

Selection and application of annatto (*Bixa orellana* L.) dye formulations in some traditional sweetmeat and savoury products

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Sweetmeats such as *kesari bhath*, coconut *burfi*, *double-ka-meeta*, and savoury products such as *murukulu*, *besan* coated peanuts and vegetable *manchurian* were prepared by using annatto dye water soluble carbonate formulation (norbixin, 11.24%), water soluble sugar formulation (norbixin, 10.6%) and oil soluble formulation (bixin, 1.39%) at concentrations in the range of 12.5 to 200 mg/kg to impart the required colour shade to replace synthetic colours. Sweetmeats and savoury products were prepared using different annatto dye formulations. Lovibond Tintometer colour readings were measured for the prepared samples and compared with the colour values of commercial samples prepared with synthetic dyes for identifying the suitable annatto dye formulations along with concentrations required to impart the matching colour shade of commercial products. Oil soluble formulation at 20 and 25 mg/kg concentrations in *kesari bhath* and coconut *burfi*, respectively and carbonate formulations at 40, 100, 150 mg/kg concentrations in *murukulu*, vegetable *manchurian* and *besan* coated peanuts, respectively yielded comparable colour shades to the commercial samples. All the formulations have shown considerable stability during processing.

Keywords: Annatto dye, bixin/norbixin, Water soluble carbonate formulation, Water soluble sugar formulation, Oil soluble formulation, Sweetmeats, Savoury products

Introduction

Food colours are used in food industry to enhance eye appeal of food products. Synthetic colours are hazardous to human health and are being restricted to use in most of the developed nations. Five synthetic colours are being permitted according to Food Safety and Standards Act, 2006 in India. There is a demand for natural food colours as they do not cause any ill-health and in addition, they enhance health benefits. Annatto dye is obtained from the outer layer of the seed coat of the tropical tree *Bixa orellana* L. Annatto (*Bixa orellana* L.) food colour is one of the oldest known natural colour for its application in various food preparations. The principle colouring pigment in annatto, namely bixin, is an oil soluble diapocarotenoid 9'-cis-bixin which is present in the resinous coating surrounding the seed¹. Bixin can be converted into water soluble norbixin by treating with alkali². Annatto dye is widely used in dairy products such as butter, cheese, ice creams and popcorn

products. Extraction methods of the dye from annatto seeds³ and analysis of bixin by high pressure liquid chromatography followed by spectrophotometric methods were reported⁴. History, trade, extraction methods, toxicology, application of annatto formulations and legislation was reviewed⁵. The stability of annatto dye towards oxidative, thermal, and light induced degradation and its effects on application in foods and food processing were thoroughly investigated⁶.

Application of annatto dye formulations in fruit and vegetable products⁷, extruded and bakery food products⁸, extrusion cooking of corn flour and in rice flour⁹⁻¹⁰ were reported. The effect of processing conditions on the stability of annatto dye formulations in some traditional foods were reported¹¹⁻¹³. Various popular traditional sweetmeat products have great demand in local markets and are being exported in recent times. Adding colour to commercial samples is a trade secret. It depends on single colour or combination of two or three colours. In the market, the sweetmeat and savoury products are mostly coloured with synthetic yellow (Tartrazine) and orange

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red (sunset yellow) dyes for marketing. The present study was more emphasized on addition of natural annatto dye formulations in lieu of synthetic food colours. An attempt was made to incorporate natural water and oil soluble annatto dye formulations into traditional sweetmeats such as *kesari bhath*, coconut *burfi*, *double-ka-meeta*, and savoury products such as *murukulu*, *besan* coated peanuts, vegetable *manchurian* to match the colour of commercial samples. The compatibility and colour stability in the products during preparation and storage periods were evaluated.

Materials and methods

Annatto seeds were purchased from M/s. Girijan Co-operative Corporation Ltd., Visakhapatnam. Analytical grade chemicals and solvents were purchased from M/s Sd Fine Chemicals Ltd., Mumbai, India. Refined wheat flour (*maida*), *sooji*, refined sunflower oil, sugar, coconut, rice flour, peanuts, corn flour, bread, vegetables, *maida* and salt were purchased from a local super market. Commercial samples of *kesari bhath*, coconut *burfi*, *double-ka-meeta*, *murukulu*, *besan* coated peanuts, vegetable *manchurian* etc. were purchased from sweet shops in Hyderabad, India.

