FINAL REPORT

Development of functional carambola juice incorporated with anthocyanins from bran of pigmented rice varieties of Assam

Submitted to

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1. Introduction

Rice bran composed of pericarp, seed coat, nucellus, aleurone layers and germ is a byproduct of rice milling and constitutes about 10% of the brown rice. Rice bran is known as a good source of proteins and other biologically active phytochemicals such as oryzanols, tocopherols, tocotrienols, and phenolic compounds (Iqbal *et al*., 2005; Yu *et al*., 2007 and Sookwong *et al*., 2007). These phytochemicals have been found to have antioxidative and disease-preventing properties. Their important functions include free radical scavenging, immune system enhancement and reduction in the risk of cancer and heart disease (Chotimarkorn *et al*., 2008; Butsat and Siriamornpun, 2010; Chen *et al*., 2010; Bhupathiraju and Tucker, 2011; Kannan *et al*., 2010 and Leardkamolkarn *et al*., 2010). Pigmented rice bran additionally is rich in anthocyanins.

Anthocyanins are a class of water soluble natural pigments that belong to the large family of flavonoids, responsible for the red, purple, and blue colors of many plant materials (Kowalczyk *et al*., 2003). There are six types of anthocyanidins that are widespread in fruits and vegetables round the world such as pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (De Pascual-Teresa *et al*., 2002; Di Paola-Naranjo *et al*., 2004 and Lohachoompol *et al*., 2008). Cyanidin-3-glucoside (C3G) is the primary anthocyanin constituent in black rice (Ichikawa, 2001). Increasing evidence suggests that anthocyanins are potent antioxidants, which are associated with protective effects observed against inflammation, atherosclerosis, carcinoma, and diabetes (Timberlake, 1996; Bagchi *et al*., 2004). Purified C3G has been found to reduce adipose inflammation and hepatic steatosis in high-fat diet-fed mice (Guo et al, 2006), as well as hyperglycemia in diabetic mice (Sasaki et al, 2007; Nasri et al, 2011). These pigments can exist in different structural forms and many factors have effect on their actual color and stability (Delgado-Vargas and Paredes-Lopez, 2003). Health promoting anthocyanins are not stable and are easily destroyed by a number of factors such as pH, light, oxygen, enzyme, ascorbic acid, thermal treatment, sulfur dioxide or sulfate salts, metal ions and co-pigments (Gradinaru, *et al*., 2003; Tiwari, *et al*., 2009; Zhang *et al*., 2008). The colour stability of anthocyanins mainly depends on the pH and appears red in acidic, violet in neutral, and blue in basic aqueous solution (Goto & Kondo, 2003). Preventing of anthocyanins degradation is an important factor for both processors and consumers. Numerous studies have suggested that natural compounds in food can be important

modulators in the prevention of a variety of chronic diseases (Cheetham and Katz, 2011; Yao et al, 2011).

Of late, consumer interest in anthocyanins pigment has increased due to their potential health benefits. The natural anthocyanin pigment is commercially used in candy, bakery products, juice powder and gelatins. It is basically used in food with lower pH values (up to 3.5) because of it greater stability in acidic conditions (Barros and Stringheta, 2006). In this first year report, extraction of anthocyanins from a coloured rice bran, its identification and properties were studied.

2. Materials and Methods

The experiment was conducted in the Department of Food Engineering and Technology, Tezpur University, Assam, India.

2.1 Chemicals

Anthocyanin standard (C3G) also was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Maltodextrine (Dextrose equivalent, DE 20%) from maize starch was from HiMedia Laboratories Pvt. Ltd, Mumbai, India. Other solvents and reagents used were of analytical grade and from laboratories stock.

2.2 Raw material

Pigmented rice cultivar was purchased from Assam Agricultural University, India. Paddy was air dried before processing. A local non pigmented paddy variety was procured from the local market nearby Tezpur University, Tezpur.

2.3 Sample preparation

Rice milling

Paddy was dehusked in a dehusker and brown rice was passed through an abrasive polisher to remove bran from brown rice and passed through a 60 mesh screen sieve. The collected pigmented bran was used for anthocyanin extraction. The non pigmented paddy was similarly milled as for pigmented rice. The collected non pigmented rice bran was used for protein extraction.

