Studies on application of annatto (*Bixa orellana* L.) dye formulations in dairy products

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Abstract Annatto is often used to add color to dairy products such as butter, cheese, or puddings. In India usage of annatto is restricted to butter and cheese, however there are no tailor made formulations available to obtain standardized colour shades for the products. Hence a study was initiated to develop the appropriate annatto dye formulations and level of application in few dairy products (butter, cheese, paneer, biscuit cream, icing cream). Dye extracted from annatto seeds was used for the preparation of water soluble potassium carbonate formulation (nor-bixin, 11.24 %), oil soluble formulation (1.35 % bixin) and an oil/water soluble propylene glycol formulation (PG formulation, 1.31 % bixin). Lovibond tintometer colour units of the commercial butter, cheese and biscuit cream samples were measured for standardizing the colour concentration in the experimental products. The present study evaluates the application and stability of these formulations in butter, cheese, paneer, biscuit cream, icing cream. The products were evaluated for stability of colour and bixin during storage period. The oil/water soluble propylene glycol formulation was found to be tailor-made for all the dairy products studied though the standardized levels varied between 3.75 and 5.0 mg/kg for butter, 3.75 mg/kg for cheese, 5.0–400 mg/kg for biscuit cream, 12.25 and 25 mg/kg for paneer, 5.0–500 mg/kg for icing cream. Increasing concentration in the range of 30–500 mg/kg yielded products with light cream to orange shades, useful for decorating cakes. The average recovery of bixin from the products immediately after processing was 90–98 %. Significant changes ($P \leq 0.05$) were

noticed in the colour units and recovery of bixin in all the products during storage of 6 months.

Keywords Annatto dye · Water soluble potassium carbonate formulation · Oil soluble formulation · Oil/water soluble propylene glycol formulation · Dairy products

Introduction

Colour is an important property of foods that adds visual appeal to the enjoyment of eating. The primary objective of adding colours to foods is to offset colour loss due to exposure to light, air, extremes of temperature, moisture and storage conditions, compensate for natural or seasonal variations in food raw materials or the effects of processing and storage to meet consumer expectations and to enhance colours that occur naturally but at levels weaker than those usually associated with a given food. Addition of colour to commercial samples is practiced with a single color or in combination of two or more colours. Increasingly, natural food colours are being used in processed foods. These are usually pigments from vegetables or fruits or from seeds or spices and from insect base. Lac dye was applied in comminuted meat products to obtain a bright red colour replacing synthetic dyes (Divya et al. 2011). Annatto extracts are natural yellowish orange colors obtained from the outer coats of the seeds of the tropical shrub *Bixa orellana*. The colouring components of annatto are the apo-carotenoids bixin ($C_{25}H_{30}O_4$) and the norbixin ($C_{24}H_{28}O_4$). Annatto is available in oil-soluble “Bixin” and water-soluble “Norbixin” forms. The solubility differences between bixin and norbixin are the main determinants for selection of an annatto formulation for a particular application. The biggest application of annatto is in the dairy industry for coloring cheese and other dairy products. For more than a

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hundred years annatto extracts have been added to numerous foods such as sweets, ice cream, dressings, and dairy products to produce an intensive color. Because of its use in butter, margarine and cheese, annatto was formerly declared as “butter color” or “cheese color” (Collins 1992).

Annatto extract can be found in many forms in the markets. It may be in the form of powder, paste, oil or chunk. Usage of annatto colour varies from country to country due to different food cultures.

Determination of annatto in high-fat dairy products, margarine and hard candy by solvent extraction followed by high-performance liquid chromatography was studied by Lancaster and Lawrence (1995). Detection of the annatto dye norbixin/bixin in cheese using derivative spectroscopy and high performance liquid chromatography (HPLC) was studied by Luf and Brandl (1988). Pink discoloration of annatto coloured process cheese was studied with reference to cooking temperature, pH and emulsifying agents (Shumaker and Wendorff 1998). Annatto can also produce a pink color in cheese. The exact cause of this pink discoloration and defect in cheese is unknown. However, several researchers have hypothesized on the causes of pinking. Barnicoat (1950) attributed the pinking to oxidation of norbixin by sulfhydryl-type compounds. HPLC and spectrophotometric determination of annatto in cheese was carried out by Armin et al. (2002).