Preparation of Annatto Dye Water and Oil Soluble Formulations

Annatto dye (bixin) was extracted from annatto (*Bixa orellana* L.) seeds using a two solvent extraction method¹⁴. Bixin was converted into norbixin under alkaline pH by adding potassium hydroxide. Norbixin dye powder was diluted with powdered potassium carbonate and sugar powder to get water soluble annatto dye potassium carbonate powder formulation containing 11.24% norbixin (water soluble carbonate formulation) and water soluble annatto dye sugar powder formulation with 10.6% norbixin (water soluble sugar formulation) respectively. Oil soluble formulation was prepared with 1.39% bixin by using monoglyceride and vegetable oil.

Measurement of Colour in Annatto Formulations and Products

The red (R) and yellow (Y) values for water and oil soluble annatto dye formulations at various concentrations (10-100 mg) were measured using Lovibond Tintometer (Model F, UK) by serial dilution with distilled water and chloroform, respectively. The commercial sweetmeat and savoury products, which were prepared using synthetic colours were measured for R and Y units. Based on the Tintometer colour readings, the matched concentrations of various annatto dye formulations with commercial

samples were selected to impart the desired colour in respective sweetmeat and savoury products. Similarly, the experimental samples coloured with annatto dye formulations were measured for R and Y colour readings and compared the colour values of commercial products for optimizing the concentration of different formulations.

Preparation of Traditional Sweetmeats and Savoury Products

Kesari bhath is a sweet consumed quite frequently in India but named differently in different regions such as *sheera* / *halwa* / *kesari*¹⁵⁻¹⁷. In general, the procedure for the preparation of *kesari bhath* includes toasting of *sooji* in ghee, cooking with 2.5 volumes of water and addition of 1.5 times of sugar based on *sooji*. To colour the product, the dye formulations viz., water soluble carbonate formulation at 15, 20, 25 and 50 mg/kg, water soluble sugar formulation at 15, 20, 25, 50 and 100 mg/kg and oil soluble formulation at 15, 20, 25, 50 mg/kg on product basis were added during final stage of cooking to impart the desired colour shade.

Coconut *burfi* is a traditional sweet preparation, which is being frequently prepared in most of the households and sweet shops. It is prepared by cooking the coconut gratings in sugar till the *burfi* consistency attained and put into a greased tray and cut into pieces after cooling¹⁷. For the coloured samples, water soluble carbonate formulation at 15, 25, 50, 75 100 mg/kg and oil soluble formulation at 15, 25, 50 mg/kg were mixed during end of the cooking on the final product basis.

Double-ka-meeta is a traditional sweetmeat which is consumed immediately after preparation and is popular in Telangana districts of Andhra Pradesh. *Double-ka-meeta* was prepared by frying the cut bread pieces in ghee, addition of sufficient quantity of sugar syrup of 65° brix and cooking till the fried bread pieces become soft¹⁷. The annatto water soluble sugar formulation at 25, 50 mg/kg was dissolved into the sugar syrup and added at the end of cooking for uniform absorption of colour.

Murukulu is a savoury product prepared very commonly in most parts of India. The different steps involved in its preparation include dough preparation, extrusion and deep fat frying^{15, 16}. The dough was prepared by taking 800 g rice flour, 200 g black gram flour, 20 g salt and 750 g water. The dough was mixed with annatto water soluble carbonate formulation for colouring at different concentrations of 40, 50, 75 and 150 mg/kg on the final product basis. *Murukulu* were extruded in its normal shape of 3-4 concentric rings by

Table 1—Tintometer colour values of annatto dye formulations at different concentrations

