Research Journal of Chemical Sciences _____ ISSN 2231-606X
Vol. **5(4),** 1-6, April **(2015)** *Res. J. Chem. Sci.*

Nutritional, Antioxidant, Microbiological and Toxicological Studies on Red Dye Extracted from Red Beet Roots (Beta vulgaris)

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Available online at: www.isca.in, www.isca.me
Received 4th February 2015, revised 9th March 2015, accepted 17th April 2015

Abstract

Synthetic food colors are widely used in food products for attractiveness of food entities. These synthetic food colors have very ruinous effects on human health. It is now direly needed to replace artificial colors by natural food colors. The high levels of betalains are present in red beet root which have health enhancing properties and hence can be used as natural food colors and food additives. The present study aims to extract natural red dye from red beetroots for coloring the food products instead of the synthetic colors to avoid the harmfulness to humans. The unique crimson red color of red beet acts as best natural dye. The obtained nutritional analysis results suggest that the dye extracted from red beetroot can be considered a potential dye to be used as natural colorant. The prepared red dye paste exhibits the highest DPPH radical scavenging activity (33.32%) with water extract (10mg/ml). The stability of red dye depends on temperature and high temperature decreases its stability. The microbiological analysis showed that beet root extract does not allow the growth of microorganisms due to its antimicrobial activity. Animal trials showed that it is non-toxic and no mortality was found in mice.

Keywords: Betalains, food color, beet, dye, natural, antioxidant.

Introduction

Red beet (Beta vulgaris L.) roots are cultivated all over the world as a food source (figure-1) and are also helpful in development of natural red dye.

Figure-1
Beet roots

The color of red beet root is due to red betacyanin and yellow betaxanthin pigments that are placed in betalain or betanins[1] compounds group. These Beet pigments called betalains are found as natural dyes in various food products for example yougurt, processed meat, baked goods, ice cream and candies. Various *in vitro* studies have verified that betalains from red beetroots have potent antioxidant activity[2,3]. Different physicochemical factors such as temperature, pH, etc affect the pigment stability[4]. Betanins extraction from beetroot involves several processes like milling, pressing, filtration and evaporation[5].

Red beet root dye can be used in products with shorter shelf life or in products sold in dry state. Latest inclusive studies recognized the information regarding thermal degradation of betalains in their aqueous preparations[6,7] and the preliminary qualitative results on deprivation of betanins in solutions containing alcohol are also available[8].

Plants and plants products have been claimed to have many health-promoting effects such as preventing from coronary heart disease, atherosclerosis, cancer and ageing[9-11]. Betanins of red beet root are water-soluble pigments which have antioxidant, anti-inflammatory, hepatoprotective, anti-cancer properties. The aim of this study was to extract the natural water soluble dye from red beetroot and to evaluate the prepared dye regarding its Nutritional, Antioxidant and Microbiological analysis.

Material and Methods

Purchasing of Raw Material: The fresh red beet roots were purchased from the local vegetable market and were stored at 4°C.

Sample preparation: The beetroots were washed thoroughly to remove the soil. The vegetable material was cut into slices and weighed. Solid liquid extraction was carried out by blending the

sliced material with water. The samples were extracted 3 times to obtain maximum color. The juice was filtered to remove particulates. Addition of acids (0.15% Ascorbic acid, 0.1% Citric acid) in the extraction medium enhances Betanin stability. The filtrate was dried in a hot air oven at 35-45^{o}C. The concentrate was obtained which was further analyzed for its characteristics.

Nutritional analysis: The proximate analysis of red dye was carried out for moisture content, total ash, crude fat, crude fiber, carbohydrate, crude protein and energy value[12].

Antioxidant activity (DPPH radical scavenging assay): The hydrogen atom or electron donation ability of the red dye concentrate was measured from the bleaching of purple colored methanol solution of DPPH (2, 2-Diphenyl-1- picrylhydrazyl) at 517 nm. This Spectrophotometric assay uses the stable radical DPPH as a reagent [13]. The purple colored DPPH is a stable free radical, which is reduced to 2, 2-diphenyl-1-picrylhydrazine (yellow colored) by reacting with an antioxidant [14]. Hundred microliters of various concentrations of the red dye concentrate in methanol were added to 3 ml of a 0.004% methanol solution of DPPH. After a 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm by digital Spectrophotometer. The percentage inhibition of free radical (DPPH) was calculated as under:

Inhibition % (DPPH) = (A blank – A sample/ A blank) x 100

A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

Microbiological Analysis: Microbiological evaluation of beet root red dye concentrate was conducted according to the methods provided in the Manual of Food Quality Control[15]. During the storage period of 03 months, 10 gram aseptically weighed red dye concentrate was homogenized monthly with 90 ml of butterfield's phosphate buffer to give $1:10^{-1}$ dilution from which serial dilutions up to $1:10^{-6}$ were prepared. Total aerobic plate counts were enumerated by using 1 ml of each dilution and pouring plate count agar. Yeasts and molds were isolated and determined on potato dextrose agar after adding 1 ml of each dilution in plates. *Staphylococcus aureus* was enumerated by using 0.3 ml and 0.4 ml of serial dilutions on bared parker agar. Appeared colonies were counted and averaged to express microbial count as cfu/g sample. 1ml of serial dilutions was used to enumerate total coliforms, fecal coliforms, *E. coli and Pseudomonas spp.* by 3 tube Most Probable Number (MPN) method. *Salmonella spp*. were estimated by a pre-enrichment step in lactose broth at 35°C for 24 hours followed by selective enrichment in 10ml of tetrathionate broth and streaking on bismuth sulphite agar, hektoen enteric agar and xylose desoxycholate agar. For the enumeration and detection of microorganisms, standard media and reagents were purchased from LabM (United Kingdom) and Oxoid (United Kingdom).

