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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/100 g 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 hand made 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.

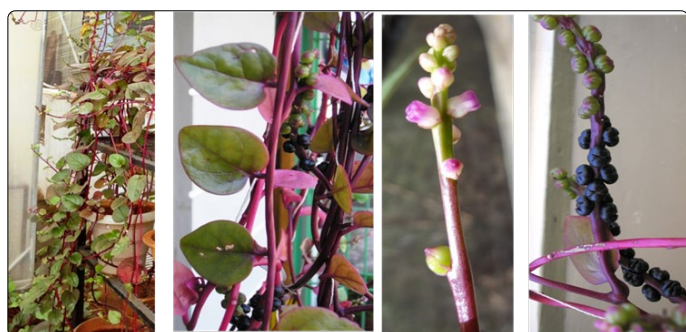


Figure 1. *Basella rubra* twines, colored leaves & stalk, inflorescence and fruits.

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 2.5 mL/min with an atomization air flow 246 L/h, and the drying air flow was 36 m³/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 vulgaxanthin I with molar extinction coefficients of 60,000 L mol⁻¹ cm⁻¹ at 535 nm and 48,000 L mol⁻¹cm⁻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 capsules 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 100 mL of 0.9% saline. This solution was serially diluted up to 10⁻⁴ g/mL. Later, 0.1 mL 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 5 mL egg yolk tellurite emulsion/100 mL (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/100 g) with 296.6 betacyanins and 51.6 mg/100 g 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].

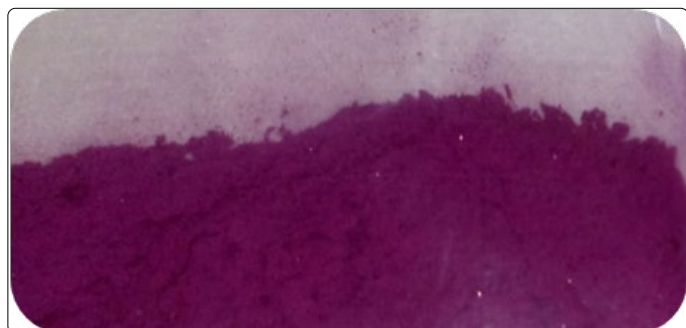


Figure 2. Maltodextrin encapsulated betalain powder from fruits of *B. rubra*.

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.

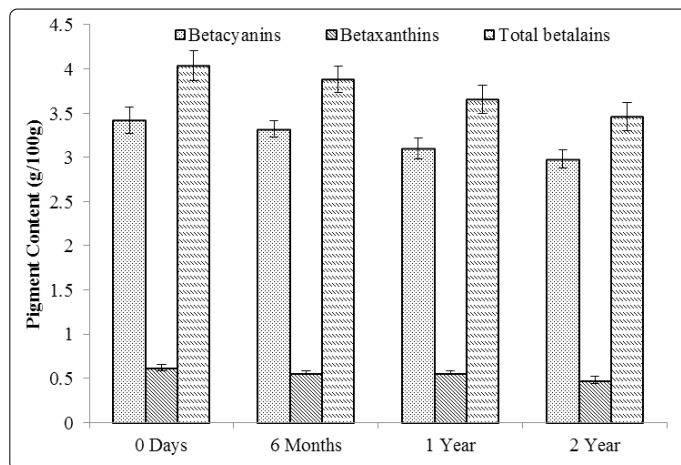


Figure 3. Total betalain content of maltodextrin encapsulated betalain powder from fruits of *B. rubra*. Values are mean ± SD of three replicates.

