Betalains rich *Rivina humilis* L. berry extract as natural colorant in product (fruit spread and RTS beverage) development

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Abstract *Rivina humilis* L. (Phytolaccaceae) or pigeon berry accumulates betalains in its berries. It is reported that the berries are safe to consume, rich in nutrient content and exhibit efficient biological activity. In this report, *Rivina* berry extract was used as natural colorant in fruit spread and beverage to evaluate its effect on physicochemical properties and acceptability of the product. Results showed that 68 % color retained in *Rivina* banana spread after 6 months of storage at 5 °C, though there was reduction in *L*, *a* and chroma values. *Rivina* banana beverage lost redness completely during processing. Microbial analysis of the products indicated that they were safe for consumption. The spread had good overall sensorial quality and was liked by consumers indicating that addition of *Rivina* berry extract did not alter product quality.

Keywords *Rivina humilis* L. (Phytolaccaceae) · Fruit spread · Color · Sensory profile · Consumer test

Introduction

Owing to changing lifestyle, there is an increasing demand for convenient and functional food products. Stimulated by this, food scientists have been putting in constant effort to develop new as well as conventional products from diverse sources such as minor fruits that remain under-utilized, although they have the potential of application in processed food products. Till date these fruits continue to be forest waste mainly because such fruits are not suitable for fresh market. Hence, these fruits could be utilized for preparation of processed products. In recent times, different value added products have been developed from minor fruits such as *Syzygium cumini* (Kannan and Thirumaran 2004), wood apple (Sakate et al. 2004), bael fruit (Kenghe 2008), *Borassus flabellifer* L., *Passiflora edulis* Sims, and *Artocarpus heterophyllus* Lam (Chakraborty et al. 2010).

Preparation of fruit spread is one way of utilization of fruits. Some popular fruit spreads, preserved by sugar and thickened or gelled to some extent, are jelly, jam, preserves, conserves, marmalades and fruit butters. The fruit imparts unique flavor and color to spreads and fruit juice serves as the medium to dissolve the other ingredients such as sugar, pectin and acid. Spreadability is achieved through the right combination of fruit, pectin, acid and sugar. Banana base has been used widely to provide the desirable consistency in fruit spreads (Adeva et al. 1968; Rekha et al. 2006).

Bright red pigeon berry (*Rivina humilis* L., Phytolaccaceae) is an un-utilised forest produce which could be of use as a

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source of betalains pigment. Studies on dietary safety, and bioactivity with reference to pigment as well as other phytoconstituents have been reported (Khan et al. 2011a, 2012). We report here our study on usage of *R. humilis* berry juice extract for preparation of spread using banana base.

Materials and methods

Ripened berries of *Rivina humilis* L. (red variety) were collected during September–November, 2010 from shady areas of the environs of CFTRI, Mysore (India) located geographically between 12°18'26" north latitude and 76° 38' 59" east longitude. Herbarium sheets of this plant were deposited at Herbarium Collection Centre (SKU–accession no. 11198), Sri Krishnadevaraya University, Anantapur, India. Berries were stored at –20 °C until use.

Chemicals Baird Parker agar base and egg yolk tellurite emulsion were purchased from Microexpress™ (A division of Tulip Diagnostics Pvt. Ltd), Goa, India. Routine media chemicals were purchased from HiMedia, Mumbai, India. All other chemicals were of analytical grade obtained from Sisco Research Laboratory (SRL), Mumbai, India. Double distilled water was used.

Preparation of ready to serve Rivina banana spread Ripened cavendish banana was purchased from the local market. The peel was removed and 1.8 kg of the pulp was taken. *Rivina humilis* berries (250 g) were extracted with 500 mL water. Banana pulp and *Rivina* berry extract were mixed together and to this, 2.2 kg granulated white sugar, 24 g citric acid (0.6 %), 28 g pectin (0.7 %), 1 g sodium benzoate (250 ppm) was added and heated to boiling in a container. *Rivina* banana spread thus prepared was stored at 5 °C.

Preparation of Rivina banana beverage Banana pulp (1.6 kg) was treated with 1 % pectinase and was kept for 3 h at room temperature (Rekha et al. 2006) to get required softness. Inactivation of enzyme was done by holding at 70 °C for 5 min. The juice was expelled through manual compression and filtering through muslin cloth and around 600 mL of free flowing juice was collected. Four litre batch of beverage was prepared supplemented with banana juice (400 mL), water (3.4 L), citric acid (10.04 g), granulated white sugar (521.24 g). *Rivina humilis* berry extract (200 mL) prepared as mentioned earlier was added and the product temperature was raised to 85 °C for 15 min for pasteurization and the product was hot filled in pre-sterilized glass bottles. The *Rivina* banana beverage was kept for cooling overnight in ambient temperature and stored at 5±1 °C.