Screening of published literature on application of the dye or formulations have shown many products namely fruits and vegetable products like tutty-fruity, jams and jellies (Satyanarayana et al. 2006); extruded and bakery foods such as wafers, noodles, sugar cones (Prabhakara Rao et al. 2007); beverage model systems (Prabhakara Rao et al. 2002). The toxicological aspects of annatto in humans and animals was studied by Preston and Rickard (1980). It was found that annatto pigments were metabolizing into blood stream and are removed from the body very rapidly. The toxicological work has helped FAO/WHO in bringing out an expanded monograph on annatto. The ADI values of bixin/norbixin in foods suggested by Sixty Seventh meeting of the Joint FAO / WHO Expert Committee on Food Additives (JECFA 2006) were bixin: 0–12 mg/kg body weight; norbixin: 0.6 mg/kg body weight. As per Food Safety and Standards Regulations (FSSR, India 2010) the permissible limits of annatto in milk products are 20 mg/kg for butter and 10–50 mg/kg for cheese.

In India, the extraction of pigments is carried out by heating the annatto seed in vegetable oil and straining which is normally added to dairy products (Aparnathi and Sharma 1991). The quantity or quality of dye extracted is not uniform as there is wide variation in the dye content of the seed and the conditions during extraction such as temperature, seed to oil ratio etc (Satyanarayana et al. 2010). Literature on the use of annatto formulations to obtain standardized colour shades in dairy products is not available. The present

group has established the extraction methods for annatto dye and preparation of different types of formulation for easy application in various foods.

In the present study, an attempt is made to prepare annatto dye formulation for dairy products (butter, cheese, paneer, biscuit cream and icing cream) to obtain desirable colour shades of commercial samples. Different formulations such as water soluble potassium carbonate formulation, oil soluble formulation and oil/water soluble propylene glycol formulation were applied at different concentrations to impart yellow shades in butter and cheese and yellow-to-orange shades in paneer, biscuit cream and icing cream. The products were assessed for visual colour, colour stability during processing and storage to identify the suitable tailor made formulation for dairy products.

Materials and methods

Annatto seeds were procured from M/s. Girijan Co-operative Corporation Ltd., Vishakhapatnam, India and the quality of the seed was analysed. Chemicals and solvents of analytical grade were procured from M/s Sd Fine Chemicals Ltd., Mumbai. Raw materials like plain butter and plain cheese, milk (for preparing paneer), margarine, sugar and icing sugar (for preparing biscuit cream) and whipped icing cream were purchased from a local bakery in Hyderabad, India. Commercial butter and cheese sample, containing annatto as declared by the manufacturer and commercial cream biscuits were purchased from local super market.

Preparation of annatto dye formulations The dye content of the seed was analysed by the spectrophotometric method (Reith and Gielen 1971) and annatto dye was extracted from the seed by patented process (CSIR 2004) in 1.5 kg soxhlet extractor. The high bixin dye collected from 10 batches was pooled and assessed for bixin quality. Bixin was converted into norbixin under alkaline pH by adding potassium hydroxide (KOH). 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 (K_2CO_3 formulation) containing 11.24 % norbixin. Oil soluble formulation was prepared with 1.35 % bixin using monoglyceride and vegetable oil. Oil/water soluble formulation was prepared with 1.31 % bixin in propylene glycol. Similarly, a traditional oil soluble formulation was also prepared by extracting the pigment from annatto seeds by boiling it in sunflower oil (1part seed: 2 parts oil) and filtering it. The filtered oil containing the pigment was used for application in plain butter and cheese.

