

Application of annatto (*Bixa orellena* L.) dye formulations in Indian traditional sweetmeats: *Jilebi* and *jangri*

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Adding colour to food materials like commercial, traditional and street foods is mainly intended to attract the consumer since time immemorial. Usage of synthetic food colours lead to public health issues. Among the sweetmeats available in the market, *jilebi* and *jangri* are mostly added with yellow and orange red synthetic colours, respectively or vice-versa. Bixin (colour pigment) extracted from annatto seeds was used for the preparation of water soluble annatto dye potassium carbonate formulation (nor-bixin, 11.24%) and water soluble annatto dye sugar formulation (norbixin, 10.6%). Lovibond Tintometer colour units of the commercial traditional sweetmeats applied with synthetic colours was measured for standardizing the colour concentration in the experimental products by using the water soluble annatto dye formulations. The present study deals with the application of water soluble annatto dye formulations at various concentrations (2.5-40 mg norbixin/kg product) in preparation of *jilebi* and *jangri*. Method of applying norbixin and the effect of frying temperature on the colour units were also studied. The products were evaluated for colour stability and % recovery of norbixin during a period of 3 days. Water soluble annatto dye sugar formulation at respective concentrations was standardized in preparation of *jilebi* and *jangri*.

Keywords: Annatto dye, Water soluble annatto dye potassium carbonate powder formulation, Water soluble annatto dye sugar powder formulation, *Jilebi*, *Jangri*

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Colours are used in the food industry to enhance eye appeal of many food products. Consumers have become aware of the hazards of synthetic additives in foods and are looking for foods with natural ingredients. Few synthetic dyes to yield red, yellow, blue and green colours are permitted in India to an extent of 100 ppm in foods namely biscuits including biscuit wafer, pastries, cakes, confectionery, thread candies, sweets, savouries and to an extent of 200 ppm in processed papaya, canned tomato juice, fruit syrup, fruit squash, fruit crushes, fruit cordial, jellies, jam, marmalade, etc. according to Food Safety and Standards Regulations 2010¹. Under such circumstances, there is an imperative need for search of natural food colours. Annatto (*Bixa orellena* L.) food colour is one of the oldest known natural colours for its application in various food preparations and is recognized as a safe. The principle coloring component of annatto color is the diapocarotenoid 9'-Cis - Bixin. Annatto is available in the states of Andhra Pradesh, Orissa, Karnataka, Tamilnadu and

West Bengal as forest produce and annatto dye is widely used in dairy products, butter and cheese. Review on extraction methods of the dye from annatto seeds² and analysis of bixin by high pressure liquid chromatography followed by spectrophotometric methods³ was described earlier. Literature review on history, trade, extraction methods, toxicology, application of annatto formulations and legislation was reviewed⁴. According to FAO/WHO the ADI limit for bixin is 0-12 mg /kg body weight and norbixin 0-0.6 mg/kg body weight⁵. As per the Food Safety and Standards Regulations of India, the maximum permissible limit for annatto in foods is 200 ppm, unless other wise mentioned¹. The stability of annatto dye towards oxidation, thermal, and induced light and its effects on application in foods and food processing, and the analysis of foods and beverages were discussed in details with the mechanistic, thermodynamic and kinetic aspects. Analytical techniques such as spectrophotometry, nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC) and mass spectrometry were reviewed⁶. A systematic

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study was conducted on the application of annatto dye formulation in fruits and vegetables⁷, extruded and bakery foods⁸, extrusion cooking of corn flour and in rice flour^{9, 10}. Studies on stability of oil soluble bixin in dye powder and oleoresin, stability of water-soluble annatto dye in ready-to-serve beverage, and effect of processing and stability of annatto dye formulations in traditional foods were carried out earlier¹¹⁻¹³.

Jilebi and *jangri* are popular Indian traditional sweetmeats found in various shades of red and yellow and served during many functions. Addition of colour to commercial samples is a trade secret and the colour shades vary from one trader to other. It depends on single colour or combination of two or three colours. *Jilebi* is a sweet, crispy, glossy and juicy product prepared from fermented batter of refined wheat flour. Some studies on batter preparation indicated that moisture content (62-65%), addition of curd (2-5%) and fermentation time (19-24 hrs) showed lower apparent viscosity necessitated for very easy flowability of batter in preparation of *jilebi*¹⁴. Deep fat fried *jilebi* are generally dipped in sugar syrup, sometimes in syrup made up of jaggery. Preparation of *jangri* made up of black gram batter including rice and corn flour requires experience and skill to make gritty and bity texture. Occasionally, the *jangri* batter is allowed at room temperature for 24 hrs to increase the flowability. In the market, these products are highly coloured with synthetic yellow and orange red dyes and sold hot or kept for marketing for a very short period of 3 to 7 days.