Analysis of physicochemical characteristics Parameters such as betalains content, TSS, pH, carbohydrate, total sugars,

reducing sugars, phenols, color and titrable acidity were analysed as reported earlier (Khan et al. 2011b, 2012), whereas color was recorded as mentioned earlier (Khan et al. 2011c).

Microbiological assays After 6 months of storage at 5 °C in dark, 10 g sample was dissolved in 100 mL 0.9 % saline. This solution was serially diluted upto 10^{–4} g/mL. One hundred millimeter of diluted sample was aseptically spread onto prepared plates containing plate count agar (for total aerobic count), dichloran rose bengal agar (for yeast and mould count), Baird Parker agar supplemented with 5 mL egg yolk tellurite emulsion/100 mL (for *Staphylococcus* count) and violet red bile agar (for *E. coli* count). Triplicate plates were used for each dilution (10^{–1}, 10^{–2}, 10^{–3} and 10^{–4}). Plates for bacterial count were incubated at 37 °C and colonies were observed after 24 and 48 h, whereas fungal count plates were incubated for 5 days at 28 °C before colonies were counted.

Sensory evaluation Sensory analysis of *Rivina*-banana products was carried out in two phases, namely Descriptive analysis and Consumer acceptance study. Descriptive analysis was chosen because the new products developed-fruit spread and beverage- required in-depth sensory testing to assess the suitability of betalains as natural colorants, whether their presence adversely affected overall quality, if so, more importantly, how these problems could be overcome before subjecting them to consumer acceptance study. Panelists were trained by providing with several samples of beverages and fruit spreads similar to *Rivina* banana products. 'Free choice profiling' was used initially to develop descriptors, where each panelist listed relevant quality attributes such as colour (red), appearance (glossy), set, texture (spreadability), etc. Based on these descriptors, a suitable score card was designed. Panelists were also oriented to use terminology to describe specific perceptions related to the products being evaluated in order to achieve homogeneity of responses. Adequate care was taken to minimize psychological bias. This led to coherence in the group's judgment, as a unit. Following this training, quantitative descriptive analysis (QDA) described by Stone and Sidel (1998) was carried out by the panel (with more than 50 h experience in sensory evaluation of fruits and vegetable products) for evaluating *Rivina* banana products after 6 months of storage at 5 °C in dark. Evaluations were conducted under white fluorescent light, in sensory booths maintained at 22±2 °C and relative humidity of 50±5 %. A suitable score card was developed using "free-choice profiling" method selecting suitable terminology. The sample was presented along with a slice of bread in porcelain plates coded with 3-digit random numbers, to trained panelists. A glass of water was also presented to cleanse the palate in between the samples. Panelists were asked to mark on a scale of 0–15 cm to indicate the intensity of each attribute listed on the score

card. The scale was anchored at 1.25 cm on either end, representing ‘recognition threshold’ and ‘saturation threshold’ respectively. The scores given for all the attributes for each sample were tabulated. The mean value was calculated for each attribute of the sample, representing the panel’s judgment about the sensory quality of the product. The score was depicted graphically as ‘sensory profile’.

Another objective of the study involved testing the overall acceptance of the product with a larger consumer group who were untrained. The group included students and researchers (67 in number, 37 female and 30 male), hailing from different parts of the country. Selection was also based on their health (ensuring that they did not have any defects in sensory perception such as ‘Anosmia’ or ‘Ageusia’), general sensitivity and their prior experience in sensory evaluation of food products. Consumer study was carried out using “Hedonic test” on a 7-point scale ranging from “like very much” to “dislike very much” with “neither like nor dislike” as midpoint. Further, the selected panelists were briefed about subtle and finer points to be paid attention to such as the delicate aroma.

Statistical analysis All analyses were carried out in triplicates. Results are presented in mean $\pm$ SD of the analyses. One Way ANOVA test was used to compare data at 5 % significance level. QDA was conducted in triplicate and the average scores from these evaluations were used in developing sensory profilograms. Since there was only one test sample i.e., *Rivina* banana spread, evaluations were carried out in triplicate and statistical analysis involved calculation of mean/average scores along with standard deviation from three different sessions.