Comparison of commercial and experimental samples for colour readings Commercial butter, cheese and biscuit

cream samples were measured for colour readings of red (R) and yellow (Y) units in a Lovibond Tintometer (Model F, UK) and the colour measurements were compared and matched with the colour readings of the different formulations applied in the samples at different concentrations. The annatto formulations which yielded samples matching to commercial samples were selected to impart the desired colour in respective products and used in the study.

Application of annatto dye formulations in butter The principal constituents of a normal plain salted butter are fat (80–82 %), water (15.6–17.6 %), salt (about 1.2 %) as well as protein, calcium and phosphorous (about 1.2 %). Annatto dye was incorporated in butter by weighing a known quantity of plain butter and mixing calculated volume of annatto dye single strength PG formulation in various concentrations i.e. 2.5–20 mg/kg for obtaining the desired colour shade of butter comparable to commercial sample. The sample was then packed in MPE pouches for storage under refrigerated conditions. The stability of annatto formulation in butter was determined by measurement of colour readings and also by extraction of the dye over a period of 6 months.

Application of annatto dye formulations in cheese Cheese is the fresh or ripened product obtained after coagulation and whey separation from milk, cream or partly skimmed milk, buttermilk or a mixture of these products. Soft cheeses are the cheeses with high moisture content. The popular cheese products in this category are Cottage Cheeses, Mozzarella, Paneer, Chhanna and so on. The constituents of cheese are protein, fat, water and mineral matter.

A known quantity of plain cheese was weighed and calculated volumes of annatto dye single strength PG formulation was added at various concentrations i.e. 2.5–10 mg/kg for obtaining the desired colour shade of cheese comparable to the colour measurement values of commercial sample. The sample was packed in MPE pouches and stored under refrigerated conditions. The measurement of colour values and the extractable bixin from the products were continued during the storage period of 6 months.

Preparation of biscuit cream and application of annatto dye formulations Biscuit cream preparation involved the addition of Shortening (Margarine), Lecithin (Monoglyceride), Icing sugar and Milk powder (Manley 2000). All the materials were added to the melted shortening and mixed thoroughly again. The product was smooth without any grittiness. A known quantity of the prepared biscuit cream was weighed and calculated volume of annatto dye single strength PG formulation was added in various concentrations i.e. 6.75–1,000 mg/kg for obtaining the desired colour

Table 1 Quality of dyes and formulations used in the study

Annatto dye/formulation	Bixin/norbixin (%) ^a
Annatto dye	91.0±2.95
Norbixin potassium salt	76.0±2.93
Water soluble carbonate formulation, single strength	11.2±1.42
Oil soluble formulation	1.3±0.06
PG formulation, single strength	1.3±0.06
Oil soluble traditional formulation	0.60±0.07

PG propylene glycol

^a Values are mean of triplicate analyses ± SD

shade of biscuit cream comparable to commercial samples. The samples were packed in MPE pouches for storage at room temperature. The colour readings and extractability of the dye were measured over a storage period of 6 months.

Preparation of paneer and application of annatto dye formulations Paneer is a type of soft, crumbly cheese native to India, although it is used in some parts of the Middle East as well. It is one of the most common cheeses used in Indian cuisine, and it has a simple, fresh, versatile flavor which makes it highly useful in an assortment of recipes. The procedure involved adding calculated volume of annatto dye single strength PG formulation at various concentrations i.e. 12.5–25 mg/kg to a known volume of milk and boiling it. After the addition of the dye, paneer was prepared by coagulating the milk with 1 % citric acid solution (Vijaya 2001). It was kept under refrigerated conditions for a few hours. The paneer can be cut into chunks and used as required. The colour readings and extraction of the dye were measured for 0 days storage for this product. Application of annatto dye formulations in paneer yielded products with orange

Table 2 Colour measurement in commercial butter, cheese and biscuit cream samples

