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MICROENCAPSULATION OF ANTHOCYANIN EXTRACTS FROM MUSA ACUMINATA BRACTS AND ITS APPLICATION AS A POTENT BIOCOLOUR

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The significance of biocolours such as anthocyanins had been known for decades. However, studies on anthocyanins from banana flower bracts are limited. Banana inflorescence bracts are underexploited and can be a cheap source of anthocyanin as they are usually discarded while cooking the flower. The vulnerability of anthocyanin in terms of stability is well known, hence, studies indicate that microencapsulation by spray drying is the best method to tackle this problem. The anthocyanin content in *Musa acuminata* bracts were assessed spectrophotometrically and the anthocyanin extract was microencapsulated using maltodextrin DE20 by spray drying. Sensory evaluation of the yoghurt with spray dried microencapsulated anthocyanin powder (SMAP) was conducted using 9 point hedonic scale and compared to a synthetic dye. Spectrophotometric analysis at 535 nanometer showed that the SMAP from *Musa acuminata* bracts when incorporated to plain yoghurt at a concentration of 0.67%, was very much comparable to commercial brand strawberry yoghurt. Results indicated that even though tinctorial strength of SMAP was very low, yoghurt with SMAP was more preferred over the sample with synthetic dye and the control sample which was devoid of colourant ($p < 0.05$); thus proving the potential of banana bract anthocyanins as a natural food colourant.

Keywords: Biocolours, Anthocyanins, Microencapsulation, Spray drying, Sensory evaluation

INTRODUCTION

Colour has an intrinsic aesthetic value and is well appreciated by all. Colour is the first notable characteristic of a food and often establishes our expectation of flavour and taste. We use colour as a way to identify a food and a way to judge the quality of a food. Food colours play an important role in food industries as additives and are essential for the determination of quality of finished product as well as acceptability by consumers. In India, according to The Prevention of Food Adulteration Act of India, the use of eight synthetic colors, i.e., carmoisine, ponceau 4R, erythrosine, tartrazine and sunset yellow, fast green FCF, brilliant blue FCF and indigotine in specified food commodities at a uniform level of maximum 100 parts per million is permitted.

Synthetic colourants tend to impart undesirable taste and are harmful to human beings as they are responsible for the intolerance and allergenic reactions. Hence, this has elicited a worldwide interest in biocolours or natural food colours which have health-promoting properties. Also market for natural food colours is estimated to increase by approximately 10% annually (CBI Market Survey, 2010).

Anthocyanins are responsible for the attractive blue-violet-red-orange colour of flowers and fruits. These compounds are water-soluble and have high colour intensities at pH less than 4.0 which facilitates their incorporation into aqueous food systems. Naidu and Sowbhagya (Naidu and Sowbhagya, 2012) have cited Fuleki's work concluding that anthocyanins absorb UV and visible light in a range 250-650 nanometer with an λ

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max (absorption maximum) at 535 nanometer. Thus, they are considered as replacements to the artificial dyes especially the coal tar dyes and are hence imparted to colour a number of foods with acidic pH such as beverages, yoghurt, jams, jellies, candies, etc. However, its stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, and presence of enzymes, flavonoids, proteins and metallic ions.

Over the years, studies have been conducted on anthocyanins extracted from grape skins, cranberry, miracle fruit, blueberries, red cabbage, cherry-plum, purple corn, bilberries, berries of *Viburnum dentatum*, purple sweet potato, black chokeberry, olive skins, calyces of roselle, dried flowers of *Clitoria ternatea*, and morning glory flower.

Recently, anthocyanin pigments in banana bracts were evaluated for their potential application as natural food colorants. It was concluded that the bracts proved to be a good and abundant source of anthocyanins with the presence of all six most common anthocyanidins (delphinidin, cyanidin, pelargonidin, peonidin, petunidin and malvidin). Jenshi *et al.* (2011) reported that banana inflorescence bracts (*Musa acuminata* and *Musa acuminata x balbisiana*) have anthocyanin content ranging from 14-32 mg anthocyanin/100 g bracts, mainly comprising of cyanidin-3-rutinoside.

However, there are only a few studies relating to extraction of anthocyanin from banana flower bracts. Since banana flower bracts are easily available and the outer, tough purplered layers are usually discarded during cooking, their use as biocolours could make it cost-effective on a commercial scale.

Cai and Corke (2000) state that microencapsulation by using spray drying is an economical method for preservation of natural colorants which are usually adversely affected by light, oxygen and free radical degradation. This is a technologically advanced method of coating products, which allow the release of the encapsulated materials at controlled rates and under specific sets of conditions, thus yielding a more stable product.