Concentrations (mg/l)	Water soluble carbonate formulation			Water soluble sugar formulation			Oil soluble formulation		
	Y	R	Y/R	Y	R	Y/R	Y	R	Y/R
10.0	30±0.0	4.0±0.26	7.50	40±0.0	4.3±0.06	9.30	20±0.0	5.0±0.17	4.00
15.0	30±0.0	5.1±0.15	5.88	40±0.0	5.6±0.15	7.14	20±0.0	7.0±0.26	2.85
20.0	30±0.0	6.0±0.20	5.00	40±0.0	6.7±0.17	5.97	20±0.0	8.0±0.36	2.50
25.0	30±0.0	7.0±0.06	4.28	40±0.0	7.7±0.26	5.19	20±0.0	9.0±0.17	2.22
50.0	30±0.0	10.9±0.15	2.75	40±0.0	11.0±0.26	3.63	20±0.0	13.0±0.18	1.53
100.0	30±0.0	16.0±0.21	1.87	40±0.0	17.0±0.40	2.35	20±0.0	17.0±0.40	1.17

±SD, n=3

taking the dough into hand extruder and were deep fried in oil at around 160° C till moisture is removed.

Besan coated peanuts is a savoury product, available in most of the sweet shops and *Swagruha* foods. The method involves preparation of batter using 70 g *besan* (Bengal gram flour) and 50 g water, 65 g peanuts, pinch of salt and chilly powder¹⁷. The annatto dye water soluble carbonate formulation was mixed in the batter at 100, 150 and 200 mg/kg on the final product basis. The *besan* coated peanuts were deep fried in a vegetable oil at around 160° C till moisture loss ceased.

Vegetable *manchurian* is a popular side dish/starter in the Indian meal and is available in most of the hotels, restaurants and fast food centers. It is prepared by using *maida*, corn flour and vegetables (cabbage, carrot shreds, capsicum and spring onions) and addition of 50 g water. The resultant thick batter was coloured with annatto water soluble carbonate formulation with 50, 100, 150 and 200 mg/kg, made into small balls and deep fried in a vegetable oil at around 160° C till moisture loss ceased¹⁷.

Analysis of Bixin/Norbixin in Formulations and Products

Bixin/norbixin content was measured in the dyes and formulations spectrophotometrically by using the $E_{1\%}^{1\text{cm}}$ values as reported earlier² in a Shimadzu double beam UV-Visible spectrophotometer UV-160 A (Shimadzu Corporation, Kyoto, Japan). The bixin in oil soluble formulations and experimental products prepared by using oil soluble formulations were analysed by dissolving/extracting into chloroform, which is suitably diluted to read optical densities in the range of 0.3-0.8 at 470 nm. Similarly, norbixin in the water soluble formulations was measured at 452 nm by dissolving in 0.1 M NaOH. The norbixin in the experimental samples prepared using water soluble formulations was analysed by a modified method.

In this method, the products were extracted by taking a weighed quantity of product in 15 ml chloroform containing 5% acetic acid and collecting the extract. The extraction was repeated for four times. The pooled extracts were dried by passing through a bed of anhydrous sodium sulphate and made up to 100 ml. The absorbance of the extract was measured at 474 nm using 5% acetic acid in chloroform mixture as the blank¹². The stability of formulations towards processing conditions was assessed by extracting/recovering the dye (bixin/norbixin) immediately after preparation of products.

Sensory Analysis and Storage Studies

Sweetmeat and savoury products prepared were subjected to sensory analysis by a panel of 8 trained judges using hedonic scale and the best scored products (> 7) were selected for further studies in terms of bixin/norbixin analysis, the R and Y units immediately after processing and during storage.

Results and discussion

The annatto dye formulations are known to impart yellowish orange to orange red colour shades. The yellow (Y) and red (R) units are very much dependent on the dye source, purity, type of formulation, etc¹⁸. Hence, the Y and R colour units were measured in Lovibond Tintometer and computed Y/R values for both water soluble and oil soluble formulation at various concentrations by diluting with distilled water and chloroform respectively (Table 1). The oil soluble formulation has shown low Y (20), and Y/R (1.17-4.0) colour units when compared to water soluble formulations of both carbonate and sugar base which have shown 30 and 40 Y units and almost similar R units, respectively. The colour values of the formulations were compared to the colour readings of commercial samples for selection and use to impart the required colour shade in the products.