Commercial samples	R	Y
Butter sample 1	1.2±0.26 ^b	4.0±0.36 ^e
Butter sample 2	1.5±0.15 ^b	4.1±0.44 ^e
Cheese sample 1	2.0±0.36 ^d	4.8±0.36 ^e
Cheese sample 2	1.6±0.26 ^d	4.6±0.26 ^e
Biscuit cream sample 1 (orange)	11.0±0.79 ^f	6.0±0.62 ^g
Biscuit cream sample 2 (p.apple)	1.1±0.21 ^h	10.0±0.70 ⁱ
Biscuit cream sample 3 (mango)	3.0±0.36 ^j	20.0±0.79 ^k
Biscuit cream sample 4 (butter scotch)	2.9±0.26 ^l	4.0±0.98 ^m

Values are mean of triplicate analyses ± SD, values with non-identical superscripts differ significantly ($P \leq 0.05$)

R Red; Y Yellow colour units in a lovibond tintometer

shade. However these formulations can be applied at different levels based on consumer preference.

Application of annatto dye formulations in icing cream Stabilized whipped cream is a type of icing known for its delicate sweet taste. It has a thin to medium consistency that keeps its soft texture on a decorated cake. Once iced, the cake must be refrigerated. It holds its shape well and can be used to create flowers, borders and writing on cakes and other types of bakery. A known quantity of plain whipped cream was weighed and calculated volume of annatto dye single strength PG formulation was added in various concentrations i.e. 4–500 mg/kg for obtaining the desired colour shade of icing cream. Stabilized whipped icing cream does not sit well at room temperature and

needs to be used immediately. The colour readings and extraction of the dye were measured for 0 days storage.

Measurement of bixin in products Analysis of bixin and norbixin was carried out spectrophotometrically as per the $E_{1\%}^{1\text{cm}}$ values reported by Reith and Gielen (1971) in a Shimadzu UV-160 A, UV-Visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan). In case of all the experimental dairy samples (except butter), the annatto dye was extracted into chloroform. The optical density of the extract was measured at 474 nm using chloroform as the blank. In case of butter, the dye was extracted into chloroform containing 5 % acetic acid. The optical density of the extract was measured at 474 nm using 5 % acetic acid chloroform mixture as the blank.

Table 3 Colour measurement of experimental butter and cheese samples prepared with annatto dye formulations

Formulation and concentration of bixin/norbixin applied (mg/kg)	Storage period in days (0 days)			
	Butter		Cheese	
	R	Y	R	Y
Oil soluble formulation (1.35 %)				
2.5	0.6±0.10	1.4±0.21	0.9±0.10	2.1±0.20
3.75	0.8±0.17	2.0±0.26	1.1±0.10	4.0±0.17
5.0	1.0±0.17	3.0±0.26	1.3±0.26	3.3±0.20
7.5	–	–	1.6±0.26	4.5±0.20
10	1.1±0.10	3.5±0.17	1.8±0.26	5.5±0.26
12.5	1.3±0.17	4.4±0.36	–	–
15	1.3±0.10	5.0±0.20	–	–
20	1.5±0.26	6.0±0.26	–	–
Oil soluble traditional (0.6 %)				
2.5	–	–	1.3±0.26	4.2±0.10
3.75	–	–	1.7±0.10	5.5±0.26
5.0	1.2±0.15	3.8±0.06	1.8±0.26	6.9±0.36
7.5	–	–	2.4±0.20	8.1±0.29
10.0	1.9±0.06	5.4±0.06	2.8±0.10	9.7±0.44
50.0	–	–	5.0±0.20	17.0±0.53
Water soluble carbonate (11.24 %)				
2.5	0.8±0.26	2.0±0.36	1.1±0.20	2.1±0.20
3.75	0.9±0.10	2.5±0.26	1.2±0.18	2.5±0.26
5.0	1.0±0.10	3.3±0.20	1.2±0.20	3.0±0.30
7.5	–	–	2.1±0.20	4.3±0.26
10	1.0±0.34	3.4±0.10	2.2±0.20	5.2±0.17
12.5	1.3±0.20	4.8±0.20	–	–
15	1.6±0.20	6.0±0.53	–	–
20	2.2±0.17	6.4±0.36	–	–
Oil/water soluble PG formulation (1.31 %)				
2.5	1.0±0.26	2.5±0.30	1.0±0.12	2.0±0.12
3.75	1.1±0.17	4.0±0.46	1.4±0.12	4.6±0.21
5.0	1.5±0.26	6.0±0.36	1.8±0.10	5.0±0.21
10	2±0.36	8.5±0.26	2.2±0.10	5.2±0.20

