Stabilization of bioactive betalain pigment from fruits of *Basella rubra* L. through maltodextrin encapsulation

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**Abstract**

The ripened fruits of *Basella rubra* are rich in betalains that can be used as food grade natural colorants. Betalains were extracted from de-seeded fruits and encapsulated with maltodextrin and spray dried. The total betalains content of the fruit extracts was 348.2 with 296.6 betacyanins and 51.6 mg/100g of betaxanthins. Further, betacyanins and betaxanthins were confirmed by HPLC. The optimization of *B. rubra* fruit juice powder was devised using the spray drying parameters of inlet air temperature (IAT) (150 °C), maltodextrin (MD) addition rate 50%) and feed flow rate (FFR) (2.5 mL/h). Encapsulated betalains with maltodextrin showed about 10 fold increase in betalains content compared to normal fruit juice. Encapsulated betalain powder from fruits of *B. rubra* preserved at 4°C for two years and subjected to microbial analysis at six month interval and the quality was found good without any significant microbial count.

**Keywords:** Betalains, gomphrenin I, encapsulation, stability studies

**Introduction**

Betalains are the water soluble natural red and yellow colored indole derived pigments found in the vacuoles of plant cells and are having great potential as a natural dye in food processing industry and in pharma as antioxidant, anti-inflammatory, and detoxifying agents [1]. These pigments are well documented from plants of Caryophyllales and other plant species [2] [3].

Recent reports on Malabar spinach (*Basella rubra* L.) belongs to Basellaceae indicates the potential benefits of pigment rich fruit extracts in food applications [4] [5]. Moreover, the pink colored tender twines and leaves of *B. rubra* are known as a leafy vegetable [6]. Similarly, the phytonutrient composition and stability of betalain extracts using stabilizing agents have been reported [7]. During the last two decades significant contributions have been made towards understanding the betalains biosynthesis, chemistry, stability and physiological aspects, apart from various applications [8]. At pH range of 3-7, extracted form of betalains are fairly stable [9]. However, the stability of the pigment extract in food matrix is a concern while making appropriate formulations as it varies with different physical and physiological aspects, such as water activity, temperatures, exposure to oxygen, and light. In addition, upon application of colors to food, it should impart uniformity of color across batches of a product [10]. In this regard, encapsulation based formulations offer good stability to the pigment and also advantageous in view of their low water activity and easier transport and storage [11] [12]. Moreover,
encapsulated pigments serve as alternative for the substitution of artificial colorants for natural colorants [13]. Maltodextrin is one of the most suitable matrix for the betalains encapsulation when compared to chitosan and inulin etc. which was reported earlier for application in food industry [14]. In the present communication, we report the maltodextrin encapsulation of betalains pigment extract from the ripened fruits of *B. rubra*, along with stability studies of powder over a period of time.

**Materials and Methods**

**Materials**

Maltodextrin and HPLC grade methanol were procured from Himedia Chemicals (Mumbai, India). Trifluoroacetic acid (TFA) was purchased from Sigma-Aldrich Co (St. Louis, MO, USA). All other chemicals used were of analytical grade. For HPLC analysis, de-gassed and 0.22 mm membrane filtered Milli-Q water was used.

**Source of fruits**

*Basella rubra* L. ripened red-violet fruits were collected from 3-month-old twine of greenhouse maintained potted plants (Figure 1) at CFTRI, Mysore during March-April. The Herbarium specimen was deposited at the Herbarium Collection Centre, University of Mysore (Reference No. 02/08/05/13) upon confirmation of its taxonomical features. The fresh fruits were cleaned under running tap water, followed by ethanol disinfection and blotting on handmade filter paper to remove water and then subjected to extractions. For experiment purpose, 1.5-2 Kg fresh fruits were collected from the single plant and from this lot pigment extractions, spray drying and encapsulated betalain powder analyses were performed.

**Figure 1: Basella rubra** twines, colored leaves & stalk, inflorescence and fruits.

**Pigment extraction**

Manually de-seeded fruit pulp (1.5 - 2 Kg) was extracted using distilled water (1 L) with a mortar and pestle until the macerate becomes colourless. Then, the macerate was centrifuged at 12,000 rpm for 10 min. All clear supernatants were pooled and stored at -20 °C until use. The pigment extracts have been subjected to fermentation with *Saccharomyces cerevisiae* to reduce the fermented sugar constituents in the fruit extracts as reported earlier [15]. The clear juice obtained after fermentation was subjected to centrifugation at 12,000 rpm for 20 min and used for further experiments.