The present study was more emphasized on addition of a natural annatto colour in lieu of synthetic food colours. An attempt was made to incorporate natural water soluble annatto dye formulations into traditional sweetmeats such as *jilebi* and *jangri* to obtain desirable colour shades of commercial samples. The studies also evaluated the methodology on the addition of annatto dye either in batter or in syrup and frying temperature on the colour units of the products.

Methodology

Annatto seeds were procured from M/s Girijan Co-operative Corporation Ltd., Visakhapatnam, India. Chemicals and solvents of analytical grade were procured from M/s Sd Fine Chemicals Ltd., Mumbai. Raw materials like refined wheat flour, black gram dhal, refined sunflower oil and sugar were purchased from a departmental store at Hyderabad. Commercial

samples, viz. *jilebi* and *jangri* were purchased from various sweet shops in Hyderabad, India.

Preparation of annatto dye water soluble formulations and measurement of colour readings

Natural dye (bixin) was extracted from annatto (*Bixa orellana* L.) seeds using a dual solvent method. In brief, the extraction procedure involves initial washing of the seed material with a non-polar solvent such as hexane followed by using a medium polar solvent like acetone to recover the dye. The dye was converted into norbixin under alkaline pH by adding potassium hydroxide (KOH). Annatto norbixin dye powder was diluted with powdered potassium carbonate (K_2CO_3) by dry grinding to get water soluble annatto dye potassium carbonate powder formulation (WSACF) containing 11.24% norbixin (CFTRI patent, 2004)¹⁵. WSACF (0.445 gm) was dissolved in 100 ml water to obtain a concentration of 500 mg/kg. This stock solution was serially diluted to prepare various concentrations ranging from 2.5 - 100 mg norbixin/kg. Similarly, water soluble annatto dye sugar powder formulation (WSASF) was prepared containing 10.6% norbixin with sugar powder. WSASF formulation (0.4716 gm) was dissolved in 100 ml and it was serially diluted to prepare various concentrations ranging from 5.0 -100 mg norbixin/kg. The coloured solutions were measured for red and yellow units in a Lovibond Tintometer (Model F, UK).

Measurement of colour readings for commercial samples

Few commercial *jilebi* and *jangri* samples purchased from local market were measured for red (R) and yellow (Y) units in a Lovibond Tintometer (Model F, UK) and matched with readings of serially diluted annatto dye formulations. Based on the matched concentrations of annatto formulation, the concentration range was fixed and prepared *jilebi* and *jangri* to impart the desired colour to the products.

Preparation of *jilebi*

Jilebi were prepared¹⁶ by applying various concentrations of WSACF and WSASF dissolved in water as mentioned below:

1. Addition of 2.5, 5.0 and 10.0 mg norbixin (WSACF)/kg into batter and frying the product at $172 \pm 2^\circ\text{C}$.

2. Addition of 5.0 and 10.0 mg norbixin (WSACF)/kg in sugar syrup.
3. Addition of 10.0 and 20.0 mg norbixin (WSACF)/kg into batter and frying the product at low temperature ($142\pm 2^{\circ}\text{C}$).
4. Addition of 5.0 and 10 mg norbixin (WSASF)/kg into batter and frying the product at $172\pm 2^{\circ}\text{C}$.

In general, the procedure for the preparation of *jilebi* involves addition of 1 kg *maida* with 400 gm curd in 1 kg water to make batter followed by fermentation for 24 hrs at room temperature. If required, a pinch of sodium bicarbonate is added to batter or incubated at 50°C for 1 hr before making *jilebi*. The batter is mixed with known quantities of water soluble annatto dye (WSA) formulations to get required amount of norbixin and passed through a fine hole (2 mm) of a thick muslin cloth by hand pressing into concentric rings interlinked with a small thread. The *jilebi* rings were deep fat fried in vegetable oil at $172\pm 2^{\circ}\text{C}$ or for 3-4 min and at low temperature ($142\pm 2^{\circ}\text{C}$) for 12-15 min. *Jilebis* were removed from oil and immediately dipped in hot ($67\pm 3^{\circ}\text{C}$) sugar syrup (75-80 brix) solution for 1 min. In general, 1 kg batter yields 2 kg *jilebi*. The yield of the product depends upon the size and thickness of the *jilebi* and incorporation of sugar syrup into rings. Control *jilebis* were prepared following a similar procedure, without addition of colour.

