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Nutritional Analysis and Stability Studies of Some Natural and Synthetic Food Colourants

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Abstract: The pH, titratable acidities, proximate and mineral compositions of two natural colourants and three synthetic colourants were determined. The pH and titratable acidity were determined over a period of 14 days at 24 h intervals. The natural colourants were the calyxes of *Hibiscus sabdariffa* (SL) and the stem of *Sorghum bicolour* (KD), while the synthetic colourants were Egg Yellow (EY), Chocolate Brown (CB) and Dark Orange (DO). The pH of all samples increased as the number of days increased. The pH of SL was considerable lower than that of other colourants in this study (from 1.6 on day 1 to 2.7 on day 14) thus suggesting high acidity while the other colourants had basic pH (5.6-6.2 on day 1 to 7.4-8.4 on day 14). The natural colourants had higher moisture, lipid, carbohydrate and fibre contents while the synthetic colourants were very high in ash content. The protein contents of the colourants were fairly uniform irrespective of their sources. The synthetic colourants were particularly high in K and Mg while the natural colourants were rich in Ca.

Key words: pH, titratable acidity, proximate composition, mineral content, food colourant

INTRODUCTION

Colour is an important factor in the acceptability of food products and food quality is first adjudged on the basis of its colour. People associate certain colours with certain flavours (Delwiche, 2004) and the colour of food can influence it perceived flavour. In fact, the colour of a food influences not only the perception of flavour, but also attraction and quality and subsequently, consumption (Abd El-Galeel, 2002). Food manufacturers therefore often add colourings to their products to simulate or enhance a colour that is perceived by the consumer as natural, to mask natural variations in food colours, to offset colour loss due to light, extremes of temperature, moisture and storage conditions. In addition food colourants provide identity to foods, by colour impartation on foods which would otherwise be colourless. And sometimes they are added just for effect or decorations purposes (Henry, 1996; Food Advisory Committee, 1987; Abd El-Galeel, 2002).

Food colourants are either natural or synthetic depending on source. Natural colourants are extracted from renewable sources such as plant materials, insects, algae, etc, while the synthetic colourants are manufactured chemically and are the most commonly used dyes in the food, pharmaceutical and cosmetic industries. The safety of synthetic colourants has previously been questioned, leading to a reduction in the number of permitted colourants. Due to this limitation and worldwide tendency towards the consumption of natural products,

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the interest in natural colourants has increased significantly (Huck and Wilkes, 1996). Of special interest to the food industry is the limited availability of red pigments (Hallangan, 1991; Lauro and Francis, 2000), therefore research into natural sources of red pigments have increased recently.

Recent controversy and criticism about the safety of food colourants have been directed towards the synthetic colourants. The natural colourants have been relatively free of criticism may be due to the belief that most are derived from food sources that have been consumed for many years (Francis, 1989). Beside the colour attributes, anthocyanins and other phenolic colourants from plant sources have been reported to be beneficial to health with potential physiological effects, such as antineoplastic, radiation-protective, vasotonic, vaso-protective, anti-inflammatory, chemo- and hepato-protective effects (Kamei *et al*., 1995; Mazza and Miniati, 1993; Minkova *et al*., 1990; Wang *et al*., 1997). Synthetic colourants on the other hand possess an intensive colouring strength, good solubility, high stability and they are easier to process than natural food colourants. The application of natural food colourants are limited and they have some disadvantages compared with the artificial colourants, though they are relatively safe in terms of toxicological check and incompatibility reactions (Abd El-Galeel, 2002).

Due to perceived safety and physiological advantage of the natural colourant over synthetic ones, interests are being geared into search of new natural colourants and the verification of the safety of existing ones. *Hibiscus sabdariffa* (also known as Roselle) is a tropical plant of considerable economic potential. Its calyxes have been suggested as food colourants for food industries; emulsifier for carbonated drinks, jam manufacture, juices and natural food colourant (Duangmal *et al*., 2004; Francis, 1989). The calyxes are rich in anthocyanin, ascorbic acid and hibiscus acid (Pilando *et al*., 1985; Ibrahim *et al*., 1971). It is water soluble with brilliant and attractive red colour and with sour and agreeable acidic taste which aid digestion. The other health benefits of this plant include diuretic and choloratic properties, intestinal antiseptic and mild laxative actions. It is also used in treating heart and nerve disorder, high blood pressure and calcified arteries (Asolkar *et al*., 1992; Chopra *et al*., 1956, 1969).