This study is presented to extract the anthocyanin pigments from banana flower bracts and microencapsulate it using a spray drier to yield a more stable biocolour; and to evaluate its application in food products.

MATERIALS AND METHODS

Processing of Sample

Fresh banana flowers were collected from a local wholesale market in Virar, District-Palghar. The outermost whorls of bracts (dark coloured) were plucked from the flower washed thoroughly and allowed to dry. Further, they were roughly chopped and weighed. The pieces were then finely ground using a blender.

Methanol Extraction of Anthocyanin (Rodriguez *et al.*, 1998)

The previously weighed, ground bracts were added to 2 times the volume (w/v) of acidified methanol (0.15% HCl in methanol). The material was allowed to extract overnight under refrigerated conditions. The slurry was then filtered using Whatman no: 1 filter paper by vacuum suction using a Buchner funnel.

Solvent Evaporation (Arueya and Akomolafe, 2014)

Filtered extract was further treated in Equitron® ROTEVA rotary vacuum evaporator at a temperature of 40 °C for the evaporation of the solvent.

Estimation of Anthocyanin

Anthocyanin content of the extract was determined by Du and Francis (1973) method. The filtered extract was diluted to 100 ml with methanol to yield an optical density within the optimum range of the instrument. The diluted extract was stored in the dark for 2 h; absorbance was measured at 535 nm using spectrophotometer. The total anthocyanin content referred to as cyanidin was calculated using the following equation.

$$\text{Total anthocyanins (mg / 100 g)} = \frac{\text{Absorbance} \times \text{dilution factor}}{\text{Sample weight} \times 51.56} \times 100$$

Microencapsulation of Anthocyanin (Ersus and Yurdagel, 2007)

Maltodextrin with a dextrose equivalent of 20 (maximum) was added to the pigment concentrate (7% solid content) and stirred to homogeneity. Maltodextrins were added until reaching to the 20% final solid content or 20°Brix which was determined using a hand refractometer. For further processing, 1 liter of feed mixture was prepared.

The feed mixture was then subjected to spray drying in a LU-22 Mini Spray Drier with an inlet temperature of 110 °C

and an outlet temperature of 65 °C. Feed flow rate was 5 ml/min. The powder thus obtained was immediately transferred to air-tight amber coloured bottles and stored in a cool, dark place. 45 g of spray dried microencapsulated anthocyanin powder (SMAP) was obtained from 1200 g of banana flower bracts.

Application

The potency of the SMAP as a biocolour was evaluated by incorporating it into plain yoghurt and compared with commercial yoghurt containing synthetic dye, i.e., cochineal. The colour intensity of commercially available strawberry yoghurt was checked using spectrophotometer at 535 nm and was used as the reference sample; a method by Ayala *et al.* (1997). To 450 g of plain yoghurt, a few drops (4-5) of strawberry essence and 65 grams of powdered sugar were added. The amount of colourant to be added in the samples was standardized according to the reference sample spectrophotometrically. Hence to Sample A (150 g), 0.67% of SMAP was added and homogenized; to Sample B (150 g), 0.13% of commercially available, food grade cochineal dye with a synthetic dye content of 3% was added and homogenized and to Sample C (150 g), no colour was added.

Organoleptic qualities of the 3 samples viz. yoghurt with SMAP, yoghurt with cochineal and the control sample were evaluated by a semi-trained panel of 15 members. The sensory characteristics scored were colour, appearance, aroma, consistency, taste, texture, mouthfeel, aftertaste and overall acceptability of the samples using a 9-point hedonic scale ranging from dislike extremely (1) to like extremely (9). Panel members used standard procedures for sensory evaluation to test the product.

Mean values and standard deviation of all the sensory attributes were calculated using Microsoft Office Excel 2007. Data was analyzed for variance using SPSS No. 16.

RESULTS AND DISCUSSION

In the present study, acidified methanol was used, which is reported to be an efficient way of anthocyanin extraction. On spectrophotometric analysis, the anthocyanin content of the *Musa acuminata* bracts was found to be 32.97 ± 1.18 mg/100 g of bracts. This is very much in accordance with the reported value of 32.3 mg/100 g bracts by Pazmino-Duran *et al.* (2001).

