Design and Development of Eco- Friendly Colourants for specific Food and Cosmetic Applications

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Abstract— *The Objective of this study is to develop the colourants from natural sources such as Annatto seeds and Red Beetroot for food and cosmetic applications. The natural colourants prepared were applied for making cake & cream and the product was found to be acceptable for human consumption. These same colourants can also be used for the preparation of cosmetic items such as Lipsticks, Soap and Shampoo. This study also aims to quantify the trace metal elements (Cr, Cu, Pb, and Fe) in natural colourants and synthetic food colourants (Tartrazine, Sunset yellow, Cryptoxanthin and Yellow 2G) available in local market Tamilnadu using Inductively Coupled Plasma- Optical Emission Spectrometer (ICP-OES). For this purpose, 50mg of synthetic sample was digested using 3 ml of concentrated hydrochloric acid and 1 ml of concentrated nitric acid. These digests were diluted to 50 ml, 10 ppm solution and the analytes were determined by ICP-OES. The concentration of the Cr, Fe, Pb and Cu in synthetic colourants were found to be available in the range of 0.01-0.37 mg/kg, 0-1 mg/kg, 0-0.09 mg/kg and 0-0.5 mg/kg respectively. Similarly for the extracted natural colourants the concentration of Cr, Fe and Pb were in the range of 0-0.02 mg/kg, 0.24-0.65mg/kg, and 0.03-0.08 mg/kg respectively. Copper was not found to be available in any of the natural colourants extracted. The results were compared with the maximum limit established by the Joint Expert Committee on Food Additives /World Health Organization (JECFA/WHO) and U.S Department of Health and Human Science. The level of expected metal intake by a person per week was determined by means of dose and intake calculations. The metal concentrations in synthetic colourants were found to be less than the prescribed limits of WHO. In natural colourants metal limits (Cr, Pb and Cu) were much lower than the synthetic colourants. In Extracted Natural food Colourants some of the metals (Cr, Pb) were in concentration of zero mg kg^{-1} or Close to Zero. When compared with synthetic food colourants, the percentage reduction in metal concentration was 14 -100 %.*

Keywords— Food colourants, Natural Colourants, Heavy metals ,ICP-OES, dose and weekly intake.

I. INTRODUCTION

The colours have been an important factor for acceptability of products like textiles, cosmetics, food and other items. Synthetic colours derived from minerals (lead chromate, copper sulphate) may cause serious health problems. Some of the synthetic food colours have been removed from both national and international lists of permitted food additives because of their mutagenic and carcinogenic activity [16]. Like other countries, India has stringent rules like Prevention of Food Adulteration Act of 1954 for additives to be used in food manufacturing [15]. The permissible colours are also not totally safe, even repeated exposure of permitted synthetic colours may cause health hazards [16]. For each of the permitted colours the joint FAO/WHO expert committee of food additives has set an Acceptable Daily Intake (ADI). The Acceptable Daily Intake (ADI) has been defined as the amount of a substance that can be consumed everyday throughout the lifetime of an individual without any appreciable health effects. There were different studies conducted by researchers on metal toxicity in cosmetics in various countries [4], [3] & [12]. The concentration of trace metals in colourants added in the food items should not exceed its permissible limits. Food and Agricultural Organization /World Health Organization (FAO/WHO) suggests the metal limit allowable per day on our regular human dietary system. The limit of ADI for Cadmium, Lead and Zinc should not exceed 0.8 µg/kg body weight (kg b.w), 0.02 µg/kg b.w and 0.3 µg/kg b.w respectively. The maximum allowable limits of ADI for Copper and Iron are at the level of 2-3 mg/day and 9 mg/day respectively. The U.S Department of Health and Human Science prescribed the maximum limit for chromium as 15.1µg/day. As like food additives, the chemical and heavy metals present in the cosmetic items causes various health risks to the users [11]. The trace metals such as copper, Chromium, Cadmium, Iron, Lead, Arsenic, Nickel and Aluminium were found to be available in different cosmetic items [19].

The permissible limits of heavy metal concentration in the cosmetic products are mentioned in the Indian and other National regulations. The concentration of lead allowed in the lipstick should not exceed 20 ppm and the arsenic should not exceed 2 ppm [5]. The colourant and additives added in cosmetics must comply with Drugs and Cosmetics Act, 1940; Drugs and Cosmetics Rules 1945.Other metals present in the cosmetic should not exceed cumulatively 100 ppm and risk associated with these metals are considered less significant when compared with lead, arsenic, cadmium, mercury and antimony [2]. The results obtained from ICP spectroscopy were

compared with limit prescribed by these organization. This study calculated the concentration of metal entering through ingestion and it was assessed using data obtained from ICP-OES measurement. Inductively coupled plasma optical emission spectrometry is widely used to determine heavy metal concentration in given liquid sample.