Values are mean of triplicate analyses ± SD, all values differ significantly ($P \leq 0.05$)

PG propylene glycol

Sensory evaluation and storage studies The products were evaluated for visual colour and overall acceptability by a panel of six semi-trained judges who were provided with commercial samples as controls. Butter, cheese and biscuit cream were analysed immediately after incorporation of the dye and also during storage at 0, 2, 4 and 6 month intervals for bixin stability. The red (R) and yellow (Y) colour units were also studied during storage using a Lovibond tintometer. The butter and cheese samples were stored under refrigerated conditions whereas biscuit cream was stored at room temperature (28 ± 2 °C). The product samples paneer and icing cream were analysed for 0 days storage.

Statistical analysis All analyses were conducted in triplicate and the mean values with standard deviation (mean $\pm$ SD) are presented in tables. Statistical analysis was carried out for individual groups of butter, cheese, biscuit cream, icing cream and paneer samples independently using SPSS 15.0 to evaluate the significance at $P \leq 0.05$. Independent sample T-Test has been employed for application at two concentration levels and for more than two concentrations ANOVA single factor analysis has been used.

Results and discussion

The quality of extracted annatto dye and different formulations used in the present study along with dye concentrations are presented in Table 1. Few commercial samples of butter, cheese and biscuit cream were analysed for colour units of R and Y in the lovibond tintometer (Table 2). It was observed that colour units among butter and cheese samples did not differ significantly ($P \leq 0.05$) though the source of samples were different. However, in case of biscuit cream samples significant differences were observed. Annatto formulations namely water soluble potassium carbonate formulation (nor-bixin, 11.24 %), oil soluble formulation (1.35 % bixin) and oil/water soluble propylene glycol formulation (1.31 % bixin) were tested for suitability in butter, cheese, paneer, biscuit cream and icing cream for obtaining yellow–orange colour shades comparable to commercial samples. Annatto emulsions are not considered in application of dairy products as annatto solutions are more stable than emulsions during application and processing (Shumaker and Wendorff 1998). The Y and R units were measured for experimental dairy samples prepared by incorporating different formulations at various concentrations (Tables 3 and 4) by using a Lovibond tintometer. It was observed that the increase in the

Table 4 Colour measurement of experimental biscuit cream, icing cream and paneer samples prepared with annatto dye formulations