Preparation of *jangri*

Jangri samples were prepared¹⁷ with 20-40 mg norbixin/kg product to obtain required colour shade in the product. The procedure involves thorough washing and soaking of black gram dhal (1.0 kg) and rice (50 gm) for 1 hr and followed by wet grinding to get batter. 100 gm corn flour and required level of water was added to the batter and whipped continuously by hand for 10 min. The batter was mixed with annatto dye formulation to impart colour and passed through a fine hole in a cloth into *jangri* rings (art of experience) and deep fat fried in vegetable oil at low temperature ($142\pm 2^{\circ}\text{C}$) for 12-15 min. The samples were removed from oil and dipped immediately in hot ($67\pm 3^{\circ}\text{C}$) sugar syrup (75-80 °brix) solution for 2 min. Generally 1 kg black gram yields 6-7 kg *jangri*.

Stability of norbixin in product and sensory analysis

Norbixin content was measured in the dyes and formulations, viz. WSACF and WSASF spectrophotometrically by using the $E_{1\text{cm}}^{1\%}$ values

reported earlier¹⁸ in a UV-Visible spectrophotometer (UV-160 A, Shimadzu Corporation, Kyoto, Japan). The annatto dye in the samples were extracted into 15ml 5% acetic acid solution of chloroform, passed through a bed of anhydrous sodium sulphate and finally made up to 100 ml. The optical density of the extract was measured at 474 nm using the blank and calculated the norbixin by using $E_{1\text{cm}}^{1\%}$ values. Commercial *jilebi*, *jangri* and annatto dye incorporated products were evaluated for sensory parameters, viz. colour, taste, flavour and overall acceptability by a panel of 10 trained members who were asked to score on a Hedonic scale¹⁹.

Results and discussion

Table 1 shows R and Y units measured in a Lovibond Tintometer of various commercial *jilebi* and *jangri* samples collected in Hyderabad (Fig. 1). The R and Y units of commercial *jilebi* samples were in the range of 1.7 to 4.1 and 9 to 20, respectively. Similarly R and Y values for commercial *jangri* samples were in the range of 9.1 to 10.8 and 20 to 20.7, respectively. Tintometer colour values of various concentrations of norbixin in aqueous media for WSACF and WSASF are presented in Table 2. The R values showed an increasing trend upon increasing concentrations of norbixin and constant in the case of Y units. The R and Y units of 2.5 and 5.0 mg/kg concentration of WSACF were nearer to the colour values of commercial *jilebi* (*jilebi*-3). Similarly, the R and Y units at 40 mg/kg concentration level of both WSACF and WSASF formulations are matching to the R units of all most all commercial *jangri* samples with slightly higher values of yellow. However, 2.5 to 20.0 mg/kg norbixin (WSACF) were used to incorporate the desired colour shade in *jilebi* and 20 to 40 mg/kg norbixin (both formulations) were used for the preparation of *jangri*.

The deep fat frying of *jilebi* without colour followed by dipping in hot sugar syrup containing WSACF resulted in uneven distribution of colour. Similarly deep fat frying of *jilebi* at low temperature of $142\pm 2^{\circ}\text{C}$ resulted in dark coloured *jilebi* with higher red units (5.0 and 8.0) when 10 mg/kg and 20 mg/kg. *Jilebi* with 5 to 10 mg norbixin (WSASF) per kg product along with control sample and *jangri* prepared with 20 to 30 mg norbixin (WSASF) per kg were shown in Fig. 2 and Fig. 3, respectively. Table 3 showed the colour readings for *jilebi* and *jangri* prepared with WSACF in the concentration ranges of

Table 1 — Tintometer colour values of commercial *jilebi* and *jangri*

Sample	Red	Yellow	Colour*
Jilebi -1	1.7	9.0	5.6 ± 0.70
Jilebi -2	3.0	16.1	6.3 ± 0.48
Jilebi -3	3.0	21.0	7.8 ± 0.79
Jilebi -4	3.1	11.0	7.5 ± 0.50
Jilebi -5	4.1	20.0	8.2 ± 0.63
Jangri -1	9.1	20.1	7.2 ± 0.63
Jangri -2	10.0	20.0	8.0 ± 0.67
Jangri -3	10.0	20.1	8.0 ± 0.67
Jangri -4	10.1	20.7	8.2 ± 0.63
Jangri -5	10.8	20.0	7.1 ± 0.32

*Values are mean of 10 replicates ± SD

Table 2 — Tintometer colour values of various concentrations of norbixin in water solution prepared from WSACF and WSASF