Sorghum bicolour stem, the second natural colourant been consider in this study is most extensively cultivated in the drier Northern Guinea, Sudan Savanah and Greenland of Africa, plains of India and the great plains of United State of America (FAO, 1988). It is the staple food of the world's poorest people in Sahel zones of Africa, Middle East, India and China (Kochar, 1981). Three major cultivars are grown in Nigeria; the Guinea, Kaura and Farafara. *Sorghum bicolor* stem is sweet and contain some sugars and minerals which make it useful for syrup manufacture (FAO, 1988). The dried stem is used as fuel in the tropics, apart from the cereal being used as food beverage (Ihekoronye and Ngoddy, 1992; Odetokun, 1997). The mature black purple shealth of the stem (locally known as karandafi or poporo) generally sold in small bundles is used as colour additives in cooking meals and also taken as beverages when steeped or boiled in water in many homes in Nigeria (Adetuyi, 2004). The resultant solution is bright red and the flour of the stem give similar colour when applied to food items. It is also used as a food preservative. The antibacterial activity of the extract has been proven by growth inhibition of *Pseudomonas aeruginosa*, *Lactobacillus* species, *Bacillus* species and *Carynebacterium bpedes* (Adetuyi, 2004). It is known to be the fourth most important cereal crop after wheat, rice and maize.

The nutritional quality of three synthetic and two natural colourants were undertaken and the advantages of one type over the other were analyzed. The stability of the resultant solution from the colourants was also considered.

MATERIALS AND METHODS

Collection of Samples

Three synthetic colourants: Egg Yellow (EY), Chocolate Brown (CB) and Deep Orange (DO) and two natural colourants: the calyces of *Hibiscus sabdariffa* known as Sobo Leaves (SL) and the stem of *Sorghum bicolor* known as poporo or karandafi (KD) were used. The *Hibiscus sabdariffa* calyxes and *Sorghum bicolor* stem were purchased from Pata market in Ilorin, Nigeria while the synthetic colourants were purchased in a departmental store, also in Ilorin, Nigeria. The three synthetic colourants were produced by Preema Internal Ltd., United Kingdom. The EY is a mixture of sodium chloride, E102 tartrazine and E110 sunset yellow FCF; CB is sodium chloride, E155 brown HT, E102 tartrazine and E122 carmosine, while DO is sodium chloride and E110 sunset yellow FCF. The study was carried out between the months of March and July 2008 at the Organic/Food Chemistry laboratory of Chemistry department of the University of Ilorin.

Sample Preparation

A 10% solution of each sample of the synthetic colourants were prepared while in the case of natural colourants, 10 g each of dried SL leaves and KD stems were separately soaked overnight in 90 mL of hot water (to make 10% solution). The mixtures were then decanted to remove the shafts and obtain clear coloured solutions.

Stability Studies

The stability of the colourant solutions was monitored by measuring the pH at 25°C for 15 days at 24 h intervals. The titratable acidity was also determined where possible by titrating 10 mL of each sample with 0.1 M NaOH solution using 1% phenolphthalein as indicator. The titration was carried out on a daily basis for a period of 15 days. The titratable acidity of some solutions could not be determined by simple titrations because of the intensities of the colours of the solutions.

Nutritional Analysis

Standard methods of the Association of Official Analytical Chemists (AOAC, 1984) were used to determine the crude protein content, crude fat, total ash, crude fibre and moisture content of each sample. Crude protein (Total nitrogen (%)×6.25) was determined by the Kjeldahl method, using 2 g samples; Crude fat was obtained by exhaustively extracting 5 g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40-60°C) as the extractant. Ash was determined by the incineration of 2 g samples in a muffle furnace maintained at 550°C for 5 h. Crude fibre was obtained by digesting 2 g of sample with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace maintained at 550°C for 5 h. Moisture content was determined by heating 2 g of each sample to a constant weight in a crucible placed in an oven maintained at 105°C. Total carbohydrate content was obtained by difference.

Mineral Content Determination

The mineral contents of the colourants were determined by atomic absorption spectrophotometry after wet ashing of the samples. One gram of each sample was digested using a mixture of perchloric, nitric and sulphuric acids according to the method of Oshodi *et al.* (1999). The digests were then diluted with distilled water and the mineral contents (Ca, Mg, Mn, Fe, Cu and Zn) of the solutions were determined by atomic absorption spectrophotometry using a Buck Scientific 200-A model AAS. Na and K were analyzed by flame photometry using a Dunmow Essex PFP 7 model flame photometer.

