical analysis of betalains

Table 2 shows the chemical characteristics and tinctorial strength of the *B. rubra* fruit extract prepared with different solvent systems such as water, methanol, ethanol, methanol:water (60:40) and ethanol:water (60:40). The TSS ($^{\circ}$ Brix) values in ethanol and ethanol:water (60:40) solvents were higher than water. Similarly the refractive index (RI), which indicates the presence of sugars, ethanol and ethanol:water (60:40) was marginally higher than that of water extract. The pH values were in the range of 5.6 to 7.24, which are in agreement with pH values reported in *Opuntia ficus-indicus* fruits (Saenz 2000). The colour of different solvent

extracts was monitored by measuring L, a^* and b^* values. Colour of *B. rubra* fruits from different solvents were given in Fig. 2a. The water extract showed higher chroma (48.74) values than alcohol extract (35.29).

Colour measurement

In addition to the photometric characterization, it is a common practice to measure colour in terms of L, a^* and b^* values. Khan et al. (2011) also reported the variation in colour measurement in ontogeny stage where pigment content increased in ripened *Tinospora cordifolia* fruit. Colour of *B. rubra* fruits from different ontogenial stages was given in Fig. 2b. The C^* (Chroma) of matured (35.92) fruits was highest and early stage (3.98) fruits were low which indicates the high content of betalains in matured fruits than early stage fruits. The L (lightness) and h^* (Hue angle) was more in case of early (63.28) and intermediate (0.03) stages of fruits.

Pigment characterization

The UV spectrum showing betalain pigments in the fruits of *B. rubra* ontogeny is shown in Fig. 3(1). The photometric results obtained for total betalains (betacyanins and betaxanthins) shown in Table 3 exhibits maximum pigment content in ripened fruits with a total betalain content (143.76 mg/100 g f. wt.), followed by intermediate (87.71±5.37) and early stage fruits having lowest (22.32±1.56). According to Lin et al. (2010), the major betalain pigment characterized in *B. alba* fruits was gomphrenin I the concentration of which increases on fruit maturity. The gomphrenin I pigment content quantified by HPLC was 26.06, 2.15 and 0.23 mg/100 g fresh weight of fruits in ripened, intermediate and early stage respectively (Fig. 3(2)). However Lin et al. (2010) had reported 36 mg/100 g of gomphrenin I pigment from ripened *B. alba* deseeded fruits on fresh weight basis.

Fig. 4 Total carbohydrate **a** content (g/100 g f. wt.) and texture measurement **b** of *B. rubra* fruits during ontogeny stages. Values are mean±SD. Significance was tested by Duncan Multiple Range Test at p 0.05, and values with different superscript were found to be significant difference from each other

Fig. 5 Ice cream prepared with pigment rich *B. rubra* fruit juice
a. control without pigment and b. ice cream with pigment

Kumar et al. (2014) had reported an increase in betalain content by fermentation using *Saccharomyces cerevisiae*.

It was suggested that changes in colour during development of fruits that contain vacuolar pigments may give valuable information on deciding the right time of harvest for market acceptability (Nergiz and Ergonul 2009). In this study, it was found that betalains accumulation starts in the intermediate stage (half-done red violet), which resulted in losing lightness (*L*) and yellowness (*b*), but increased chroma was observed due to increase in redness (*a*) from green to red violet stages of *B. rubra* fruits. Decrease in lightness and increase in redness of fruits on maturation owing to accumulation of more pigments (Nergiz and Ergonul 2009) and wax (Zheng et al. 2009) has been reported for various fruits, such as olive fruits and currant. Breakdown of chlorophyll and subsequent accumulation of pigments, such as carotenoids, anthocyanins and betalains have been associated with fruit colour change during ripening (Koning 1994; Khan and Giridhar 2014a).

Fig. 6 Consumer acceptance study of control ice cream and samples

Determination of chlorophyll content

The chlorophyll content during ontogeny of *B. rubra* fruits showed decrease in chlorophyll content during maturity stages as shown in Table 4. The chlorophyll content was high in green fruits with more or less equal ratio of *chl a* and *chl b*. But in ripened fruits as betalains were the dominant pigments, chlorophyll was not detected, indicating that the rate of photosynthesis was decreased on maturation (Khan et al. 2011).

