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Assessment of banana cultivars for pigment extraction from bracts, its suitability and stability as food colourant

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SUMMARY : Bracts from six commercial banana cultivars *viz.,* Grand Naine, Ney Poovan, Poovan, Karpuravalli, Red Banana and Virupakshi were used to extract and estimate the anthocyanin content, assess their antimicrobial properties and their suitability as food colourant. The results revealed that Red Banana recorded the highest anthocyanin, phenolic and flavonoid contents (89.73mg/ 100g bracts, 238.93 mg pyrocatachol per 100 ml and 333.37mg quercetin / 100 ml, 333.37mg quercetin / 100 ml, respectively). The anthocyanin extract from banana bracts exhibited antimicrobial activity against bacteria and fungi. Banana bract extract in amla squash showed the highest stability under refrigerated condition (8°C) for 28 days, as observed from the lowest degradation rate (12.91%).

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Colour is one of the most important qualities of foods and food colour determinants the acceptance of a food item. The advent of synthetic dyes caused rapid decline in the use of natural dyes, which were completely replaced by the former within a century (Singh and Singh, 2002). However, research has shown that synthetic dyes are suspected to release harmful chemicals that are allergic, carcinogenic and detrimental to human health. But, natural dyes exhibit better biodegradability and have better compatibility with the environment. Now -a- days most foods are processed in some way or the other before reaching the consumer and manufactures have a need to replace colour lost during processing or to colour products which would otherwise be colourless and unappealing. With increasing public concern over the safety of synthetic colourants, natural colourants are assuming greater prominence.

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Anthocyanins are bright attractive colours, nontoxic and water soluble pigment. New sources of anthocyanins with high stability and low cost are desired characteristics as natural food colourant (Francis, 1975). The potential of plants as commercial sources of anthocyanin is generally limited. During banana harvest 300 kg of coloured bracts per hectare are disposed as residues. Since bracts of banana are widely available and has been traditionally used as food without toxic effect, they could be a potential source of anthocyanins.

EXPERIMENTAL METHODS

Assessment of banana cultivars for pigment extraction from bracts :

Banana male flowers buds of six commercial cultivars *viz.,* Grand Naine, Ney Poovan, Poovan, Karpuravalli, Red Banana and Virupakshi were collected from the germplasm maintained at orchard of Horticultural College and Research Institute, Coimbatore. The male buds were collected immediately after completion of female phase. The 8 whorls of coloured bracts were used for anthocyanin extraction.

Preparation of anthocyanin extract :

The anthocyanin was extracted using 0.15 per cent HCl in methanol (Rodriguez *et al.,* 1998). Five gram of bracts were ground and filtered on a filter paper (Wathman No.1).The filter cake residue was re-extracted until a

clear solution is obtained. One ml of alcohol extract and three ml of HCl in aqueous methanol were taken in a test tube. One ml of anthocyanin samples were added in the above solution and incubated under dark for 15 min. and measured in UV-VIS spectrometer at 525 nm.

Analysis of total phenolics content :

The total phenolics content was determined according to the protocol of Chandler and Dodds (1993). 250 mg plant material was ground in two five ml portions of 80 per cent ethanol and centrifuged. The supernatant was pooled in a standard flask and make up to 10 ml. The ethanol extract was evaporated on a water bath. The anthocyanin extract was mixed with 3.75ml of distilled water and 0.25 ml of 50 per cent folin- ciocalteu reagent. The mixture was allowed to react for five minutes and one ml of 20 per cent Na_2CO_3 was added. Thereafter it was thoroughly mixed and placed in the dark for 30 minutes and the absorbance was measured at 660 nm using UV-VIS spectrometer. A pyrocathecol standard curve (ranging from 0.1 mg per ml to 0.001 mg/ml) was prepared for the calculation of phenolics content. The concentration of phenolics was expressed as milligram of pyrocathecol equivalents per 100 ml of the extract.

Analysis of total flavanoids content :

Aluminium chloride colorimetric method was used for flavonoids determination as described by Chang *et al.* (2002). One ml of suitably diluted extract (10 mg/ml in distilled water) was mixed with three ml of methanol, 0.2 ml of 10 per cent aluminium chloride, 0.2 ml of 1 M potassium acetate and 5-6 ml of distilled water. The mixture was measured at 415 nm with UV-VIS spectrometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations (12.5 mg to 100mg/ml) in methanol. The concentration of flavonoids was expressed as milligram of quercetin equivalents per 100 ml of the extract.