Concentration (mg/kg)	WSACF		WSASF	
	Red	Yellow	Red	Yellow
2.5	2.0	20.0	ND	ND
5.0	3.0	20.0	3.1	40.0
10.0	4.0	30.0	4.3	40.0
15.0	5.1	30.8	5.6	40.0
20.0	6.0	30.0	6.7	40.0
25.0	7.0	30.0	7.7	40.0
30.0	8.0	30.0	8.6	40.0
40.0	10.0	30.0	10.0	40.0
50.0	18.0	30.0	11.0	40.0
60.0	22.0	30.0	13.0	40.0
80.0	30.0	30.0	15.1	40.0
100.0	30.0	30.0	17.0	40.0

ND = not determined as the products were of very low colour and rejected

2.5 to 40 mg norbixin/kg and with WSASF in the range of 5.0 to 30 mg norbixin/kg and sensory score for colour parameter alone to judge physically.

It was observed that the tintometer readings of R and Y of WSASF were almost similar to the commercial sample of *jilebi* (*jilebi*-4). Similarly the R values of *jangri* prepared using 40 mg/kg of the same formulation was same as the commercial sample of *jangri* (*jangri*-4) and the R value was lower. On the other hand, *jilebi* with 5.0 mg/kg concentration of WSASF was almost nearer to the colour values of few commercial *jilebi* samples. All most all commercial *jilebi* samples have shown high Y values (9-21) and the Y values were higher for WSASF formulation than WSACF (Table 3). The overall sensory quality of *jilebi* and *jangri* samples was found to be good. The sensory score for both *jilebi* (5 mg/kg) and *jangri* (30 mg/kg) using WSASF was higher than the samples prepared using WSACF at concentrations of 2.5 mg/kg for *jilebi* and 40 mg/kg in *jangri* (Table 3). The colour of *jilebis* prepared with annatto dye

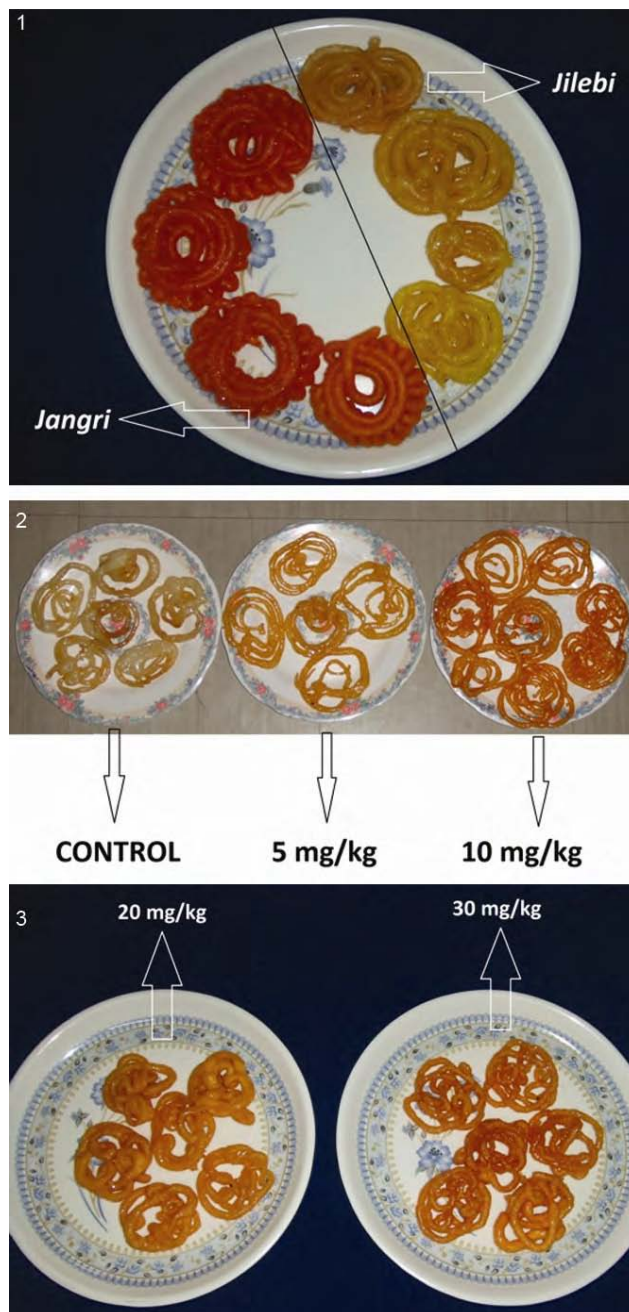


Fig. 1—Commercial *jilebi* and *jangri*; (2)—*Jilebi* with 5 & 10 mg norbixin in Kg along with control; (3)—*Jangri* with 20 & 30 mg norbixin per Kg

formulations could not be compared with commercial *jilebi* as they are applied with synthetic yellow colour. The higher Y values and lower R values at lower concentrations of WSASF has given a scope to provide a comparable shade to the *jilebi* and more specific to *jangri*. *Jangri* samples prepared with annatto dye formulations were almost comparable visually with commercial samples as they are orange-red in colour.