Defatting of rice bran

Rice bran was defatted before extraction of anthocyanin. Rice bran (5g) and diethyl ether (25 mL) were mixed (1:5 ratio) together in an air tight jar. The air tight jar was placed in a shaking machine set at 180 rpm at 20 °C. The solvent was separated from solid part by decanting after 24 h. The defatted rice bran was air-dried for 24 h and stored at 6-8^{o}C until further studies.

Extraction of anthocyanin from black rice bran using ethanol extraction method

A modified method of Daungmal *et al*. (2008) was followed to extract anthocyanin (pigment). Black rice bran (10 g) was soaked in 100 mL of ethanol containing 2 % citric acid at pH 2. The mixture was kept in the shaking incubator (Sartorius, USA) at 20^{o}C and at the speed of 180 rpm for 24 h. After incubation, the ethanol extract was filtered with Whatman no. 4 filter paper and the filtrate was concentrated using rotary vacuum evaporator at 30^{o}C to remove the ethanol. The anthocyanins extract from coloured rice bran (AECB) was stored at 4^{o}C until further use.

Purification of the anthocyanin extract in Amberlite XAD-16 column chromatography

For further purification of anthocyanins extract column chromatography with Amberlite XAD 16N resins was used. At first, the ethanol extract was filtered through Whatman filter paper and centrifuged again for 10 min. Aliquots of 100 mL of the extracts were applied to the Amberlite XAD-16 resin (Kammerer *et al*. 2005), which was washed with 50 mL of distilled water. The extract was subsequently rinsed with 125 mL distilled water and with 50 mL acidified water at a flow rate of 3 mL/min in the glass column. Acidified ethanol (200 mL) was used for elution of the pigments. During sample application, washing and elution of the pigments, the fractions of the dark red color was collected. This isolated anthocyanin extract was used for further study.

Extraction of protein from local rice bran

Defatted non pigmented rice bran (10g) was mixed with 75 mL of water and pH was adjusted at 5.0. Enzymes viz. xylanase (1g, activity 2500unit/g) and phytase (0.1g, activity 0.04 unit/mg) were mixed with the sample and incubated for 2 h at 55 °C. The enzymes were then inactivated by raising the pH to 10.0 and the mixture was centrifuged for 20 min at 6000 rpm. The supernatant was decanted and pH of residue was adjusted at 4.0. The mixture was centrifuged for 5 min at 3500 rpm and residue was collected. The residue was neutralized on bringing the pH to 7.0 and stored under refrigerated conditions (Wang *et al*., 1999). This isolated protein residue was used for microencapsulation of anthocyanins extracted from rice.

2.4 Preparation of anthocyanin rich microencapsulates

Preparation of feed mixture

Two feed mixtures, viz. one comprising of 100 g maltodextrin (DE 20) and 50 ml AECB extracts and the other mixture of 100 g maltodextrin, 250 ml protein extracts and 50 ml AECB extracts, were spray dried to obtain anthocyanin encapsulated powders EP1 and EP2 respectively. The mixtures were stirred to homogeneity with IKA T25 digital Ultra-

Turrax, Germany, for 10 min. The final total soluble solid (TSS) content of the mixture before spray drying was lowered to 11 °Brix.

Spray drying conditions

The feed mixtures were spray dried in a Lab Spray Drier (SD-Basic LabPlant, England). The drier was operated at 175 °C inlet and 76±2 °C outlet temperature, respectively. The pump pressure was kept 75 psi to maintain feed flow rate 6 mL/min, and air blowing and compressor capacities were kept at maximum.

2.5 Analytical methods

Determination of total anthocyanin content (TAC)

TAC was determined by the pH-differential method described by AOAC (2005), using two buffer systems: potassium chloride buffer at pH 1.0 (0.025 M) and sodium acetate buffer at pH 4.5 (0.4 M). An aliquot of the extract was transferred to a 10 mL volumetric flask and made up to 10 mL with corresponding buffer and the absorbance was measured at 520 and 700 nm by UV-VIS spectrophotometer (CECIL, CE 7400, 7000 Series). The reason for measuring the absorbance at 700 nm is to correct for haze. TA was calculated as cyanidin-3-glucoside according to the following equation:

$$TA\ (mg/L) = (\Delta A / \varepsilon) \times l \times M \times 10^3 \times D$$

Where $\Delta A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$; ε (molar extinction coefficient) = 26,900 L/mol/cm for cyanidin-3-glucoside; l, path length in cm; M (molecular weight) = 448.8 g/mol for cyanidin-3-glucoside; D, dilution factor; 10^3, conversion from gram to milligram.