Concentration of bixin/ norbixin applied (mg/kg)	Storage period in days (0 days)					
	Biscuit cream		Icing cream		Paneer	
	R	Y	R	Y	R	Y
Oil soluble formulation (1.35 %)						
4.0	–	–	0.1 $\pm$ 0.06	0.4 $\pm$ 0.10	–	–
6.75	1.0 $\pm$ 0.10	3.8 $\pm$ 0.21	1.1 $\pm$ 0.17	5.5 $\pm$ 0.31	–	–
25	–	–	–	–	5.5 $\pm$ 0.15	20 $\pm$ 2.00
50	–	–	–	–	6.9 $\pm$ 0.21	20 $\pm$ 1.15
30	2.3 $\pm$ 0.26	9.0 $\pm$ 0.40	3.8 $\pm$ 0.25	15.0 $\pm$ 0.55	–	–
100	2.5 $\pm$ 0.20	10.0 $\pm$ 0.55	4.1 $\pm$ 0.29	15.0 $\pm$ 0.50	–	–
500	–	–	7.0 $\pm$ 0.21	7.0 $\pm$ 0.20	–	–
1000	7.7 $\pm$ 0.26	30.0 $\pm$ 2.0	–	–	–	–
Oil/water soluble PG formulation (1.31 %)						
5.0	2.1 $\pm$ 0.20	5.0 $\pm$ 0.38	1.7 $\pm$ 0.15	5.0 $\pm$ 0.31	–	–
12.5	–	–	–	–	4.0 $\pm$ 0.31	12.1 $\pm$ 0.25
25	2.8 $\pm$ 0.17	11.1 $\pm$ 0.26	–	–	5.9 $\pm$ 0.21	19.1 $\pm$ 0.30
30	–	–	2.2 $\pm$ 0.21	7.0 $\pm$ 0.26	–	–
50	4.0 $\pm$ 0.17	15.2 $\pm$ 0.35	4.0 $\pm$ 0.21	11.1 $\pm$ 0.21	–	–
300	–	–	7.0 $\pm$ 0.21	16.1 $\pm$ 0.36	–	–
400	7.1 $\pm$ 0.20	17.2 $\pm$ 0.31	–	–	–	–
500	–	–	8.1 $\pm$ 0.20	17.0 $\pm$ 0.31	–	–
Water soluble carbonate (11.24 %)						
25	–	–	–	–	7.4 $\pm$ 0.21	10 $\pm$ 0.31
50	–	–	–	–	5.2 $\pm$ 0.15	10 $\pm$ 0.21

Values are mean of triplicate analyses $\pm$ SD, all values differ significantly ($P \leq 0.05$)

PG propylene glycol

level of application of different formulations had significant effect ($P \leq 0.05$) on the colour values of red and yellow in all samples namely butter, cheese, biscuit cream, icing cream and paneer. Products prepared by application of annatto dye formulations in icing cream and paneer were not compared with commercial products, as these products could be coloured based on consumer preference. The colour readings and percent recovery of bixin immediately after processing and during storage for experimental dairy samples prepared with annatto dye PG formulation are presented in Tables 5 and 6 respectively. Significant changes ($P \leq 0.05$) were noticed in the colour units and recovery of bixin in all the products during storage of 6 months.

Butter Among the various concentrations applied, it was observed that the Y, R values of single strength oil/water soluble PG formulation at 3.75 and 5.0 mg/kg respectively are almost nearer to the values of the commercial butter samples which imparts creamy-yellow colour shade and was preferred by most of the panellists. The PG formulation has provided the required colour shade (Y& R values) at lower concentrations of 3.75 and 5 mg/kg, whereas the other formulations provided similar colour values at higher concentrations (Table 3). It was observed that recovery of bixin

was maximum (98 %) initially, which reduced to 88 % (Table 6) after storage for 6 months under refrigerated conditions. The recovery of dye was found to be higher in butter than cheese during processing and storage and similar observations were also made by Reith and Gielen (1971). Sachindra and Mahendrakar (2010) also observed that processing did not affect the stability of carotenoid pigments applied in fish sausages. During the storage period of 6 months under refrigerated conditions, a decrease in Y and R values was observed which might be due to degradation of bixin (Table 6). Traditional oil soluble annatto dye formulation applied in butter at 5.0 and 10.0 mg/kg showed colour values with the R value almost comparable to that of a commercial sample. However, visually they were not comparable and also the colour was less appealing.

Cheese Among the various concentrations applied, the Y value (1.5) of single strength oil/water soluble PG formulation at 3.75 mg/kg was almost similar to the colour (creamy-yellow) and Y value (1.6) of one of the commercial cheese slice sample (Table 3). The R value (4.6) at this concentration was similar to that of commercial sample by using the same formulation. The PG formulation at a concentration of 3.75 mg/kg yielded creamy yellow shade when compared to

Table 5 Colour measurement (lovibond) during storage for butter, cheese, paneer, biscuit cream and icing cream