II. ICP Anlysis (Synthetic Colourants)

The concentration metals in synthetic and extracted natural colours were determined by ICP-OES. This study focused on quantification of four important metals such as Chromium, Iron, Lead and Copper. The study conducted by *Pratima Rao* pointed that the consumption of colours like tartrazine and sunset yellow were an excessive consumption in India during festival times and also they also concluded as, it is responsible for Indian government to take consideration on maximum permissible limits [13].

A. Sample Collected

Totally four synthetic colour samples were collected from local markets. The first two samples listed in table 1 were collected from Cake Baking Industry and the rest of the samples collected from local market. The collected samples were subjected to ICP analysis in order to quantify the trace metals.

B. ICP-OES Analysis

ICP Spectrometer is widely used for quantifying the unknown metals present in a sample. When plasma energy is given to the samples, then its atoms are excited to conduction band. The excited atoms release spectrum rays when it returns to valence band and the corresponding photon wavelength are measured. The following steps were followed in order to analyse the samples

- 2% HNO_3 Solution is considered as a blank solution. A 10 ml of Concentrated HNO_3 was taken in a 500 ml Standard Measuring Flask (SMF) and the space remaining was filled up with pure Millipore water. The prepared solution was mixed well in order to get homogenous solution.
- The collected sample was exactly weighed up to 50 mg by using electronic weighing machine.
- Weighed samples were taken in the beakers and Aqua regia (concentrated HCl: HNO_3 = 3:1) was added (3 ml HCl with 1 ml HNO_3).
- After a rhythmic agitation of this acid mixture, it was then kept in a hot- plate at the temperature of 100^0C until all liquid gets evaporated.
- The residues settled on the bottom of the beaker were rinsed with Blank Solution and transferred into 50 ml SMF.
- From this 50 ml solution, 0.5 ml was taken in another 50ml SMF and the remaining space filled up with Blank Solution. This was the final solution, considered as a 10 mg/L sample solution and ready to be injected in ICP Spectroscopy.
- The Merck Standard solution was used as a reference solution. This solution has twenty three heavy metals in the equal concentration of 10 mg/L which also includes Cr, Cu, Fe and Pb.
- 0.5 ml of this standard solution was taken in a 50ml SMF and then it was filled up with Blank Solution.
- The blank solution was injected into the ICP spectroscopy by means of the nebulizer. The instrument was kept at 4-6 bar pressure. The blank solution may contain trace amounts of metal elements. These concentrations were set to zero value in the instrument.

TABLE I. Heavy Metal Detected in Synthetic Colourants by ICP Spectrometer

Sample ID	Sample	Common Industrial Name	E No	Level of Detection (mg/kg)			
				Cr	*Fe*	*Pb*	*Cu*
I	Bakery-Yellow	Tartrazine	E102	0.01	0	0.04	0
II	Bakery-Orange	Sunset Yellow FEF	E110	0.23	0.7	0	0.5
III	Kesari Powder-Red Orange	Cryptoxanthin	E161c	0.37	1	0.09	0
IV	Kesari Powder-Lemon Yellow	Yellow 2G	E106	0.11	0.1	0.05	0

- The standard solution was next injected to the instrument and the 23 metals in this standard solution were set to 10 mg/L in the ICP Spectroscopy. Finally, the sample solution was injected and results were observed. This result was in mg/L and can be converted into mg/kg by multiplying the dilution factor of 10.

The level of metals analysed in synthetic colourants is shown in table 1. The chromium was found in all synthetic samples taken in the

concentration range of 0.01 – 0.37 mg/kg. The limit of iron is in the range of 0-1mg/kg. Lead was found to be available in most of the synthetic colourants taken and it is in the concentration range of 0-0.09 mg/kg. The metal Copper was only found in Cryptoxanthin colour mostly used in the preparation of Kesari and it is at the level of 0.5 mg/kg.

C. Dose and Intake Calculation

Totally three cases were assumed here. In case I it was assumed that each week a child/ person consumes 250 gram of coloured cake and ice cream. In case II and III it was assumed that a person consumes 500 gram, 1000 gram of coloured food items weekly. In the local Bakery industry, 15 gram of colour powder were added per kg of cake dough. On the basis of data collected the dose and intake calculation were determined and the expected level of metal entering through ingestion was calculated for synthetic colourants and it was listed in table III. Dose calculation for Chromium present in the synthetic colour Tartrazine is derived below.

Case I: Taking 250 gram of coloured food per week

Detected 'Cr' concentration in sample I (Tartrazine)
= 0.01 mg/kg,
'Cr 'present in 1 gram of sample I
= (0.01 mg)/1000
= 0.00001 mg
Amount of colourant added to 1 kg of food produced (Cake) is around 15 gram, so totally 3.75 gram is added to 250 gram of cake/ice cream.