RESULTS

The results obtained for the pH values of the samples showed that the pH for all samples increased i.e. the solutions became more acidic as the days progressed (Table 1). A sharp increase was observed after the 4th or 5th day for all the colourants. Three synthetic colourants had similar pH values from day to day and the values ranged between 5.6 and 7.9. The results also showed that both the most acidic as well as the most basic colourants were natural; therefore the acidity or alkalinity of colourants was not dependent on whether the colourants were natural or synthetic. The results of titratable acidity shown in Table 2 showed the relationship between the pH and the titratable acidity of the colourant solutions. Samples with high pH values had low titratable acidity and as the pH increased, the acidity decreased. KD with the highest pH after 14 days also had the least titratable acidity. The titratable acidity of SL and CB could not be determined, because the solutions of these colourants were very darkly coloured and it was not possible to observe any change in colour during simple acid-base titration.

The proximate composition of the colourants (Table 3) showed that the synthetic colourants had very high ash contents (79.50-82.50%) compared to the natural colourants (15.75-22.28%). The reverse was the case for carbohydrate and fibre contents of the colourants. The synthetic colourants had very low carbohydrate and fibre content (between 2.39 and 3.99%) while the natural colourants had values between 44.20 and 68.69%. SL and KD had the highest lipid and moisture contents. The protein contents of the colourants were fairly uniform but that of KD (7.66%) was low compared to others.

Table 4 shows the mineral composition of the colourants. In all the minerals considered in this study, K and Mg had the highest values, Zn and Fe had the least while Mn and Cu were not detected in the synthetic colourants but were detected at low concentrations in the natural colourants.

Table 1: pH stability of colourant samples

Colourant samples	pH values/days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
EY	6.2	6.3	6.6	6.7	6.9	6.9	7.3	7.6	7.7	7.7	7.9	7.9	7.9	7.9
CB	6.1	6.2	6.3	6.5	7.1	7.1	7.2	7.3	7.4	7.4	7.4	7.4	7.4	7.4
DO	5.6	6.2	6.2	6.3	7.1	7.2	7.3	7.3	7.4	7.4	7.4	7.4	7.4	7.4
SL	1.6	1.7	2.0	2.1	2.4	2.6	2.6	2.6	2.6	2.7	2.7	2.7	2.7	2.7
KD	6.2	6.4	6.6	6.8	6.9	7.7	7.8	7.9	8.0	8.2	8.3	8.4	8.4	8.4

Table 2: Titratable acidity of some colourant samples

Colourant samples	Titratable acidity ($\times 10^{-3}$ M)/days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
EY	8.3	5.0	5.0	4.0	4.0	3.5	3.5	3.0	2.5	2.5	2.5	2.0	2.0	2.0
DO	5.2	4.8	4.5	4.0	3.0	3.0	2.5	2.5	2.5	2.0	2.0	2.0	2.0	2.0
KD	8.3	5.0	4.0	3.0	2.5	2.0	2.0	2.0	1.5	1.5	1.0	1.0	1.0	1.0

Table 3: Proximate Composition of colourant samples

Colourant samples	Proximate composition (%)				
	Moisture content	Lipid content	Protein content	Ash content	Carbohydrates and fibre content
EY	2.66	2	9.85	81.50	3.99
CB	1.98	3	10.13	82.50	2.39
DO	2.23	4	11.38	79.50	2.89
SL	16.24	8	9.28	22.28	44.20
KD	3.90	4	7.66	15.75	68.69

Table 4: Mineral composition of the colourant samples

Colourant samples	Mineral composition (g mL^{-1})										
	K	Na	Ca	Mg	Fe	Zn	Mn	Cu	Na/K	Ca/Mg	K/(Ca+Mg)
EY	6.20	1.86	1.59	12.00	0.03	0.01	ND	ND	0.30	0.13	0.46
CB	12.10	1.09	0.57	13.10	0.02	0.01	ND	ND	0.09	0.04	0.88
DO	1.88	1.72	ND	1.65	0.03	0.01	ND	ND	0.91	-	1.14
SL	12.90	2.46	10.5	14.40	0.01	0.01	0.68	ND	0.19	0.73	0.52
KD	10.40	2.75	0.81	9.03	0.04	0.24	0.01	5.7	0.26	0.09	1.06

ND: Not detectable

DISCUSSION

We also found that the synthetic colourants were higher in ash than the natural, while the natural colourants contained more of the other nutrients. The sharp increase observed in the pH of the colourant solutions after the fourth or fifth day was attributed to breakdown of colourant constituents. Sobo leaves (the calyxes of *Hibiscus sabdariffa*) had the lowest pH values (1.6-2.7), which indicated high acidity. The sample also had the least variation in the pH values from day 1 to day 14 (1.1) and this indicated the stability of this colourant above others. The low pH and high stability was due to the presence of natural organic acids and antioxidants which have been shown by other workers to aid longer shelf life of the products in addition to possessing biological advantages to consumers (Ibrahim *et al*., 1971). It should therefore be applied with caution for individuals that are susceptible to stomach and peptic ulcer. The other natural colourant KD had the highest pH values (between 6.2 and 8.4). This makes it the least acidic colourant as well as the most unstable because of the high difference (2.2) recorded between the pH at day 1 and that of day 14. The synthetic colourants had similar pH values and the variation in pH between day 1 and day14 ranged from 1.3 in CB to 1.7 in EY and 1.8 in DO.