Table 2—Application of annatto dye formulations in *kesari bhath*

Concentration Applied (mg/kg)	Tintometer colour readings		Recovery after processing	
	R	Y	mg	%
Water soluble carbonate Formulation				
15	5.0±0.36	20.0±1.07	13.23±0.48	88.20±3.17
20	6.1±0.44	20.0±0.80	18.41±0.81	92.05±4.07
25	7.9±0.62	24.0±1.15	23.0±1.40	92.01±5.58
50	8.9±0.36	33.1±1.71	47.15±1.15	94.30±2.29
Water soluble sugar Formulation				
15	5.0±0.87	20.0±1.92	13.51±0.77	90.06±5.15
20	6.0±0.30	20.1±0.75	19.02±0.65	95.10±3.27
25	6.2±0.36	30.1±1.06	26.89±0.00	100.00 ±0.0
50	7.3±0.44	30.0±1.18	42.77±1.08	85.55±2.15
100	10.2±0.62	31.0±0.71	87.22±0.99	87.22±0.99
Oil Soluble Formulation				
15	5.0±0.36	20.0±0.75	13.81±0.65	92.0±4.31
20	5.3±0.35	20.0±1.21	18.90±0.87	94.5±4.33
25	5.4±0.56	32.0±1.57	23.95±0.87	95.0±3.48
50	5.9±0.61	32.0±0.82	43.30±0.34	86.61±0.68
Commercial sample 1	5.8±0.46	20.0±1.35	NA	NA
Commercial sample 2	6.6±0.15	20.0±1.18	NA	NA

±SD, n=3, NA= Not applicable

Table 3—Application of annatto dye formulations in coconut *burfi* and *double-ka-meeta*

Concentration applied (mg/kg)	Coconut <i>burfi</i> (oil soluble formulation)				<i>Double-ka-meeta</i> (Water soluble sugar formulation)			
	Tintometer colour readings		Recovery after processing		Tintometer colour readings		Recovery after processing	
	R	Y	mg	%	R	Y	mg	%
15	3.3±0.26	13.0±0.36	9.44±0.39	62.90±2.62	-	-	-	-
25	3.9±0.36	14.0±0.72	16.89±0.34	67.56±1.36	8.7±0.46	22.1±0.72	22.0±0.32	88±1.26
50	4.3±0.53	20.5±0.46	29.57±0.53	59.14±1.06	11.1±0.26	23.9±0.70	24.2±0.44	97±0.88
Commercial sample I	5.1±0.26	13.9±0.46	NA	NA	8.1±0.53	21.0±0.95	NA	NA
Commercial sample II	1.9±0.20	11.3±0.56	NA	NA	-	-	-	-

±SD, n=3, NA: Not applicable

The commercial *kesari bhath* samples showed Lovibond Tintometer values ranging between 5.8-6.6 R units and 20 Y units. The water soluble carbonate formulation, which has shown lower Y colour units than the water soluble sugar formulation, was selected to impart the colour to *kesari bhath*. Similarly, oil soluble formulation was selected as it is not only matching with the colour units of commercial samples, but also compatible for use in the preparation of *kesari bhath*. It was observed that both the formulations at 20 mg/kg concentration has yielded products with 5.0-6.0 R and 20.0-20.1 Y units, which were almost comparable to colour units of 5.8-6.6 R and 20 Y units in commercial sample (Table 2). However, the product coloured with oil soluble formulation at 20 mg/kg concentration was more appreciated by the panelists due to bright and glossy

appearance. The recovery of dye in the products after processing was 85.5 to 100.0% (Table 2) prepared by using different formulations at various concentrations indicated that the dye (bixin/norbixin) was quite stable during processing.

In case of coconut *burfi*, oil soluble formulation, which is possessing slightly high R (4 - 17) and low Y (20) colour values at different concentrations was selected as the commercial sample colour values were falling in the same range (R 5.1 and Y 13.9). The Tintometer colour readings of coconut *burfi* coloured with oil soluble formulation at different concentrations were presented in Table 3 along with commercial samples. The annatto oil soluble formulation at 25 mg/kg provided the colour shade nearer to the commercial sample with colour values of R 3.9 and Y 14.0. It was

Table 4—Application of annatto dye water soluble carbonate formulation in *Murukulu* and *besan* coated peanuts