Results and discussion

Physicochemical properties of *Rivina* banana spread analysed immediately after preparation and after 6 months of storage at 5 °C are presented in Table 1. The data showed slight increase in pH followed by reduction in total titrable acidity, carbohydrate and reducing sugar whereas there was significant increase in non-reducing sugar content. Initially, total soluble solids content of 70.6° Brix was achieved in the *Rivina* spread but decreased after 6 months (66.1° Brix). There was slight increase in reducing sugar for few months of storage (data not shown) but trend was reversed at the end of storage period (6 months). During storage, sucrose might have hydrolyzed and resulted in an increase of reducing sugar and it mainly depends on the inversion of sucrose in presence of citric acid or any other organic acid. High sugar content helps in flavor retention, gelling and inhibition of microbial growth (University of Missouri Extension 2010). There was no significant change in protein and fat content. Betalains content has been correlated with phenols content due to phenolic ring

Table 1 Physicochemical parameters of *Rivina* banana spread analysed on day 0 and after six-month storage at 5 °C

Parameter	Day 0	After 6 months
pH	3.6 $\pm$ 0.01	3.9 $\pm$ 0.1*
Titrate acidity (g citric acid eqv./100 g)	1.5 $\pm$ 0.05	0.96 $\pm$ 0.04*
TSS (° Brix)	70.6 $\pm$ 2	66.1 $\pm$ 2.2
Carbohydrates (%)	70.5 $\pm$ 3.4	47.3 $\pm$ 2.1*
Total sugars (%)	na	64.5 $\pm$ 3.2
Total invert sugars (%)	na	58.3 $\pm$ 3.0
Reducing sugars (%)	48 $\pm$ 2.1	33.7 $\pm$ 2.1*
Non-reducing sugars (%)	22.5 $\pm$ 1.4	29.2 $\pm$ 1.8*
Proteins (%)	1.8 $\pm$ 0.2	1.2 $\pm$ 0.1
Oils (%)	0.05 $\pm$ 0.003	0.05 $\pm$ 0.004
Total phenols (mg GAE/100 g)	83.0 $\pm$ 4.6	32.1 $\pm$ 4.0*
Pigments (mg/100 g)	0.22 $\pm$ 0.02	0.15 $\pm$ 0.02*
Color		
<i>L</i>	8.5 $\pm$ 0.15	17.4 $\pm$ 2.4*
<i>a</i>	17.2 $\pm$ 0.56	7.4 $\pm$ 0.67*
<i>b</i>	4.3 $\pm$ 0.19	5.6 $\pm$ 0.49
Chroma	17.5 $\pm$ 0.89	9.3 $\pm$ 0.65*
Hue angle	0.19 $\pm$ 0.02	0.64 $\pm$ 0.01*

na not analysed, GAE gallic acid equivalent, *L* lightness, *a* redness, *b* yellowness. Chroma and hue angle are colour parameters calculated based on *L,a,b* values. Values are mean $\pm$ SD (*n*=3). **P*<0.05

present in them (Wu et al. 2006). Therefore, loss of phenols content was reflected in reduced betalains content (68 %) after 6 months storage at 5 °C. The stability of betalains in this study was better than that of anthocyanins in strawberry jam (Patras et al. 2011). The present observation was in agreement with our finding that *R. humilis* berry juice betalains content reduced by 15 % (thereby retaining ~85 %) during storage at 5 °C in dark over a period of 3 months (Khan and Giridhar,

Fig. 1 *Rivina* banana spread before (a) and after storage for 6 months at 5 °C in dark (b)

Table 2 Microbial profile of *Rivina* banana spread after six months of storage at 5 °C

Microorganism	Viable count (cfu/g)
Total aerobic bacteria	75±5
<i>Staphylococcus aureus</i>	nil*
<i>E. coli</i>	nil*
Yeast and mould	nil*

Values are mean ± SD ($n=3$)

*No colonies were observed in 10^{-1} dilution

unpublished). Loss of betalain pigments was further supported by observed changes in color of the sample during storage at 5 °C (Fig. 1). Increase in lightness (L) and decrease in redness (a) resulting in reduction in chroma and increase in hue angle. *Rivina* banana beverage lost betalainic color during processing due to exposure to heat as betalains are thermo-labile. Because of this, it was assumed that *Rivina humilis* L. berry juice may not be suitable for such beverages although there was regeneration of color to some extent during storage at 5 °C (data not shown). Hence, physicochemical properties of the prepared beverage were not analysed.