HPLC analysis of anthocyanins

Identification of anthocyanins was carried out with an HPLC system (Waters, USA) equipped with Symmetry ® C18 (5 μm, 4.6 X 250 mm) column and a 535 nm (UV/Visible, Waters 2489) detector, using an injection volume of 20 μL, and operated at room temperature with a flow rate of 1 mL/m. The experimental conditions were as described by Chen et al. (2012). Elution was carried out with $H_2O/CH_3CN/CH_3OH/CH_3COOH$ = 40/29.5/29.5/1, v/v/v/v (solvent A) and H_2O/CH_3COOH = 99/1, v/v (solvent B) as follows: 7 min, 7% A and 93% B; 35 min, 25% A and 75% B; 10 min, 65% A and 35% B. Two anthocyanin standards: cyanidin-3-glucoside (C3G) and peonidin-3-glucoside (P3G) were used for qualitative analysis of the AECB samples. The peak times were compared to those of the standard anthocyanins. All the samples were analysed in triplicate to ensure reproducibility.

Determination of DPPH radical scavenging activity

DPPH free radical scavenging activity of the AECB extract was measured according to the method of Brand-Williams, Cuvelier, and Berset (1995). Precisely, 100 µL of extracts was added to 1.4 mL DPPH radical methanolic solution (10^{-4} M). The absorbance at 517 nm was measured at 30 min against blank (100 µL methanol in 1.4 mL of DPPH radical solution) using a UV-Vis Spectrophotometer (Cecil Aquarius 7400). The results were expressed in terms of radical scavenging activity.

Radical scavenging acitivity (%) = [(Ao-As)/ Ao] × 100

Where, Ao is absorbance of control blank, and As is absorbance of sample extract.

Determination of ferric reducing antioxidant property (FRAP)

FRAP activity of the samples was measured by the method of Benzie and Strain (1996). Briefly, a 40 µL aliquot of properly diluted sample extract was mixed with 3 mL of FRAP solution. The reaction mixture was incubated at 37°C for 4 min and the absorbance was determined at 593 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400) against a blank that was prepared using distilled water. FRAP solution was pre warmed at 37°C and prepared freshly by mixing 2.5 mL of a 10 mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution in 40 mM hydrochloric acid with 2.5ml of 20mM ferric chloride and 25 mL of 0.3M acetate buffer (pH 3.6). A calibration curve was prepared, using an aqueous solution of ferrous sulfate (1-10 mM). FRAP values were expressed as µM of ferrous equivalent Fe (II) per 100 mL of sample.

Determination of soluble protein content

Lowry's method (Niamke *et al.,* 2005) was followed. Four reagents were used, namely reagent A containing of 2% sodium carbonate in 0.1N sodium hydroxide; Reagent B containing 0.5% copper sulphate in 1% potassium sodium tartrate; Reagent C containing mixed 50 mL of reagent A and 1 mL of reagent B prior to use; and Reagent D comprising of Folin-Ciocalteau reagent. A volume of 0.1 mL of the sample extract was taken in two test tubes to which water was added and volume was made up to 3 mL in all the test tubes. Other two test tubes with 3 mL of water were taken to served as blank. Then, 5 mL of reagent C was added to each tube including the blank. All the tubes were vortexed and allowed to stand for 10 min. Finally, 0.5 mL of reagent D was added, vortexed and incubated for 30 min at room temperature in the dark place to allow blue color development. Absorbance of mixtures was measured at 660 nm with a UV-VIS spectrophotometer (CECIL, CE 7400, 7000 Series) against control blank samples. The absorbance was converted to mg/g of protein with standard graph of bovine serum albumin.