Enumeration of bacteria and fungi and microbial load of anthocyanin powder :

Commonly used method for the enumeration of bacteria is nutrient agar media and fungi are Martin's Rose Bengal agar media (Martin, 1950).

One gram of anthocyanin sample was taken and added to ten ml of sterile water blank. Emulsion was shaken well for 10-15 min to obtain homogenized suspension of microorganisms. This gave a dilution of 1:10 (10^{-1}). One ml from (10^{-1}) this dilution was transferred to nine ml of sterile water blank with a sterile one ml pipette, which gave a dilution of 10^{-2}. The process was repeated up to 10^{-6} dilutions with the sterile water blank. Each time sterile one ml aliquots from 10^{-3} and 10^{-6} dilutions were transferred to the sterile Petridishes for the enumeration of fungi and bacteria, respectively.

Appropriately, 15 -20 ml of molten and cooled medium (45°C) for the respective organisms were added to each Petridish and the plates were rotated in clockwise and anticlockwise and anticlockwise directions to have uniform dispersion of colonies. The plates were then incubated at room temperature for 24-28 hr for bacteria and three days for fungi, respectively. After the incubation the period, the colonies were counted and the number of organisms (bacteria and fungi) per gram sample was calculated by applying the formula,

Number of colony forming units (cfu's) per gram of the sample :

$$\text{cfu} = \frac{\text{Mean number of cfu's X dilution factor}}{\text{Quantity of sample on weight basis}}$$

Evaluation of anthocyanin extract of banana bracts as food colorant :

Spray dried anthocyanin extract of banana bracts extracted from fresh flowers was used to colour freshly prepared amla squash.

Banana bract anthocyanin extract in amla squash :

Five hundred milligram of banana bract anthocyanin was added to 100 ml amla squash along with benzoate preservative (750 ppm). The squash was filled in sterilized bottles, sealed and stored at room temperature. The squash was diluted to3 times with water and served for organoleptic evaluation.

Organoleptic qualities :

Sensory characteristics such as colour, appearance, consistency, flavour, taste and overall acceptability of banana bract anthocyanin coloured amla squash were evaluated using untrained judges using nine point hedonic scales.

Stability of anthocyanin extract in amla squash at different temperatures :

Amla squash coloured with banana bract extracts was taken in screw-capped glass vials and kept in different temperatures *viz.,* 8°C ambient condition and 40°C. The remaining anthocyanin content was measured on alternate days or first 7 days and at weekly intervals upto 20 days. The anthocyanin was measured using UV-VIS spectrometer (525 nm). The anthocyanin reduction from initial content and expressed in per cent.

Statistical analysis :

Statistical analysis was done to study the effect of different parameters on all dependent variables by using the statistical software AGRES. Analysis of variance (ANOVA) was performed to determine the significant difference in anthocyanin content, stability of anthocyanin using the statistical analysis software AGRES. All the treatments and their instructions were compared at $P< 0.05$ level using the least significant difference (LSD) test which was also performed by statistical analysis software AGRES.

EXPERIMENTAL FINDINGS AND ANALYSIS

The highest anthocyanin content was recorded in Red banana bracts (89.73 mg/100g). The anthocyanin content in bracts of different banana cultivars is presented in Table 1. The phenolic and flavonoid content of anthocyanin extracts derived from different banana cultivars are presented in Table 2. Significantly difference in the phenolics content was observed. The anthocyanin extract of red banana recorded the highest phenolics content (238.93 mg pyrocatachol /100ml) followed by Ney Poovan (217.51 pyrocatachol mg/100ml). Among the cultivars the highest flavonoids content of 333.37 mg quercetin/100 ml was recorded in anthocyanin extracts of Red Banana bracts followed by Ney Poovan (303.48mg quercetin/100 ml). Initially the banana cultivars were selected by visual observation by naked eye. The Red Banana bracts were looking bright red in colour. This implied that anthocyanin biosynthesis depended on cultivars, which may be due to genes involved in biosynthesis. These results corroborate the findings of Kasipong kitdamrongsont (2008) that flavonoids caused bluing of colour associated with anthocyanin pathway.