Toxicological Studies: The toxicology study was conducted on healthy mice in order to detect the toxic effects of extracts (figure-4). 6 to b weeks old Mice were purchased and were housed in boxes at room temperature 25±2^{0}C and 12 hour dark/light cycle with food and water.

Results and Discussion

Red beet dye is obtainable as a concentrate which is formed by vacuum evaporation of beet juice to a total solids content of 40–60%. These dyes can be obtained in powder form by spray-drying of concentrate[16]. The stability of powder dye is more as comparable to concentrate due to low degradation of the pigments.

Figure-2
Color extraction from Beta vulgaris

Betalain are water soluble pigments and the beet extracts also contains 80% of the nitrogenous compounds and fermentable carbohydrates. The presence of free sugars cause impulsive fermentation of beet extract and caramelization also takes place when food is processed at elevated temperatures[17]. When Betanins are subjected to heat, light and oxygen these degrade; therefore, these dyes are mostly used in products with short shelf life, frozen products, or products sold in dry state.

Betalains are highly affected by temperature which is considered as most influencing factor[18]. Betalains degrade spontaneously by increasing temperature. Temperature of 40°C is most favorable for the extraction of betalains from red beet. A marked decrease in the yield of the obtained betacyanins and betaxanthins is observed when these are subjected to boiling and roasting[19].

Betanine % age was studied after 15 days interval for 3 months at different temperatures 4°C, 25°C and 45°C. It was observed (table-1) that at 4ºC after 90 days the % age of betanine was maximum i.e. 0.46% but as the temperature increased Betanin % age decreased.

Table-1
Stability of Color Content (% of Betanin) of Freeze Dried Beet Red Color

Sr. No.	No. of Days	Betanine % at 4 ºC	Betanine % at 25 ºC	Betanine % at 45 ºC
1.	0	0.50	0.50	0.50
2.	15	0.48	0.46	0.27
3.	30	0.47	0.45	0.25
4.	45	0.47	0.45	0.23
5.	60	0.46	0.44	0.21
6.	75	0.46	0.43	0.19
7.	90	0.46	0.42	0.18

Red beet concentrate was analyzed for the basic nutritional compositions i.e. moisture, ash, protein, total dietary fibre, carbohydrates and energy (table-2).

The moisture and ash contents were found to be 87.20% and 1.02% respectively. Protein contents were 1.35% in beetroot red dye. 0.2% fat and 0.87% fibre contents were found in prepared red dye. The carbohydrate contents in red beetroot dye were high i.e. 9.36%. Food energy of red dye extracted from beetroot was found to be 44.64 Kcal/100g. The obtained nutritional analysis results suggest that the dye extracted from red beetroot can be considered a potential dye to be used as natural colorant.

Table-2
Nutritional Facts of Red Beet Root

Sr. No.	Parameters	Value (%)
1.	Moisture	87.20
2.	Ash	1.02
3.	Protein	1.35
4.	Fat	0.20
5.	Fiber	0.87
6.	Carbohydrates	9.36
7.	Energy (Kcal/100g)	44.64

Results of the activity of free radical scavenging of beet root concentrate are presented in table-3. Results showed that water extract (10mg/ml) contained the highest DPPH radical scavenging activity (33.32%), followed by water+EtOH (10mg/ml) having DPPH radical scavenging activity (24.39%).

In vitro the strong antiradical scavenging activity of betalains has been confirmed by many researchers[20-22]. It was found that there are certain active components present in red beet root like betacyanins and betaxanthines which act as free radical hunters and avoid free radical-mediated oxidation of biological molecules. Presently, Kapadia et al.[23] demonstrated the effectiveness of betanin for long-term local inhibition of skin and liver tumors stimulated by different carcinogens in mice.