The high ash contents of the synthetic colourants compared to the natural is as a result of their composition. Synthetic colourants are made from inorganic compounds which account for the high ash content while the natural colourants are made of organic matters, hence the lower ash and higher carbohydrate contents of the natural colourants. The high carbohydrate and fibre content helps digestibility. The moisture content of SL (16.24%) was particularly high maybe because it was obtained from the calyx of a flower which has the ability to retain more water while KD is the thin covering of *Sorghum bicolor* stem. The high protein contents of synthetic colourants (9.85-11.38%) may be indicative of protein fortification in the course of synthesis while the low protein contents of the natural colourants may be due in part, to the part of the plant used (calyxes and stem sheath) and the method of extraction/preparation which involves heating, that may destroy some amino acids and result in consequent nitrogen loss (Raunio *et al*., 1978). The protein content of SL (9.28%) was comparable to those of the synthetic colourants (9.85% for EY, 10.13% for CB and 11.38% for DO) but that of KD was particularly low.

The values recorded for K and Na in all the colourants were not up to Recommended Daily Allowance (RDA) of 2,500 mg (Food and Nutrition Board, 2000) but were relatively higher in the natural colourants. Apart from giving the desirable colour to the food, the natural colourants also add reasonable quantity of K and Na that is required to maintain osmotic balance of the body fluid. Similar quantities of these minerals had earlier been reported in vegetable materials (Olaofe and Sanni, 1988; Aremu *et al*., 2005, 2006; Oshodi *et al*., 1999). Though the concentration of Fe and Zn were low with Fe ranging between 0.01-0.04 g kg^{-1} and Zn between 0.01-0.24 g kg^{-1}, the quantities are available for

biochemical functions. The daily recommended Fe requirements for humans are 10-15 mg for children, 18 mg for women and 12 mg for men. Cu was not detected in all the colourants except in KD with 5.7 g kg^{-1}, although the daily requirement is only 2 mg. Fe and Cu are present in cytochrome oxidase (enzyme) which is involved in energy metabolism (NAS, 1976). All the colourants could supply the daily body requirement of Ca of 800 mg except SL which means other sources of supply of this mineral is necessary when SL is the colourant of choice. Ca is a co-ordinator among inorganic elements, for example excess amount of K, Mg or Na in the body can be corrected by Ca and also adequate quantity of Ca in the diet assist in Fe utilization (Fleck, 1976). The Mg values in this colourants are high except for DO, a synthetic colourant which has 1.65 g kg^{-1}. Mg is an activator of many enzymes systems maintains the electrical potential in nerves (Shills, 1973; Shills and Young, 1992).

In Table 4, the Na/K, Ca/Mg and K/(Ca+Mg) ratios were also shown. Both K and Na are required for osmotic balance of the body fluid and the pH of the body, muscle regulation and nerve irritability, glucose absorption control and enhancement of normal retention of protein during growth (Food and Nutrition Board, 2000). Na/K ratio of less than one is the daily recommended dose (Nieman *et al*., 1991). All the colourants have Na/K ratio less than 1 hence they all have capacity to hinder high blood pressure. The Ca/Mg ratio is also less than 1 as recommended (Food and Nutrition Board, 2000). The K/(Ca+Mg) for all the colourants is between 0.46-1.14 milliequivalent. To prevent hypomagnesemia, the K/(Ca+Mg) must be less than 2.2 milliequivalent (Marten and Andersen, 1975), therefore all the colourants are suitable to prevent hypomagnesemia.

CONCLUSION

This study has drawn attention to the stability of the colourants, with particular attention on the acidity of one of the natural colourants, SL, which may make it unsuitable for individuals with a history or tendency of stomach or peptic ulcer. From the foregoing, it can be concluded that when considering bio-assimilation, bio-digestibility and easy elimination, the natural colourants are preferred over the synthetic ones. The higher presence of nutrients and antioxidants in the natural colourants also support their choice over synthetic colourants. However, in terms of cost, solubility, colour impact and higher mineral contents, the synthetic colourants may be preferred. Therefore, depending on the usage target or goal, any of the colourants can be used.

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