For 250 gram of food, (for a week)
'Cr' concentration
= (0.00001x 3.75) x 1000 µg
= 0.038 µg

Case II: Taking 500 gram of coloured food per week

Detected 'Cr' concentration
= 0.01 mg/kg,

For 500 gram food,
'Cr' Concentration
= (0.00001x 7.5) x 1000 µg
= 0.076µg

Case III: Taking 1000 gram of coloured food per week

Detected 'Cr' concentration
= 0.01 mg/kg,
For 1000 gram food,
'Cr' Concentration

= (0.00001x 15) x 1000 µg
= 0.15µg

TABLE II. WEEKLY METAL INTAKE FOR SPECIFIED CASES (SYNTHETIC COLOUR)

Sample ID	Metal Intake (µg/week)	Analyte			
		Cr	*Fe*	*Pb*	*Cu*
1	Case I	0.038	0	0.15	0
	Case II	0.076	0	0.3	0
	Case III	0.15	0	0.59	0
2	Case I	0.863	1.875	0	2.588
	Case II	1.726	3.75	0	5.176
	Case III	3.452	7.5	0	10.352
3	Case I	1.388	3.75	0.338	0
	Case II	2.776	7.5	0.676	0
	Case III	5.552	15	1.352	0
4	Case I	0.413	0.375	0.788	0
	Case II	0.826	0.75	1.576	0
	Case III	1.652	1.5	3.152	0

III. EXTRACTION OF NATURAL COLOURANTS

A. Extraction of Annatto Colour- Water Soluble

Ministry of Agriculture, Fisheries and Food (MAFF) pointed that Annatto is the most commonly consumed natural colour additive in the UK. The daily consumption was estimated to be 0.065 mg kg^{-1} body weight day^{-1} [9]. The colour of Annatto seed were extracted using 0.1 M KOH solvent (Seed: KOH = 1:20). This mixture was agitated uniformly using Magnetic stirrer at 70^0 Centigrade. 0.5% Tween 80 was added during the extraction for pigment stabilization. The slurry was filtered through muslin cloth, centrifuged at 3000 rpm for 30 minutes and then filtered through a filter paper (60 Mesh) [8]. The product was a pure, dark orange- red coloured solution.

B. Extraction of Beetroot Colour- Water Soluble

Commercially available beetroot was used as extraction material. The vegetable material was cut into slices having approximately 21 mm length, 5 mm width and 1-2 mm height. 0.2% Citric Acid solution was used as an extraction solvent. Since betanin are naturally alkaline, the addition of citric acid solvent facilitates neutralization. The extraction was carried out at 80^0C up to 15-20 minutes in the liquid solid /ratio of 5:1. The superficial layer of colourant extract was filtered by filter paper (60 Mesh) and centrifuged at 3000 rpm for 30 minutes. This colour gives the better result upon instant usages.

IV. ICP ANLYSIS (NATURAL COLOURANTS)

The extracted natural colour was subjected to ICP-OES analysis to find the trace metal concentration. The extract is a pure liquid solution so that it does not require any digestion by with Aqua regia. 0.05 ml of this natural colourants taken in an SMF and the space remaining was filled up with Blank solution. This is a final solution ready to be injected into ICP Spectrometer. Trace Metal concentrations were found by the procedure discussed earlier in chapter II. The result was listed in table III.

TABLE III. HEAVY METAL DETECTED IN NATURAL COLOUR SAMPLES BY ICP SPECTROMETER

Extract ID	Sample	Level of Detection (mg/kg)			
		Cr	*Fe*	*Pb*	*Cu*
i	Annatto (Water Soluble)	0.02	0.65	0.08	0
ii	Beetroot (Water Soluble)	0	0.24	0.03	0

The metal limits in natural colourants found to be comparatively lesser than synthetic colourants. Copper was eliminated in natural colourants.

A. Dose and Intake Calculation

TABLE IV. WEEKLY METAL INTAKE FOR SPECIFIED CASES (NATURAL COLOUR)

Extract ID	Metal Intake (µg/week)	Analyte			
		Cr	Fe	Pb	Cu
i	Case I	0.075	2.438	0.3	0
	Case II	0.15	4.875	0.6	0
	Case III	0.315	10.238	1.26	0
ii	Case I	0	0.9	0.113	0
	Case II	0	1.8	0.257	0
	Case III	0	3.78	0.473	0

The same three cases assumed here as like as synthetic dose and intake calculation. The dose and intake calculation were determined and listed in table IV.

V. COMPARITIVE ANAYSIS OF %OF METAL REDUCTION IN SYNTHETIC VERSES NATURAL COLOURANTS

The comparison between percentage of metal reduction in synthetic and natural colourants is shown in table V. The concentration of Chromium in natural colourants is comparatively lesser than synthetic. Other metal concentrations are also reasonably found to be lesser in natural colourants.