Moisture content of microencapsulates

The moisture content of the prepared fruit powder samples were determined by AOAC (1995) method. Briefly, 5 g of the fruit powder sample was taken in previously dried and weighed covered dishes. The sample was allowed to dry in a hot air oven (Jiotech, South Korea) at 105°C for 8 h till a constant weight was attained. The final weight of the dish containing the sample was measured both before and after drying and moisture content was calculated.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_2} \times 100$$

Where, W_1 is the weight of the sample with the dish before drying; W_2 is the final weight of the sample with dish after drying.

Determination of solubility of encapsulated powder materials

The method of Singh and Singh (2003) was followed. It consisted of determining the solubility of the spray dried sample in cold water. A 1% (w/v) powder suspension was agitated for 30 min using a shaker. The suspension was then centrifuged at 1200 rpm for 10 min and 25 mL aliquot from the supernatant was taken and deposited in a porcelain pan and subjected to a temperature of 110 °C for 4 h in a drying oven. The solubility was calculated according to the following equation.

Solubility (S) % = {(Grams of supernatant solids X 4) X 100} / (Grams of sample)

Hygroscopic moisture (HM) of encapsulated powder materials

Encapsulated powder materials (10 g) were weighed accurately and kept in desiccators with saturated sodium chloride solution at relative humidity 75%. The desiccators were kept in an incubator at temperature 30 °C for 7 days. The hygroscopic moisture (HM, %) was measured by the following equation:

% HM = (weight of water absorbed / total weight of sample) X 100

Total anthocyanin content (TAC) of encapsulated powder materials

TAC was determined by the pH-differential method described by AOAC (2005), using two buffer systems: potassium chloride buffer at pH 1.0 (0.025 M) and sodium acetate buffer at pH 4.5 (0.4 M). An aliquot of the extract was transferred to a 10 mL volumetric flask and made up to 10 mL with corresponding buffer and the absorbance was measured at 520 and 700 nm by UV-VIS spectrophotometer (CECIL, CE 7400, 7000 Series). The reason for measuring the absorbance at 700 nm is to correct for haze. TA was calculated as cyanidin-3-glucoside according to the following equation:

$$\text{TA (mg/L)} = (\Delta A / \varepsilon) \times l \times M \times 10^3 \times D$$

Where $\Delta A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$; ε (molar extinction coefficient) = 26,900 L/mol/cm for cyanidin-3-glucoside; l, path length in cm; M (molecular weight) = 448.8 g/mol for cyanidin-3-glucoside; D, dilution factor; 10^3, conversion from gram to milligram.

Determination of encapsulation efficiencies

In order to evaluate the efficiency of encapsulation, TAC and surface anthocyanins content (SAC) were determined after spray drying. For the TAC determination, the prepared encapsulated powdered (1g) was accurately weighed and dispersed in 10 mL solvent (ethanol: acetic acid: water = 50: 8: 42, v: v: v). Dispersion was agitated using a vortex for 1 min. The supernatant was centrifuged at 12896 g for 5 min and then filtered.

For the SAC determination, encapsulated powdered (100mg) was treated with 10 mL of ethanol: methanol (10: 1, v: v) solvent. This dispersion was agitated in a vortex at room temperature for 1 min and then filtered. TAC and SAC values were determined by the above-mentioned method and efficiency was calculated according to the following equation:

$$\% \text{ efficiency} = [(TAC - SAC)/TAC] \times 100$$

Scanning of encapsulated powders by scanning electron microscopy (SEM)

Morphology of the encapsulated powder was observed under a Scanning Electron Microscope (SEM, JEOL 6993V). Dried powder samples were sputter coated with platinum and the images were taken at an accelerating voltage of 15 kV and magnification of 1000 X, 2000 X and 3000 X.

Color measurement of encapsulated powder materials

The L*, a* and b* color values of the different rice flour samples were measured using Color Measurement Spectrophotometer (Hunter ColorLab Ultrascan Vis) where 'L*' indicates degree of lightness or darkness (L* = 0 indicates perfect black and L* = 100 indicates most perfect white); 'a*' indicates degree of redness (+) and greenness (−); whereas 'b*' indicates degree of yellowness (+) and blueness (−). Hue angle (H°) and Chroma (C*) were determined.