Table 2 presents data on viable microbial profile of *Rivina* banana spread after 6 months of storage at 5 °C in dark. The total aerobic bacterial count was estimated to be 75 cfu/g, which was within the acceptable limit. There was no detectable growth of *Staphylococcus*, *E. coli* and yeast and mould. Very low viable microbial count may be attributed to the high sugar content in the product. There was no detectable growth of microorganisms in *Rivina* banana spread and *Rivina*

banana beverage on day 1 of the preparation and there was no microbial count observed in *Rivina* banana beverage after 6 months of storage (data not shown). The overall microbial profile indicated that the product was safe for consumption.

Sensory profile (QDA) of *Rivina*-banana spread is presented in Fig. 2a. Overall quality of *Rivina* banana spread was more than 10 on a scale of 0–15, based on sensory attributes like sweetness, banana like aroma, fruity aroma, smoothness, spreadability, set, glossiness and redness. *Rivina*-banana spread had desirable red colour, and glossy visual appearance that refers to the amount of light reflected off the surface of the sample. Generally, glossy products have better visual appeal, especially in products such as jams and fruit spreads. Texture of the product was also perceived to be good as evidenced by higher scores for smoothness (9.5) and spreadability (10.9). The textural parameter ‘Set’ is usually used for semisolid food samples, sensed visually referring to how thick the product is, how long and to what extent it can retain its shape. For instance, jellies have a firmer set, while jams generally have comparatively not-so-firm set. Set is also an indication of the sharpness of edges and how long this sharpness is retained when the product is scooped out using a spoon. Samples with low/poor set become flat when such scooped out servings are placed on a flat surface and left undisturbed for a while, whereas those with good set retain their shape longer when treated similarly. Similarly, spreadability refers to the ease with which the product could be spread into a uniform layer on a flat surface using another flat surface. For instance, products with low spreadability can be easily spread into a thin layer on a slice of bread while those with high spreadability need more effort to do the same while

Fig. 2 Sensory profile of *Rivina*-banana spread (a), *Rivina*-banana beverage (b) and consumer acceptability of *Rivina*-banana spread (c) evaluated after six months of storage at 5 °C. LVM—like very much, LM—like moderately, LS—like slightly, NLND—neither like nor dislike, DS—dislike slightly, DM—dislike moderately, DVM—dislike very much

forming relatively thick layers. Of course, the thinness of layers can always be manipulated using our fingers and another flat surface such as butter knife. In the present study, the parameter ‘Smoothness’ was taken into consideration as it indicates the absence of perceptible uniform particles on the tongue/in the oral cavity when the product is chewed/manipulated using the tongue in the mouth (oral cavity). Also it was observed that ‘fruity aroma’(sweetish fruit like aroma note) and ‘banana-like’ aroma notes (reminiscent of banana) were balanced and well blended, adding to the appeal of the product. These aroma notes were not affected due to addition of *Rivina* berry extract as colorant, which provided redness. No off odor or taste was perceived by the sensory panelists. The product had clean sweet taste owing to retention of sugar and all these attributes contributed to a high score of 10.3 for overall quality.

Although *Rivina* banana beverage had lost redness resulting in brown color (reminiscent of colour of chocolate), overall quality of the product was good (Fig. 2b). Overall quality of these products was measured in a separate session when sensory profiling was carried out. This refers to how the limited number of trained panelists rated the overall quality of the products. The word anchors for this quality parameter are different-‘very poor’ and ‘very good’, compared to the intensity word anchors ‘low’ and ‘high’ used for measuring the perceived intensity of sensory attributes. The term ‘overall quality’ was used in this context. Loss of betalains colour is justified by the heated aroma (a note typical of fruit juices that undergo thermal processing), which could be due to longer duration of thermal processing, detected in considerable quantity in the beverage unlike in *Rivina* banana spread. Thermal processing loss of betalains has been reported (Chandran et al. 2012). The beverage had low score in terms of banana like aroma, body and sourness (taste elicited by citric acid), whereas sweetness (taste elicited by sugar or sweet taste) was the highest among all the sensory attributes evaluated. The textural parameter ‘body’ usually used for liquid samples, referring to the amount of liquid portion present, relative to suspended particles or dissolved solids. Liquids with ‘high’ body tend to have more dissolved solids or suspended particles. Such samples tend to be described as ‘thick’. On the other hand, liquid samples with low levels of dissolved solids or suspended particles tend to be described as ‘thin’.