TABLE V. CORRELATION OF %OF METAL REDUCTION IN NATURAL COLOUR VERSES SYNTHETIC COLOUR

Synthetic Vs Natural Colours									
Sample Id	**Analyte**	**L.O.D (mg/l) in Annatto**	**L.O.D (mg/kg) in Synthetic**	**%of Metal Reduction**	**Sample Id**	**Analyte**	**L.O.D (mg/l) in Beetroot**	**L.O.D (mg/kg) in Synthetic**	**%of Metal Reduction**
1	Cr	0.02	0.01	**0**	1	Cr	0	0.01	**100**
2	Cr	0.02	0.23	**91.3**	2	Cr	0	0.23	**100**
3	Cr	0.02	0.37	**94.6**	3	Cr	0	0.37	**100**
4	Cr	0.02	0.11	**81.8**	4	Cr	0	0.11	**100**
1	Fe	0.65	0	**0**	1	Fe	0.24	0	**0**
2	Fe	0.65	0.70	**7.1**	2	Fe	0.24	0.7	**65.7**
3	Fe	0.65	1	**35.0**	3	Fe	0.24	1	**76**
4	Fe	0.65	0.1	**0**	4	Fe	0.24	0.1	**0**
1	Pb	0.08	0.04	**0**	1	Pb	0.03	0.04	**25**
2	Pb	0.08	0	**0**	2	Pb	0.03	0	**0**
3	Pb	0.08	0.09	**11.1**	3	Pb	0.03	0.09	**66.7**
4	Pb	0.08	0.05	**0**	4	Pb	0.03	0.05	**40**
1	Cu	0	0	**0**	1	Cu	0	0	**0**
2	Cu	0	0.50	**100**	2	Cu	0	0.5	**100**
3	Cu	0	0	**0**	3	Cu	0	0	**0**
4	Cu	0	0	**0**	4	Cu	0	0	**0**

VI. RESULTS AND DISCUSSION

The concentration of metals in synthetic and extracted natural colours was determined by ICP-OES. This study focused on quantification of four hazardous metals such as Chromium, Iron, Lead and Copper. The study conducted by *Pratima Rao* pointed that the consumption of colours like tartrazine and sunset yellow were an excessive consumption in India during festival times and also they also concluded as, it is responsible of Indian government to take consideration on maximum permissible limits. [14]. The level of metals analysed is shown in table 1 and 3. The chromium was found in all synthetic samples taken in the concentration range of 0.11 – 0.37 mg/kg. The limit of chromium is found to be available in extracted annatto colour in the concentration of 0.02 mg/kg and it was not found in colour extracted from beetroot. The limit of iron in both the type of colours looks equal in the range of 0-0.75 mg/kg. Lead was found to be available in most of the synthetic and natural colours taken in the range of 0.03-0.09 mg/kg. The metal Copper was only found in Cryptoxanthin colour mostly used in the preparation of Kesari and it is in the level of 0.5 mg/kg and it was not found to be available in natural colours. All the metal concentration was below the prescribed limit prescribed by the World Health Organization. Heavy metals are dangerous because they tend to accumulate in the human body over a few years. The accumulation of these metals will cause serious chronic health risk, such as stomach pain, lung and throat problems. Some of the metals like Arsenic, Cadmium, Chromium, Silver, Mercury and Lead have a tendency to cause cancer when it exceeds the permissible limit [18] & [6]. The extracted natural colourants were applied in the cake colouring process in a Baking Industry, Rajapalayam.

Figure 1: Application of natural colourants on cake making process

The cake dough is prepared by the usual procedures and at last the extracted natural colourants were added shown in figure 1. The prepared cake dough is kept in an Aluminium vessel in an oven for 25 minutes at a temperature of 180^0C. In which, the annatto colourants have the ability to withstand the high temperature and the results were reasonably

accepted and appreciated by the bakery workers. The colour extracted from beetroot dose not able to withstand high temperature rather it can be most suited for cake cream colouring. This same colournats can also be used in a cosmetic items [13].

VII. Conclusion

In this study, Chromium, Copper, Iron and Lead were determined in various synthetic food colours and in natural colours. From the results, it is inferred that the levels of heavy metals present in synthetic and natural colours were in low quantities. However the continuous use of synthetic food colours cause slow accumulation on human organs and causes harmful effects to the users over time. Extensive use of such synthetic colours should be avoided. The investigation also focused on extraction of colourants from Annatto seeds and Red Beetroot that may be used instead of synthetic colourants. It doesn't mean that natural colours are most substitutes for synthetic, since it's also has its own limitations. Nevertheless, some of the metals and chemicals in synthetic colours are eliminated when we go with usage of natural colourants.

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