Concentration applied (mg/kg)	<i>Murukulu</i>						<i>Besan</i> coated peanuts					
	Initial		15		30		Initial		15		30	
	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y
40	5.0 ±	12.0 ±	4.9 ±	12.0 ±	4.5 ±	11.6 ±	-	-	-	-	—	-
		0.66	0.66	0.46	0.30	0.30	0.64			-		
50	6.4 ±	14.1 ±	5.7 ±	13.0 ±	5.6 ±	13.0 ±	-	-	-	-	-	-
-		0.44	0.44	0.36	0.26	0.36	0.62					
75	7.0 ±	18.0 ±	7.0 ±	17.0 ±	6.7 ±	16.2 ±	-	-	-	-		
		0.70	0.98	0.44	1.08	0.56	0.46					
100	-	-	-	-	-	-	5.0 ±	21.0 ±	5.0 ±	21.1 ±	5.0 ±	20.0 ±
							0.24	0.89	0.20	0.70	0.50	0.75
150	11.9 ±	18.9 ±	11.5 ±	18.7 ±	11.0 ±	18.4 ±	6.1 ±	21.1 ±	5.9 ±	21.0 ±	5.4 ±	20.0 ±
	0.75	0.46	0.87	0.52	0.56	0.52	0.22	0.70	0.36	0.70	0.46	0.26
200	-	-	-	-	-	-	7.9 ±	21.0 ±	7.4 ±	21.0 ±	7.3 ±	20.2 ±
							0.62	1.28	0.56	1.04	0.75	0.70
Commercial sample	5.7 ±	13.0 ±	2.7 ±	8.4 ±	2.5 ±	8.4 ±	6.4 ±	20.9 ±	6.3 ±	20.0 ±	6.0 ±	19.0 ±
	0.56	0.46	0.44	0.36	0.46	0.46	0.36	0.79	0.56	0.44	0.17	0.26

±SD, n=3

Table 5—Optimum concentrations of various dye formulations in sweetmeat and savoury products

Products	Formulation	Optimum concentration (mg/kg)
<i>Kesari bhath</i>	Oil soluble formulation	20
<i>Coconut burfi</i>	Oil soluble formulation	25
<i>Double-ka-meeta</i>	Water soluble sugar formulation	25
<i>Murukulu</i>	Water soluble carbonate formulation	40
<i>Besan</i> coated peanuts	Water soluble carbonate formulation	150
<i>Vegetable manchurian</i>	Water soluble carbonate formulation	100

observed that the recovery of the dye from the products was in the range of 59.1 to 67.6%. Lower recovery may be due to the binding of dye to the starch/protein matrix. Earlier workers have reported binding of annatto pigments to rice/corn starches during extrusion cooking experiments^{9, 10}.

The Tintometer colour readings of commercial *double-ka-meeta* sample and samples prepared by using annatto water soluble sugar formulation were presented in Table 3. The product prepared by using 25 mg/kg concentration has given colour values of R 8.7 and Y 22.1, which were almost nearer to the values of commercial sample (R 8.1 and Y 21.0). The samples were analysed for the norbixin content immediately after processing to know the stability and it was observed that the recovery was in the range of 88-97%, which indicated that the dye is highly stable during processing.

As the preparation of *murukulu* and *besan* coated peanuts involves deep fat frying operation, the oil soluble

dye was not selected to overcome the leaching of dye into oil medium¹³. Hence, water soluble carbonate formulation was selected for both *murukulu* and *besan* coated peanuts. The tintometer colour readings of both the samples prepared by using water soluble carbonate formulation at different concentrations along with the commercial samples at initial and during the storage were presented in Table 4.

The different formulations along with optimum dye concentrations used to colour various sweetmeat and savoury products were presented in Table 5. In general, the overall sensory quality of sweetmeat and savoury samples were found to be good and addition of colour has appealing effect and did not cause any change in taste or flavour during the processing or storage period. These studies indicated that usage of annatto dye formulations in traditional sweetmeat and savoury preparations showed promising results.

Conclusion

Studies indicated that annatto dye water soluble sugar, carbonate powder and oil soluble liquid formulation are suitable for use in the savoury and sweetmeat products for obtaining the required colour shade comparable to commercial products. This study provided scope to prepare tailor-made annatto dye formulations for easy application by diluting the formulations at recommended level of concentration for traditional sweetmeat and savoury products.

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