3. Results and Discussion

3.1 Anthocyanin content in pigmented rice bran

Pigmented rice contained 52g of bran per kg of paddy. Pigmented rice bran contained 5.09 mg anthocyanins per gram of bran (Table 1). Park *et al.* (2008) has reported total anthocyanins content of 1214.85 mg per kg of black rice. Extraction of anthocyanins was

carried out under cold conditions with ethanol containing a small amount of acids as it helps in the retention of the flavylium cation form, which is stable in acidic medium (Strack and Wray, 1989).

3.2 Protein content in non pigmented rice bran

Protein content in local rice bran was 5.24 %. This protein was used as carrier agent for encapsulation of anthocyanins (Table 1).

Table 1. Anthocyanin and protein content in rice bran

Anthocyanin content in pigmented bran	0.5%
Protein content in non pigmented bran	5.24%

3.3 HPLC analysis of AECB extracts

Cyanidin-3-glucoside is the principal anthocyanin in rice followed by peonidin-3-glucoside (Fossen et al., 2002). For identification of the anthocyanins, two standards, namely cyanidin-3-glucoside and peonidin-3-glucoside was allowed to run first (Fig. 1b and 1a). Only cyanidin-3-glucoside was detected in the AECB extracts and peonidin-3-glucoside was not detected in the AECB extracts (Fig. 1c). However, another form of anthocyanin emerged at a retention time of 54 min (Fig. 1c), which needs to be identified.

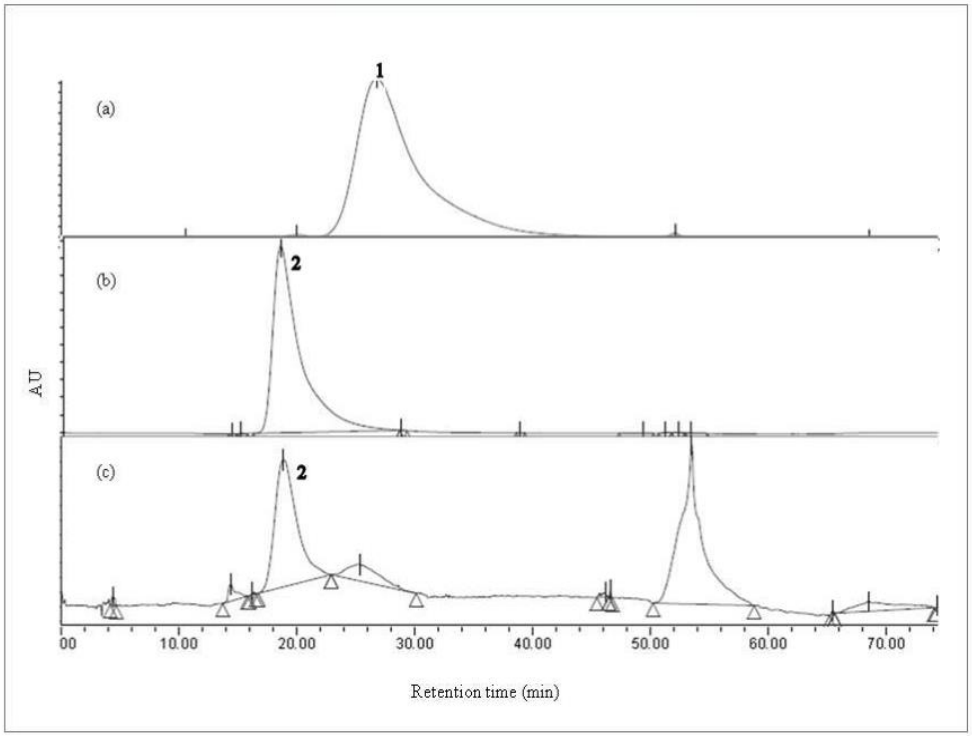

Fig.1. Typical HPLC chromatogram of standard anthocyanin standards and anthocyanin from coloured rice bran. (a) Chromatogram of Peonidin-3-glucoside standard. (b) Chromatogram of Cyanidin-3-glucoside standard. (c) Chromatogram of anthocyanin from Chakhao Poireiton. Peaks: 1, peonidin-3-glucoside; 2, cyanidin-3-glucoside.