Since main aim of this work was to assess suitability of *Rivina* berry juice as food colorant, *Rivina* banana spread, which retained about 68 % pigment without altering overall quality, was considered for consumer acceptability study. The term ‘overall acceptability’ appears in the next phase (Fig. 2c) where acceptability of the products was assessed by a larger number of untrained respondents/consumers in the next phase using ‘Hedonic test’. This is referred to as ‘overall acceptability’ in the materials and methods section. Moreover, it refers to Fig. 2c where results from consumer acceptance study are

shown. Augmenting the findings of descriptive analysis, results of consumer acceptance study (Fig. 2c) showed that the sample was rated “Like very much”, by 37 % of respondents, “Like moderately” by 43 %, “Like slightly” by 13 %, and “Neither like nor dislike” by the rest. It may be noted here that majority of the respondents liked the product and it did not receive any negative ratings. This revealed that the product was acceptable. Most of the consumers recommended addition of a flavoring agent to minimize the fruitiness and further enhance sensory appeal of the product.

Conclusion

In the present study, betalains rich red colored *Rivina humilis* berry extract was tested for its suitability to use as food colorant. *Rivina*-banana spread and beverage were prepared. Colour retention (68 %) was observed in only the spread after thermal processing and subsequent storage at 5 °C for 6 months. Data on sensory profile and consumer acceptability of the spread revealed that the addition of *Rivina* berry extract did not alter overall quality of the product and it was liked by consumers.

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

References

- Adeva LV, Payumo EM, Copez MV (1968) Studies on the preparation of banana spread. *Philipp J Nutr* 21:234–240
- Chakraborty I, Chaurasiya AK, Saha J (2010) Quality of diversified value addition from some minor fruits. *J Food Sci Technol*. doi:10.1007/s13197-010-0194-y
- Chandran J, Nisha P, Singhal RS, Pandit AB (2012) Degradation of colour in beetroot (*Beta vulgaris* L.): a kinetics study. *J Food Sci Technol*. doi:10.1007/s13197-012-0741-9
- Kannan S, Thirumaran AS (2004) Studies on the storage life of jamun (*Syzygium cumini* Rom) fruit products. *J Food Sci Technol* 41:186–188
- Kenghe RN (2008) Bael fruit processing for value addition and employment generation. *Food Pack Com* 2(8):10–12
- Khan MI, Joseph KMD, Muralidhara, Ramesh HP, Giridhar P, Ravishankar GA (2011a) Acute, subacute and subchronic safety assessment of betalains rich *Rivina humilis* L. berry juice in rats. *Food Chem Toxicol* 49:3154–3157
- Khan MI, Sri Harsha PSC, Giridhar P, Ravishankar GA (2011b) Pigment identification, antioxidant activity, and nutrient composition of *Tinospora cordifolia* (willd.) Miers ex Hook. F & Thoms fruit. *Int J Food Sci Nutr* 62:239–249
- Khan MI, Sri Harsha PSC, Giridhar P, Ravishankar GA (2011c) Berberine and lycopene profiling during the ontogeny of *Tinospora cordifolia* (Willd.) Miers ex Hook. F & Thoms fruit. *Curr Sci* 100:1225–1231

- Khan MI, Sri Harsha PSC, Giridhar P, Ravishankar GA (2012) Pigment identification, nutritional composition, bioactivity, and *in vitro* cancer cell cytotoxicity of *Rivina humilis* L. berries, potential source of betalains. *LWT Food Sci Technol* 47:315–323
- Patras A, Brunton NP, Tiwari BK, Butler F (2011) Stability and degradation kinetics of bioactive compounds and colour in strawberry jam during storage. *Food Bioprocess Technol* 4:1245–1252
- Rekha MN, Avula RY, Chauhan AS, Ramteke RS (2006) Process for preparation of shelf stable fruit spread with no added sugar. US patent no. US 2006/0246201 A1
- Sakate RJ, Bhosale DN, Patange DD, Khedkar CD, Patil MR (2004) Optimization of manufacturing technique for wood apple burfi. *Indian J Dairy Sci* 57:21–25
- Stone H, Sidel JL (1998) Quantitative descriptive analysis development, application and future. *Food Technol* 62:48–52
- University of Missouri Extension (2010) Jam and jelly basics. <http://extension.missouri.edu/explore/hesguide/foodnut/gh1461.htm>, Accessed on May 13, 2011
- Wu L-C, Hsu H-W, Chen Y-C, Chiu C-C, Lin Y-I, Ho J-A A (2006) Antioxidant and antiproliferative activities of red pitaya. *Food Chem* 95:319–327