**Encapsulation of betalains**

The above prepared betalains rich extract was mixed with 50% maltodextrin (10 DE) as a carrier agent under vigorous vortexing. Particles were prepared in a Buchi B-290 mini spray dryer (Buchi Labortechnik AG, Flawil, Switzerland). The input air temperature was 150 °C and the outlet air temperature was kept at 68°C. The flow rate was maintained at 2.5mL/min with an atomization air flow 246 L/h, and the drying air flow was 36mL/h. The particles were separated from the drying air by a cyclone. The powders were stored in a desiccator over silica gel at 4°C until analysis.

**Encapsulated powder analysis**

**Estimation of moisture content**

The moisture content in the powder sample was performed according to AOCS method [16].

**Total betalains content**

The total betalain content was analysed in triplicates with spray dried betalain powder with the above said procedure. Quantification of total betalain content was performed using a UV-visible spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan). Total betalain concentration was determined with betacyanins and betaxanthins expressed in terms of betanin and vulgaraxanthin I with molar extinction coefficients of 60,000 L mol⁻¹ cm⁻¹ at 535 nm and 48,000 L mol⁻¹ cm⁻¹ at 477 nm, respectively. While calculating total betalains, a correction factor was integrated, resulting from the absorbance of light by impurities present in the sample, on the basis of measurement of extinction at 600 nm. Total pigment content was expressed as the sum of betacyanins and betaxanthins [17].

**HPLC analysis**

HPLC analysis of betalains was conducted using an HPLC pump equipped with a C₁₈ column (Sunfire, Waters Corporation, Milford, MA, USA) of 250 × 4.6 mm i.d. The UV detector was set at 477 and 535 nm [18].

**Scanning electron microscopy (SEM)**

The betalain powders were analysed for the outer structure of the micro encapsules through SEM. The powders were coated with gold on a double sided adhesive tape mounted on SEM stubs under vacuum using an SEM coating system and examined in a LEO Scanning Electron Microscopy 435 VP (Leo Electron Microscopy Ltd., Cambridge, UK).

**Stability of encapsulated betalains assessment**

The encapsulated betalain powder was stored at 4°C in subdued light condition. A portion of the sample was withdrawn at six month interval up to two years and analysed for total betalain content analysis in triplicates with the above mentioned procedure.

**Microbial analysis**

During the course of betalain powder storage at 4°C, the microbial assay was also performed to analyse the microbial contamination in the powder. One gram of the sample was
dissolved in 100mL of 0.9% saline. This solution was serially
diluted up to $10^{-4}$ g/mL. Later, 0.1mL of the diluted sample
was aseptically spread onto prepared media plated containing
plate count agar (for aerobic bacterial count), rose Bengal
agar (for yeast and mould count) and Violet Red Bile Agar
(VRBA) (for *E. coli* estimation) and Baird Parker Agar (BPA)
supplemented with 5mL egg yolk tellurite emulsion/100mL
(for *Staphylococcus aureus*). Triplicate plates were used for
each analysis with different dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$).
The inoculated plates were incubated at 37 °C for the total
bacterial count, 28 °C for *S. aureus* and colonies were observed
after 24 and 48 h. However, the fungal plates were incubated
for 5 days at 31 °C and observed for colony growth.

**Statistical Analysis**

All values presented are mean ± SD of three analyses. The
data was 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 when $p<0.05$.

**Results and discussion**

**Pigment quantification**

The photometric quantification of betalains from fresh
de-seeded *B. rubra* ripened fruits extract results total betalain
content (348.2 mg/100g) with 296.6 betacyanins and 51.6
mg/100g of betaxanthins. The obtained values were in
concomitant with the previous reports [4].