3.4 Antioxidant assays

The ethanolic extracts were diluted with distilled water (1:5, dilution) before the DPPH free radical scavenging assay and FRAP reducing assays were done. The DPPH free radical scavenging activity of the ethanolic extracts of anthocyanin extracted from rice bran was found to be 71.93±0.02%. The antioxidant power of the ethanolic extracts of anthocyanin measured by FRAP assay was found to be 22.95±0.13 µmol ascorbic acid equivalents (AAE) per gram sample (Table 2). Chung and Shin (2007) reported the DPPH activity of the ethanolic extracts of aleurone layer of some *Oryza sativa* varieties, with values ranging from 58.8 to 85.6%. The DPPH activity of the isolated anthocyanin extracts were in the range, reflecting that the ethanolic extracts of the isolated anthocyanins showed good free radical scavenging activity and reducing power.

Table 2. Antioxidant properties of the anthocyanin extract

DPPH activity	71.93±0.02%
FRAP assay	22.95±0.13 µmol ascorbic acid equivalents (AAE) per gram sample

3.5 Parameters of the spray dried encapsulated powders

The protein content of the AECB extracts as estimated by Lowry's method was found to be 4%. In terms of yield, 44.4 g of encapsulated powder EP1 (Fig. 1a) was obtained on spray drying of mixture consisting of maltodextrin and anthocyanin extract. Similarly, 41.0 g of encapsulated powder EP2 (Fig. 1b) was obtained on spray drying of mixture consisting of maltodextrin, anthocyanin extract and protein extract.

The moisture content of the encapsulated powders is presented in Table 3. The moisture content of encapsulated powder EP2 was found to be higher than the moisture content of encapsulated powder EP1.

The hygroscopicity of encapsulated powder EP1 and EP2 are presented in Table 3. The hygroscopicity of encapsulated powder EP1 was found to be higher than that of

encapsulated powder EP2. Ersus and Yurdagel (2005) reported that the hygroscopic moisture of spray dried powders increased with increase in DE value of maltodextrin. Maltodextrins contained more hydrophilic groups because of low molecular weight (Cai & Corke, 2000).

The solubility of encapsulated powder EP1 and EP2 are presented in Table 3. The solubility power of encapsulated powder EP1 was found to be higher than that of encapsulated powder EP2. Maltodextrin of different dextrose equivalent (DE) are commonly used because of its high water solubility (Avaltroni et al., 2004) and contained more hydrophilic groups because of lower molecular weight**.**

The encapsulation efficiency of encapsulated powder EP1 and EP2 are presented in Table 3. Robert et al. (2010) reported that the encapsulation efficiencies of the maltodextrin encapsulated pomegranate juice and maltodextrin encapsulated pomegranate ethanol extracts ranged between 89.4-100 and 96.7-100%. The encapsulation efficiency of encapsulated powder EP1 and EP2 was found to be in the range and thus encapsulated powders with good encapsulation efficiency were obtained. Encapsulation efficiencies are defined as the potential of the wall material to encapsulate or hold the core material inside the microcapsule and it is also related to the shelf life of the anthocyanin content in the powder (Idham et al., 2007). The anthocyanins encapsulating efficiency was significantly better in Maltodextrin (MD) matrix. This behaviour could be related with the bioactive compounds nature (i.e. charge: positive for anthocyanins) and with polyelectrolyte structure (type and density charge: MD, a polysaccharide), conditioning the bioactive–polymer interaction (Robert et al., 2010).

Table 3. Parameters of the spray dried encapsulated powders.

Encapsulated powders	Yield (%)	Moisture content (%)	Hygroscopicity (%)	Solubility (%)	Encapsulation efficiency (%)
EP1	44.4	4.0	25.7	95	96
EP2	41.0	4.5	16.7	92	100

$C^* = (a^2 + b^2)^{1/2}$; $H° = \tan^{-1}(b/a)$; Data are average of triplicates.

The L*, a*, b*, C* and H^o values of the encapsulated powder are shown in the Table 4. The L* values of both EP1 and EP2 indicated that the powders were bright. The

a*, b* and C* values of the encapsulated powders indicated that both the powders EP1 and EP2 were purple shade of red colour (Fig.2).

Table 4. Colour properties of spray dried encapsulated powders.

Encapsulated powders	Colour values				
	L*	a*	b*	C*	H°
EP1	84.47	6.75	-1.76	6.97	-14.61
EP2	78.37	4.05	-0.43	4.07	-6.06

(a) **(b)**

Fig.2. (a) Encapsulated powder (EP1) containing maltodextrine as carrier agent and anthocyanin extract. (b) Encapsulated powder (EP2) containing maltodextrine as carrier agent, anthocyanin extract and bran proteins.