Concentration of bixin/norbixin (mg/kg)	Storage period in months							
	0 month		2nd month		4th month		6th month	
	R	Y	R	Y	R	Y	R	Y
Butter								
3.75	1.4±0.17	4.0±0.17	1.4±0.06	4.0±0.15	1.2±0.20	3.8±0.26	1.0±0.12	3.4±0.10
5.0	2.0±0.21	6±0.31	1.8±0.30	5.9±0.38	1.7±0.26	5.6±0.20	1.6±0.25	5.5±0.12
Cheese								
3.75	1.4±0.17	4.2±0.20	1.4±0.20	4.1±0.21	1.0±0.12	3.0±0.31	1.0±0.06	2.8±0.20
Biscuit cream								
5.0	2.1±0.30	5.0±0.36	2.0±0.17	5.0±0.21	1.9±0.20	5.0±0.10	1.9±0.08	5.0±0.36
25	2.8±0.17	11.1±0.30	2.7±0.21	11.0±0.45	2.6±0.26	11.0±0.35	2.5±0.17	10.9±0.26
50	4.0±0.36	15.2±0.26	4.0±0.21	15.0±0.38	3.8±0.17	14.0±0.29	3.8±0.10	13.8±0.35
400	7.1±0.44	17.2±0.36	6.9±0.36	16.1±0.26	6.6±0.26	15.7±0.26	6.5±0.15	15.5±0.31
Icing cream								
5.0	1.7±0.26	5.0±0.36	—	—	—	—	—	—
30	2.2±0.20	7.0±0.31	—	—	—	—	—	—
50	4.0±0.31	11.1±0.26	—	—	—	—	—	—
300	7.0±0.42	16.1±0.40	—	—	—	—	—	—
500	8.1±0.26	17.0±0.26	—	—	—	—	—	—
Paneer								
12.5	4.0±0.26	12.1±0.20	—	—	—	—	—	—
25	5.9±0.18	19.1±0.51	—	—	—	—	—	—

Values are mean of triplicate analyses ± SD, all values differ significantly ($P \leq 0.05$)

those obtained by other formulations at higher concentrations. The recovery of bixin reduced from the initial 90 % to 49 % (Table 6) during 6 months storage under refrigerated conditions. The lesser recovery of bixin during storage might be due to the binding of the dye to the milk proteins (Chandan et al. 2008) and also due to degradation of bixin. This can be clearly understood by observing the Y and R values which remained same up to 2 months storage and decreased slightly during further period (Table 5). A similar observation was made in cheese flavoured peanut spread. The colour of the cheese flavoured peanut based spread did not significantly change during storage at 30 °C for 60 days (Muego-Gnanasekharan et al. 1993).

Traditional oil soluble annatto dye formulation was applied in cheese in different concentrations (2.5–50 mg/kg) and observed for the colour values. The Y value was almost comparable to that of a commercial sample. But, visual colour was less appealing and was not comparable to commercial sample. During storage period of 1 month under refrigerated conditions an increase in Y values was observed indicating the shade shift towards yellowish orange.

Biscuit cream In case of biscuit cream, of all the formulations applied (Table 4), oil soluble formulation (6.75–1,000 mg /kg) and PG based formulation (5–400 mg/kg) was yielding good and attractive colours visually comparable to commercial products. Also, it was found that PG formulation was yielding attractive colours at lower concentrations when compared to oil soluble formulations applied at higher concentrations. They were comparable to commercial samples visually. However, their colour values were not comparable to commercial samples. This could be attributed to the fact that addition of colours in commercial samples might be practiced with a combination of two or three colours. Oil/water soluble PG formulation yielded a cream shade at 5 mg/kg, pineapple yellow with 25 mg/kg, mango shade at 50 mg/kg and orange shade at 400 mg/kg levels. Maximum recovery of bixin to an extent of 96 % and minimum of 54 % (Table 6) was observed during analysis on 6 months storage at room temperature.