Table 1: Anthocyanin content in the bracts of different banana cultivars

Banana cultivars	Anthocyanin content (mg/100g)
Grand Naine	10.36
Ney Poovan	72.90
Poovan	62.80
Karpuravalli	35.53
Red banana	89.73
Virupakshi	51.56
Mean	53.81
SEd	1.562
C.D (P=0.05)	3.404

Table 2: Total phenolics and flavonoids in bract anthocyanin extract of different banana cultivars

Banana cultivars	Total phenolics content (mg pyrocatachol /100ml)	Total flavonoid content (mg quercetin/100 ml)
Grand Naine	94.74	131.67
Ney Poovan	217.51	303.48
Poovan	208.44	290.83
Karpuravalli	173.15	241.59
Red banana	238.93	333.37
Virupakshi	136.35	190.24
Mean	178.180	248.53
SEd	5.523	6.722
CD (p=0.05)	12.035	14.64

The microbial load of anthocyanin powder was analysed at different dilution factors (10^{-3} and 10^{-6}). Table 3 shows that there was no microbial population (bacteria and fungi) in both the dilution in the anthocyanin powder of banana bracts. This antimicrobial activity may be associated with the high content of phenolic compounds, including low molecular weight phenolic acids, flavonoids such as anthocyanin, flavonols and proanthocyanidins (Hakkinen *et al.*, 1999). If such compounds can cross the bacterial cell wall, it could be hypothesized that crossing of bacterial cell wall by such compounds may result in their reaction with metabolism, reading the antimicrobial activity.

Table 3: Microbial load present in the banana bract anthocyanin powder

Powder	Microbial load (cfu/ml)			
	Bacteria		Fungi	
Banana bracts	10^{-5}	10^{-6}	10^{-3}	10^{-4}
anthocyanin powder	NIL	NIL	NIL	NIL

Values are mean (n=30) :

The scores for the sensory characters such as colour, appearance, flavour and consistency, taste and overall acceptability of amla squash with banana bract anthocyanin extract were evaluated (Table 4). The colour and appearance of amla squash coloured with banana bracts extract obtained the highest score of 7.56. The sample without banana bracts extract got the score of 6.56.

Table 4: Organoleptic scores of amla squash with banana anthocyanin extract

Samples	Colour and appearance	Flavour	Consistency	Taste	Overall acceptability
Squash with colour	7.56	7.53	7.23	7.37	7.83
Squash without colour	6.56	6.23	6.53	6.23	6.23

Table 5: Effect of storage temperatures on stability of banana bracts anthocyanin in amla squash

Storage condition	Anthocyanin content (mg/100g)							
	Storage days							
	0	1	3	5	7	14	21	28
Ambient temperature	100	97.05 (2.95)	94.28 (5.72)	92.01 (7.99)	90.05 (9.95)	86.59 (13.41)	82.97 (17.03)	78.24 (21.76)
8°C	100	99.48 (0.52)	98.23 (1.77)	97.61 (2.39)	96.64 (3.36)	93.46 (6.54)	90.86 (9.14)	87.09 (12.91)
40°C	100	95.67 (4.33)	90.18 (9.82)	86.27 (13.73)	81.89 (18.11)	72.68 (27.32)	55.25 (44.75)	35.16 (64.84)
Mean		97.4	94.23	91.963	89.527	84.243	76.36	66.83
SEd		1.751	1.375	2.216	1.858	1.540	2.099	1.872
CD (p=0.05)		4.284	3.358	5.423	4.547	3.770	5.138	4.580

Different storage temperatures *viz.,* 8°C, ambient and 40°C had significant effect on degradation of banana bract anthocyanin in amla squash (Table 5.). The squash stored in 8°C showed the lowest anthocyanin degradation (12.91%) at 28 days after storage. The squash stored at 40°C showed the highest anthocyanin degradation (64.84%) at 28 days after storage. The colour of anthocyanins is provided by its resonating structure, but a resonance phenomenon also confers its intrinsic stability. Moreover, it has been established that instability has a direct relation with the number of hydroxyl groups and indirect relation with the number of methoxyl groups. The results showed that stability may be positively correlated with flavonoids. This result is in agreement with the findings of Kirca *et al.* (2006) who reported that the degradation rate of anthocyanins was high at 40°C, while the highest stability was observed at 8°C revealing the fact that the elevated temperature may cause increased pigment degradation.

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