4. Conclusions

Pigmented rice bran contains anthocyanin pigments that can be a superior source of antioxidants if added to food products. It also offers a method of utilizing the by product of rice milling. Addition of protein helps in improving the properties of spray

encapsulates of anthocyanin. These encapsulates can be added to fruit juice for improving its functional properties.

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Charu Lata Mahanta

(C.L.Mahanta)

PI

Department of Food Engineering and Technology
Tezpur University

22/05/2018

Mr. Rahul Tamuli
Project Scientist
Innovation Technology Generation & Awareness
S&T Division
Assam Science Technology and Environment Council
Bigyan Bhawan, G.S. Road
Guwahati-5

Sub: Submission of Final Report & UC

Ref: Sanctioned order No. ASTEC/S&T/1614(4/1)/2014-15/3769 dated 16/2/2016

Dear Sir,

Please find enclosed herewith the Final Report of the project referred to above for favour of your necessary action. The Utilisation Certificate and Statement of Expenditure of the project are also enclosed. Kindly reimburse an amount of Rs 76,307/- for the expenditure incurred during the second year of the project.

Regards,

Yours sincerely,

Charu Lata Mahanta 22/05/2018
(Charu Lata Mahanta)

Encl as stated

UTILIZATION CERTIFICATE

FOR THE FINANCIAL YEAR 2017-2018 (01/04/2017-31/03/2018)

1. Title of the Project/ Scheme	Development of functional carambola juice incorporated with anthocyanins from bran of pigmented rice varieties of Assam
2. Name of the Institution	Tezpur University
3. Principal Investigator	Prof. Charu Lata Mahanta
4. ASTEC letter No. & date sanctioning the project	ASTEC/S&T/1614(4/1)/2014-15/3769 Dt.16/02/2016
5. Head of account as given in the original sanction letter	Non-recurring = NIL Recurring = ₹ 2,65,000/-
6. Amount brought forward from the previous financial year	(-) ₹ 20/-
7. Amount received during the financial year	i Amount ₹ NIL
	ii Letter No. NIL
	iii Date NIL
8. Total amount that was available for expenditure during the financial year	(-) ₹ 20/-
9. Actual expenditure (excluding commitments) incurred during the financial year (Upto 31st March, 2016)	**₹ 76,287/-**
10. Balance amount available at the end of the financial year	**Excess expenditure ₹ 76,307**
11. Amount to be carried forward to the next financial year	NIL

UTILIZATION CERTIFICATE

Certified that out of ₹ **NIL** of grants-in-aid sanctioned during the year 2017-18 in favour of Registrar, Tezpur University from Assam State Technology and Environment Council and excess expenditure of **₹ 76,307/-** was made for the purpose of project for which it was sanctioned during the year 2017-18.

Charu Lata Mahanta

Signature of Principal Investigator with date

Prof. Charu Lata Mahanta
Dept. of Food Engg. & Tech.
Tezpur University
Tezpur -784 028, Assam

21/5/18

Signature of Registrar/ Accounts Officer

Finance Officer
Tezpur University

Signature of Head of the Institute

Registrar
Tezpur University

UTILIZATION CERTIFICATE

FOR THE FINANCIAL YEAR 2016-2017 (01/04/2016-31/03/2017)

1. Title of the Project/ Scheme	Development of functional carambola juice incorporated with anthocyanins from bran of pigmented rice varieties of Assam
2. Name of the Institution	Tezpur University
3. Principal Investigator	Prof. Charu Lata Mahanta
4. ASTEC letter No. & date sanctioning the project	ASTEC/S&T/1614(4/1)/2014-15/3769 Dt.16/02/2016
5. Head of account as given in the original sanction letter	Non-recurring = NIL Recurring = ₹ 2,65,000/-
6. Amount brought forward from the previous financial year	₹ 1,85,000/-
7. Amount received during the financial year	i Amount ₹ NIL
	ii Letter No. NIL
	iii Date NIL
8. Total amount that was available for expenditure during the financial year	₹ 1,85,000/-
9. Actual expenditure (excluding commitments) incurred during the financial year (Upto 31st March, 2016)	₹ **1,85,020/-**
10. Balance amount available at the end of the financial year	₹ NIL
11. Amount to be carried forward to the next financial year	(-) ₹20