After spray drying, 45 g of spray dried microencapsulated anthocyanin powder (SMAP) was obtained from 1200 g of bracts. The SMAP obtained was pink in colour and very

hygroscopic in nature; hence it was necessary to immediately transfer the powder into air-tight amber coloured bottles to retain its properties.

Since the biocolour was pink, it could be easily related to the pink in strawberry yogurt. Mintz (2013) has reported that cochineal was the artificial colouring agent used in one of the commercially available popular brand of strawberry yoghurt. Liquid cochineal available in market is a synthetic food dye made up of Carmoisine Supra, Brilliant Blue and Propylene Glycol. Carmine and cochineal have long been listed on labels simply as “artificial coloring” or “color added.” Therefore, the present study used cochineal in Sample B.

While preparation of sample for sensory evaluation, the most important aspect considered was the intensity of the pink tint present in commercially available strawberry samples and that of the colour produced by both, the SMAP and the synthetic dye. Hence, the intensity of the colour was adjusted spectrophotometrically at 535 nm using commercial strawberry yoghurt as reference. This was obtained when 0.67%, i.e., 1 g of the biocolour powder was added to 150 grams of yoghurt (Sample A) whereas, in another portion of 150 grams of yoghurt, 0.13%, i.e., 0.2 ml of cochineal which is equivalent to 0.006 g of synthetic dye was added (Sample B). The concentration of the colours used in the preparation of the samples denotes that the tinctorial strength of SMAP was very low in comparison to the tinctorial strength of cochineal.

Table 1 shows the mean value and the deviation of the scores of sensory attributes for each sample.

A gradual reduction was observed in the scores from Sample A to Sample C with respect to most of the sensory attributes (except in the case of consistency and mouthfeel). Mean values were the same for Sample B and C in terms of consistency and mouthfeel, viz., 7.6 and 7.8 respectively. It was deduced that the yoghurt with SMAP was better than the other two. These observations compare favourably with those of Preethi and Balakrishnamoorthy (2011), wherein, on sensory evaluation, the banana bract anthocyanin extract incorporated amla squash, scored better than the control squash (without colour) for all the sensory attributes, viz., colour and appearance, consistency, flavour, taste and overall acceptability.

The analysis of variance by SPSS No. 16 proved that there was a significant difference in the preference of Sample A over Sample B ($p = 0.009$) and Sample C ($p = 0.000$) at a

Table 1: Mean Values of Sensory Evaluation

Attributes	Sample A	Sample B	Sample C
Colour	8.067±0.45	7.400±0.82	6.667±1.11
Appearance	8.000±0.75	7.267±1.22	6.400±1.29
Aroma	7.800±0.77	7.533±0.51	7.467±0.51
Consistency	7.933±0.59	7.600±0.82	7.600±0.63
Taste	7.800±0.56	7.333±0.72	7.267±0.79
Texture	7.867±0.51	7.533±0.63	7.400±0.63
Mouthfeel	8.000±0.37	7.800±0.56	7.800±0.56
Aftertaste	8.000±0.53	7.600±0.63	7.533±0.91
Overall acceptability	8.133±0.51	7.567±0.62	7.200±0.77

Figure 1: Spray-Dried Microencapsulated Anthocyanin Powder



Figure 2: Samples for Sensory Evaluation



level of 0.05. Therefore, from the results, it can be concluded that the yoghurt with SMAP was well accepted than the yoghurt with synthetic dye or the control sample. However, no significant difference in the preference between yoghurt with cochineal and the control sample ($p = 0.164$) was observed.

CONCLUSION

The present study was aimed at utilizing the waste of banana cultivation, i.e., banana flower bracts since immense importance to waste utilization has been given in the recent years. There has been much interest in the development of biocolours due to the growing safety concerns over continued use of synthetic or artificial colours in food. Anthocyanins are water soluble and this property facilitates their incorporation into numerous aqueous food systems, which make it as attractive natural colorants. The results of sensory evaluation show the scope of studying the incorporation of SMAP into various other food products.

Though, tinctorial strength of SMAP was relatively low as compared to the synthetic liquid cochineal dye, it should be noted that the anthocyanins from bracts are a natural source of colour and being a by-product, is cost-effective. Moreover, anthocyanins are also reported to have anti-oxidant properties and thus reduce the risk of cardiovascular disease (CVD), cognitive decline, and cancer. Due to its anti-oxidant potential, it could be developed into a health supplement. Also it has a wide anti-microbial range and could be a potent source to treat and heal a number of infections and diseases. This shows that SMAP of banana flower bract holds promise as a commercial potential source of biocolourant in food systems.

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