Total carbohydrate content

Total carbohydrate content of *B. rubra* fruits was increased on ripening as shown in Fig. 4a. The green fruits exhibited the presence of lowest carbohydrate content (0.9 g), and the

Fig. 7 Colour intensity (Hunter) of pigment rich ice cream during storage. Values are mean±SD (*n*=3). Significance was tested by Duncan Multiple Range Test at *p* 0.05, and values with same superscript were found not significant difference from each other. Separate significance was conducted for *L*, chroma and hue

ripened fruits showed 1.8 times higher carbohydrate levels than the green fruits. During ripening many biochemical changes, such as an increase in sugar content has been reported in sapota, and cherry fruits (Ishida et al. 1997; Chaughule et al. 2002). Increase in carbohydrate content in ripened fruits may be attributed to a reduction in metabolic activities and these carbohydrate skeletons further facilitate seed development. A similar observation was found in fruits of olive (Nergiz and Ergonul 2009) and *S. album* (Khan and Giridhar 2014b) which contain anthocyanins. Similarly, increase in water content and sugar content on fruit ripening has been reported in cherry and sapota fruits (Ishida et al. 1997; Chaughule et al. 2002).

Earlier studies demonstrated high positive correlation (r) between pigment content and chroma, pigment content and a values; and carbohydrate and chroma in green, yellow and red *Tinospora cordifolia* fruits from different stages of development (Wilkinson et al. 1977) and also in *S. album* berries (Khan and Giridhar 2014b). Similar observations were made in the present study of *B. rubra* fruits during developmental stages.

Texture measurement of ontogenial fruits

Textural properties is one of the key factors influencing the acceptability of fruits to the consumer. The textural characteristics change as a result of physiological and biochemical processes which occur throughout ripening (Harker et al. 2010). The texture measurement at different ontogeny stages of fruits is an important criteria and can be measured by sensory and instrumental methods. Lokesh et al. (2014) reported that the instrumental method is more sensitive and reproducible than sensory method. In the present study, the fruits were ruptured through axial punctures and the maximum force required to penetrate into the fruit was recorded in Newtons (Fig. 4b). There was a steady gain of force applied for penetration from green fruits (0.16 N/s) to ripened fruits (0.38 N/s) indicating that softness of the green fruits over ripened fruits which may be attributed to their tender nature.

Preparation of ice cream containing pigment rich extracts of *B. rubra* fruits and sensory analysis

The sensory consumer acceptance data analysis of ice-cream samples (control-without colour and treated sample-with colour (Fig. 5) revealed that treated ice cream samples were rated Liked Very Much (LVM) by 60 % of the panelists, and 35 % rated it Like Moderately (LM) while remaining 3.5 % rated it Like Slightly (LS). In the case of control samples, 35.7 % of the panelists rated as LVM, 32 % rated LM and the remaining 32 % as LS. Thus, addition of colour to the ice-cream increased the acceptance scores compared to control sample (Fig. 6). It may be worthwhile to note that majority of panelists

responded to this product, and none of them had given negative rating, indicating the overall acceptance of the product. The measurement of colour values also indicated that there was no significant decrease in colour after 180 days of storage at -20°C (Fig. 7) with retention of 86.63 % colour. Similar studies on betalains efficacy in product development by using pigment rich extracts of pigeon berry was reported (Khan et al. 2014).

Conclusion

From these findings we conclude that, gomphrenin I was detected as major pigment during *B. rubra* fruits development and its content increases during fruit development. Similarly for the first time the changes in colour measurement values, total carbohydrates, physical dimensions and weight of ontogenial fruits had been achieved and these parameter are important determinants of processing parameters as well as organoleptic properties and are attentive to the food industry. The use of this betalain rich pigment as natural colourant in ice cream product development received consumer acceptability. The measurement of colour values also indicated that there was no significant decrease in colour after 180 days of storage at -20°C with retention of 86.63 % colour.

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