Table 3 — Lovibond Tintometer colour readings of *jilebi* and *jangri* incorporated with WSACF and WSASF

Applied concentration (mg/kg product)	WSACF			WSASF		
	Red	Yellow	Colour*	Red	Yellow	Colour*
<i>Jilebi</i>						
2.5	3.4	10.4	6.7 ± 0.48	ND	ND	ND
5.0	4.3	10.1	7.0 ± 0.47	5.0	20.0	8.2 ± 0.42
10.0	5.4	14.0	7.5 ± 0.71	6.9	20.1	7.9 ± 0.32
5.0 (in syrup)	3.4	10.0	6.6 ± 0.52	ND	ND	ND
10.0 (in syrup)	4.9	10.0	6.9 ± 0.32	ND	ND	ND
10.0 (LT)	5.0	15.0	7.5 ± 0.53	ND	ND	ND
20.0 (LT)	8.0	15.0	7.3 ± 0.48	ND	ND	ND
<i>Jangri</i>						
20	ND	ND	ND	5.0	21.1	7.2 ± 0.42
30	15.2	10.7	7.1 ± 0.32	8.2	21.1	7.9 ± 0.74
40	10.1	13.0	7.5 ± 0.53	ND	ND	ND

*Values are mean of 10 replicates ± SD, ND = not determined (as the products were of very low colour/ uneven distribution of colour/darkening or browning during frying)

Table 4 — Stability of norbixin in *jilebi* and *jangri* incorporated with WSACF and WSASF during storage at RT

Applied concentration (mg/kg product/syrup)	WSACF				WSASF			
	0 days		3 days		0 days		3 days	
	Conc. extracted (mg/kg)	% recovery	Conc. Extracted (mg/kg)	% recovery	Conc. Extracted (mg/kg)	% recovery	Conc. Extracted (mg/kg)	% recovery
<i>Jilebi</i>								
2.5	1.595	63.80	1.476	59.05	ND	ND	ND	ND
5.0	3.985	79.70	3.808	76.16	3.400	68.70	3.224	64.40
10.0	5.334	53.34	5.133	51.33	5.485	54.80	4.840	48.10
5.0 (in syrup)	3.858	77.16	3.614	72.28	ND	ND	ND	ND
10.0 (in syrup)	6.901	69.01	6.861	68.61	ND	ND	ND	ND
10.0 (LT)	6.090	60.90	7.048	70.48	ND	ND	ND	ND
20.0 (LT)	14.213	71.06	13.154	65.70	ND	ND	ND	ND
<i>Jangri</i>								
20	ND	ND	ND	ND	3.400	8.20	1.489	7.44
30	11.021	36.75	6.17	27.23	5.485	8.40	1.907	6.35
40	14.110	35.30	12.60	31.50	ND	ND	ND	ND

Values are mean of triplicate determinations, ND = not determined (as the products were of very low colour/ uneven distribution of colour/darkening or browning during frying)

The stability of the dye in *jilebi* and *jangri* was assessed by recovery of the dye immediately after preparation and the data is presented in Table 4. In general, the recovery was more in *jilebi* than *jangri*. The lower recovery in *jangri* may be attributed to longer frying times and also binding of annatto dye with starch molecules in black gram and rice¹⁰. A marked decrease of dye was observed during the 3 days of storage in both the products with both the formulations.

In general, the overall sensory quality of *jilebi* and *jangri* samples was found to be good (≥ 7.0) and addition of colour did not show any adverse effects on taste and flavour during these studies. The authors felt that creating awareness among the manufacturers on

the usage of annatto colour in such traditional sweetmeats should be encouraged.

Conclusion

The studies indicated that water soluble annatto dye sugar powder formulation (WSASF) at a concentration of 5 mg/kg and 30 mg/kg were found optimum to obtain required color shades of *jilebi* and *jangri* respectively when compared to water soluble annatto dye potassium carbonate powder formulation (WSACF) which may not be useful in the preparation of *jilebi* and *jangri*. Further, preparation of tailor-made annatto dye formulations like 5 mg norbixin/g sugar powder and 30 mg/g sugar powder are easy for application in the final products.

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