Table-3
% Inhibition (DPPH) of Red Dye Paste

Sr. No.	Extracts (Concentration)	% Inhibition (Paste)
1.	Water (1mg/ml)	5.34
2.	Water+EtOH (1mg/ml)	6.11
3.	Water (10mg/ml)	33.32
4.	Water+EtOH (10mg/ml)	24.39

Microbiological analysis of the beet root dye were carried out monthly to determine the possible growth of spoilage microorganisms and food borne pathogens during the storage period of 3 months. Aerobic total plate count, total coliforms, fecal coliforms, *E. coli*, *Pseudomonas spp, Salmonella spp*., *Staphylococcus aureus*, yeast and molds were enumerated monthly (table 5). The enumerations were carried out with 10 gram aseptically weighed beet root dye. The higher limit of detection for aerobic plate count was 5.0×10^3 cfu/g and the lower was 1.6×10^2 cfu/g. The Codex Alimentarius Commission of Joint FAO/ WHO Food Standards Programme [24] allows maximum limits of 10^6 cfu/g for total aerobic plate count. In contrast, Food Additives- Coloring Permitted in Thailand; Notification of the Ministry of Public Health for Food Additive[25] set maximum limits of 5×10^3 cfu/g for total aerobic plate count. Herewith, the observed numbers of counts are within the limits of microbiological standards. However, the observed decrease in number of counts with months during storage serve as a significant indicator of the antimicrobial activity of beet root extract reported previously by Jasna et al., Maria et al., and Maria et al[26-28]. Indicator (*E. coli*) and specific microorganisms (*Salmonella spp., Staphylococcus aureus and Pseudomonas spp., yeast and molds)*, the presence of which reflect fecal contamination and lack of cleanliness in handling and improper storage, were not detected during 3 months study. Codex Alimentarius Commission and Notification of the Ministry of Public Health for food additive also specify that *E. coli, Salmonella spp., Staphylococcus aureus, Pseudomonas spp., yeast and molds* should be absent. Hereby, the provided results are coincident with the previous data which report that beet root extract does not allow the growth of microorganisms due to its antimicrobial activity and protect the food from spoilage[29] as described in table-4.

Toxicological studies were conducted on healthy mice to check the harmful effects of dye prepared. The oral dose of dye in pure drinking water was given in different quantities to animals and it was found that after 4 week the mice remained alive and also their weights were increased normally. No deaths were reported in rats given high oral doses of beetroot red dye. No significant differences were found in food intake, or gross pathological features relative to controls.

Table-4
Microbiological analysis of beet root extract

Microorganisms	**1st Month**	**2nd Month**	**3rd Month**
Total plate counts cfu/g	5.0×10^3	4.2×10^3	3.4×10^3
Total coliforms (MPN/g)	Not detected	Not detected	Not detected
Fecal coliforms (MPN/g)	Not detected	Not detected	Not detected
E. coli (MPN/g)	Not detected	Not detected	Not detected
Pseudomonas spp. (MPN/g)	Not detected	Not detected	Not detected
Salmonella spp./25g	Not detected	Not detected	Not detected
Staphylococcus aureus/g	Not detected	Not detected	Not detected
Yeast counts/g	Not detected	Not detected	Not detected
Mold counts/g	Not detected	Not detected	Not detected

Figure-3
Animal trials for checking the effect of Red Dye

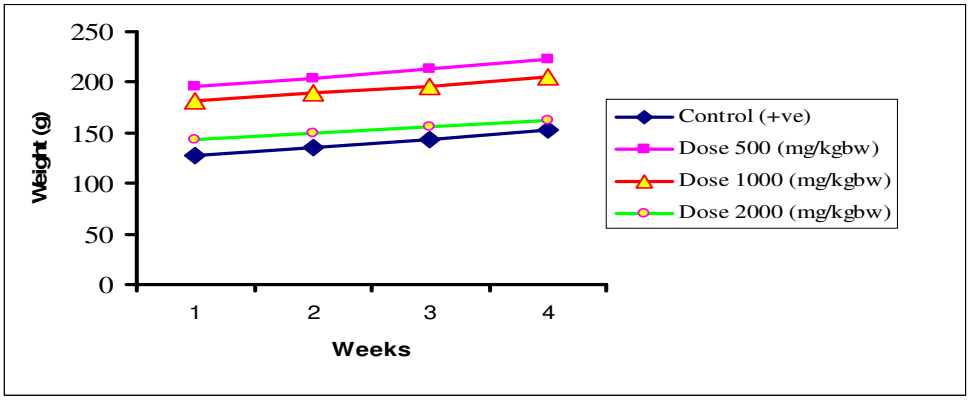

Figure-4
Body weight of rats in acute toxicity study of Beet Root Color

It was observed that by increasing dose of extracted color from 500- 2000 mg/kgbw, no mortality was observed as in table-5.

Table-5
Toxicology effect of Beet Root after 4 week treatment

Groups	Dose (mg/kgbw)	No. of Animals	Mortality
1.	-	4	Nil
2.	500	4	Nil
3.	1000	4	Nil
4.	2000	4	Nil

Similarly, the color extract was found harmless, as the body weight of the animal was not reduced. Instead its body weight was increased in 4 week study.

Conclusion

It is obvious from the study that the developed Beet root (*Beta vulgaris*) red dye is stable between the temperature range 4°C-25°C for three months. The stability of color is greatest in food products with low moisture content and can be commercialised as liquid concentrate. Moreover; it can be used in many processed foods especially in puddings, ice creams, frozen fruits, desserts, candies, confectionaries and baked foods. Betalain pigments especially red dye extracted from red beet roots (*Beta vulgaris*) provide a natural healthier alternative to synthetic dye such as EI62. The pigment betanin is a water soluble dye having potential antiviral, antioxidant and antimicrobial activities.

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