HPLC analysis

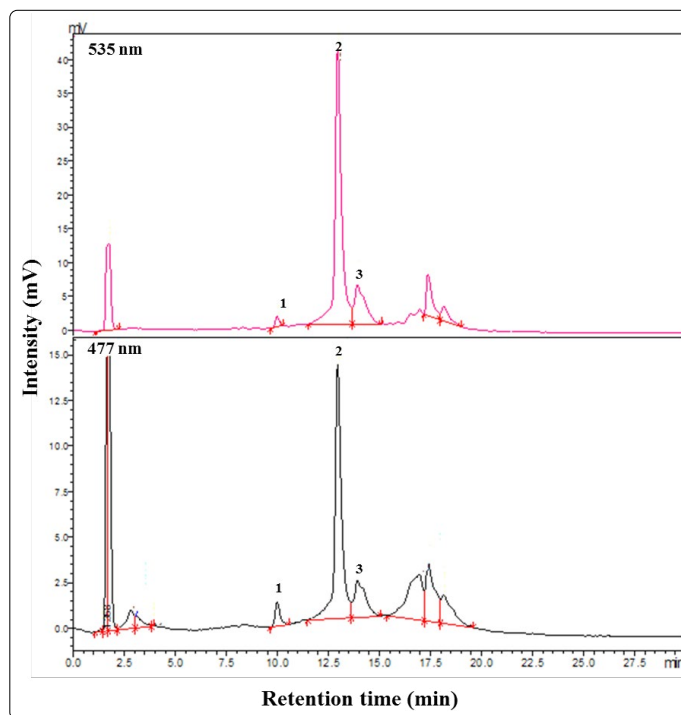


Figure 4. HPLC profile of maltodextrin encapsulated betalain powder, betacyanin (535) and betaxanthin (477) from fruits of *B. rubra*. 1 = Betanin, 2 = Gomphrenin I and 3 = unknown peaks.

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/100 g of gomphrenin I pigment from ripened *B. alba* de-seeded fruits on fresh weight basis. In the present study, the gomphrenin I pigment

content 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 gomphronein I) than the gomphrenin I pigment content in ripened fruits extract as reported earlier in fresh deseeded fruits [5].

Scanning electron microscopy (SEM)

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].

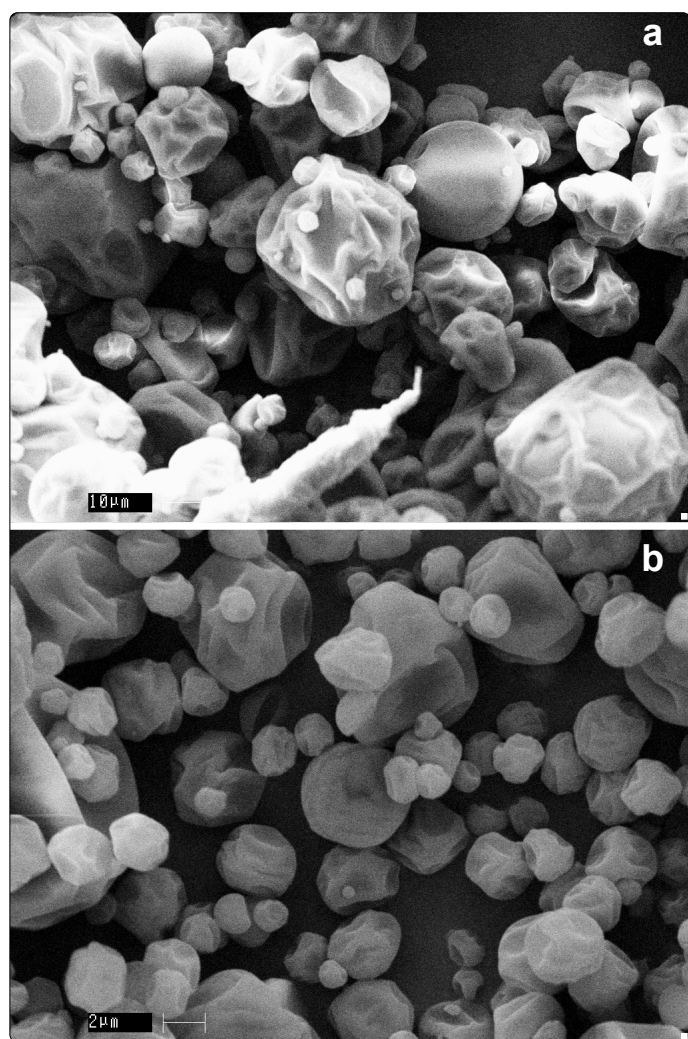


Figure 5. Scanning electron microscope (SEM) images for maltodextrin encapsulated betalain powder from fruits of *B. rubra*. The scale bar is 10 μm in (a) and 2 μm in (b).

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.

Table 1. Microbial profile of encapsulated betalain powder storage at 4°C from fruits of *B. rubra*.

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
<i>Staphylococcus aureus</i>	nil*	nil*	nil*	nil*
<i>E. coli</i>	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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