Paneer In paneer curries commercial orange shades are generally applied to paneer for attractive appearance. The dye gets coated superficially on the surface without

Table 6 Stability of dye during processing and storage in butter, cheese, paneer, biscuit cream and icing cream

Concentration of bixin applied, mg/kg	Storage period (in months)							
	0 month		2nd month		4th month		6th month	
	Recovery of bixin							
	(mg/kg)	%	(mg/kg)	%	(mg/kg)	%	(mg/kg)	(mg/kg)
Butter								
3.75	3.7±0.66	98.1±1.48	3.6±0.08	95.2±2.18	3.4±0.12	91.5±3.18	3.3±0.12	88.5±3.14
5.0	4.9±0.04	97.8±0.46	4.8±0.13	95.0±2.66	4.6±0.06	92.4±1.25	4.5±0.06	90.6±1.22
Cheese								
3.75	3.3±0.04	90.2±0.62	2.6±0.02	68.6±0.41	2.4±0.04	64.2±0.96	1.8±0.09	49.1±2.32
Biscuit cream								
5.0	4.8±0.07	96.8±1.22	4.8±0.02	95.2±0.35	4.3±0.04	86.8±0.81	4.2±0.06	83.3±1.11
25	23.4±0.19	93.6±0.50	22.2±0.09	88.7±0.34	19.2±0.03	76.8±0.13	19.1±0.06	76.3±0.22
50	43.7±2.12	87.4±0.23	40.2±0.02	80.3±0.04	35.8±0.07	71.6±0.13	35.7±0.04	71.4±0.07
400	362.7±1.79	90.7±0.32	332.2±0.52	83.0±0.13	261.0±1.07	65.2±0.27	219.2±0.74	54.8±0.19
Icing cream								
5.0	4.8±0.18	96.0±2.21	—	—	—	—	—	—
30	29.7±0.72	99.0±2.34	—	—	—	—	—	—
50	48.5±0.55	96.9±0.81	—	—	—	—	—	—
300	297.2±2.75	99.1±0.84	—	—	—	—	—	—
500	483.8±5.35	96.7±0.96	—	—	—	—	—	—
Paneer								
12.5 ^a	62.5±27.62	75.7±1.35	—	—	—	—	—	—
25 ^a	112.4±5.93	68.1±1.82	—	—	—	—	—	—

Values are mean of triplicate analyses ± SD, all values differ significantly ($P \leq 0.05$)

^a Based on milk

penetration into the pieces. Among the various formulations applied in different concentrations (Table 4), it was observed that 12.25 and 25 mg/kg of PG formulation was more appealing and attractive. When water soluble carbonate formulation and oil soluble formulations were applied in milk at concentrations of 12.5–50 mg/kg the product was not coloured uniformly. Application of dye in milk before coagulation has yielded the uniform coloured paneer when compared to application of the dye after coagulation. Coloured commercial samples are not available for comparison. Maximum recovery of bixin to an extent of 75 % was observed for 0 days storage period.

Icing cream Icing cream samples were applied with annatto dye oil soluble and PG based formulations (Table 6). The Oil/water soluble PG formulation applied in different concentrations imparted an eye appealing colour and was also preferred by the panellists. At 5 mg/kg PG based formulation yielded cream with a shade suitable for applying as base cream on cake toppings. Increasing concentration in the range of 30–500 mg/kg yielded products with light cream to orange shades, useful for decorating cakes. Application of annatto dye formulations in icing cream yielded products not comparable with commercial colours. However, these formulations can be applied at different levels based on consumer preference. Maximum recovery of bixin to the extent of 96 % was observed for 0 days storage period.

Conclusion

The studies showed that the annatto dye oil/water soluble PG formulation could effectively be used in most of the dairy products to impart an eye appealing colour. It was also observed that the formulation in all dairy samples studied yielded products with good brightness and yellow colour comparable to commercial samples when compared to other formulations where, red colour was dominant. Hence, annatto oil/water soluble PG formulation can be suggested as the tailor-made natural colourant to impart required colour shades in general for dairy products, and in particular for butter and cheese.

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