**Encapsulation of betalains**

Encapsulated Spray drying is a feasible downstream
processing technique for betalain production. The spray dried
samples of the fermented betalains extract was shown in
Figure 2. Betalains are highly stable at low water activities. This
phenomenon has been proved by a water-glucose system
where degradation was reduced by decreasing water activity
[19]. In view of this, *B. rubra* fruit pigments are a novel source
of natural colourant used in minimally processed foods, such
as desserts, drinks, yoghurts, ice creams, etc [5].

**Encapsulated powder analysis**

The moisture content of the encapsulated spray dried
betalain powder was about 3.2 ± 0.02%. Similar observations
with the bioactive compound encapsulation by maltodextrin
and inulin from cactus pear fruit was reported [12].

**Total betalain estimation**

The total betalain retention in the encapsulated betalain
powder showed that there was 96.81% pigment retention
after 2 years of storage at 4 °C (Figure 3). Hence, the spray
dried betalain powder can be further explored for its use in
food industry as a natural colourant.

**HPLC analysis**

According to earlier reports [18] the major betalain
pigment characterized in *B. alba* fruits were gomphrenin I the
concentration of which increases on fruit maturity. The
identification and characterization during ontogeny of *B.
rubra* fruit extracts by HPLC and MS was reported recently [4]
[5]. Lin et al [18]. had reported 36 mg/100g of gomphrenin I
pigment from ripened *B. alba* de-seeded fruits on fresh weight
basis. In the present study, the gomphrenin I pigment content

![Figure 2: Maltodextrin encapsulated betalain powder from fruits of *B. rubra.*](image)

![Figure 3: Total betalain content of maltodextrin encapsulated betalain powder from fruits of *B. rubra.* Vales are mean ± SD of three replicates.](image)

![Figure 4: HPLC profile of maltodextrin encapsulated betalain powder, betacyanin (535) and betaxanthin (477) from fruits of *B. rubra.* 1 = Betanin, 2 = Gomphrenin I and 3 = unknown peaks.](image)
quantified by HPLC on the spray dried betalain powder was 0.64 g/100 g (Figure 4). This study shows that spray dried betalain powder showed 25 folds more intense colour (in terms of gomphrine I) than the gomphren I pigment content in ripened fruits extract as reported earlier in fresh deseeded fruits [5].

**Scanning electron microscopy (SEM)**

The Figure 5 presents the SEM photograph of encapsulated betalain powder. The morphology of the spray dried powder was regularly spherical in shape. Similar morphology was reported for betalains in *Lampranthus productus* plant flowers [14].

![Figure 5: Scanning electron microscope (SEM) images for maltodextrin encapsulated betalain powder from fruits of *B. rubra*. The scale bar is 10µm in (a) and 2µm in (b).](image)

**Microbial analysis**

The prepared spray dried powders of betalains were free of all types of aerobic bacteria, coliforms, *Staphylococcus aureus*, yeast and moulds, etc. after two years of storage at 4°C. The total aerobic bacterial count observed was within the limit as shown in Table 1. There was no growth of any other microbes or moulds identified. This may be due to the presence of high dextrose content in the powder. The overall microbial load data showed indicates that the powder is safe for consumption and has a very good shelf life.

| Microorganism          | 0 day Viable count (cfu/g) | 6 months Viable count (cfu/g) | 1 year Viable count (cfu/g) | 2 year Viable count (cfu/g) |
|------------------------|---------------------------|-------------------------------|----------------------------|----------------------------|
| Total aerobic bacteria  | nil*                      | 38 ± 3                        | 76 ± 3                     | 114 ± 5                    |
| *S. aureus*            | nil*                      | nil*                          | nil*                       | nil*                       |
| *E. coli*              | nil*                      | nil*                          | nil*                       | nil*                       |
| Yeast and mould        | nil*                      | nil*                          | nil*                       | nil*                       |

All these observations are made by five replicates analysis over incubation.

**Conclusion**

Fruits of *B. rubra* were investigated for its encapsulation using maltodextrin to produce spray dried betalain powder. Encapsulated betalains with maltodextrin bettered by 10 folds for betalains compared to normal fruit juice. Even two years storage at 4°C did not hamper the quality of this spray dried powder. These findings provide value addition to *B. rubra* fruit betalain powder as a potential source of natural colour and as a functional food to the food industry.

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**Conflict of Interest:** The authors declare that there is no conflict of interest.

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