UTILIZATION CERTIFICATE

Certified that out of ₹ **1,85,000/-** of grants-in-aid sanctioned during the year 2015-16 in favour of Registrar, Tezpur University from Assam State Technology and Environment Council and ₹ **1,85,020/-** has been utilized for the purpose of project for which it was sanctioned and the excess expenditure of ₹ **20/-** at the end of the year has been carried forward and will be adjusted during the year 2017-18.

Charu Lata Mahanta 26/5/2018
Signature of Principal Investigator with date
Prof. Charu Lata Mahanta
Dept. of Food Engg. & Tech.
Tezpur University
Tezpur -784 028, Assam

24/5/18
Signature of Registrar/ Accounts Officer
Finance Officer
Tezpur University

Signature of Head of the Institute
Registrar
Tezpur University

UTILIZATION CERTIFICATE

FOR THE FINANCIAL YEAR 2015-2016 (16/02/2016-31/03/2016)

1. Title of the Project/ Scheme	Development of functional carambola juice incorporated with anthocyanins from bran of pigmented rice varieties of Assam
2. Name of the Institution	Tezpur University
3. Principal Investigator	Prof. Charu Lata Mahanta
4. ASTEC letter No. & date sanctioning the project	ASTEC/S&T/1614(4/1)/2014-15/3769 Dt.16/02/2016
5. Head of account as given in the original sanction letter	Non-recurring = NIL Recurring = ₹ 2,65,000/-
6. Amount brought forward from the previous financial year	NIL
7. Amount received during the financial year	i Amount ₹ 1,85,000/-
	ii Letter No. ASTEC/S&T/1614(4/1)/2014-15/3769
	iii Date 16/02/2016
8. Total amount that was available for expenditure during the financial year	₹ 1,85,000/-
9. Actual expenditure (excluding commitments) incurred during the financial year (Upto 31st March, 2016)	₹ **0.00**
10. Balance amount available at the end of the financial year	₹ 1,85,000/-
11. Amount to be carried forward to the next financial year	₹ 1,85,000/-

UTILIZATION CERTIFICATE

Certified that out of ₹ **1,85,000/-** of grants-in-aid sanctioned during the year 2015-16 in favour of Registrar, Tezpur University from Assam State Technology and Environment Council and ₹ **NIL** has been utilized for the purpose of project for which it was sanctioned and the balance of ₹ **1,85,000/-** remaining unutilized at the end of the year has been carried forward and will be adjusted during the year 2016-17.

Charu Lata Mahanta 26/3/2018

Signature of Principal Investigator with date

Prof. Charu Lata Mahanta
Dept. of Food Engg. & Tech.
Tezpur University
Tezpur -784 028, Assam

21/5/18

Signature of Registrar/ Accounts Officer

Finance Officer
Tezpur University

Signature of Head of the Institute

Registrar
Tezpur University

Consolidated Statement of Expenditure

Sr. No	Sanction Heads (I)	Budget approved (₹) (I)	Fund (installment) recd (₹) (III)	Expenditure (₹)(IV)			Total Expenditure (₹) (V)
			1st	16/02/2015 to 31/03/2016	01/04/2016 to 31/03/2017	1/4/2017 to 15/02/2018	
1	Consumables	230000	₹ 185000/-	NIL	185020	46267	230000
2	Raw materials	15000		NIL		15000	15000
3	Travel	20000		NIL	NIL	15020	16307
8	Total (₹)	**265000**		**NIL**	**185020**	76287	**261307**

Fund allocated : ₹ 265000/-
Fund Released : ₹ 185000/-
Fund Utilized : ₹ 261307/-

Balance to be released: ₹ 76307/-

Charu Lata Mahanta

Name and signature of Principal Investigator
Date: 21/5/2018

Prof. Charu Lata Mahanta
Dept. of Food Engg. & Tech.
Tezpur University
Tezpur -784 028, Assam

21/5/18

Signature of Competent financial authority (with seal)
Date:

Finance Officer
Tezpur University