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STABILITY AND USABILITY OF NATURAL COLOURANT FROM *OPUNTIA FICUS-INDICA* IN FOOD PRODUCTS

SAIBABA JAGADEESAN[1], KIRTHIGA DURAIRAJ[2], SATHYA VELUSAMY[3] & KALPANA PALANI[4]*

[1,3,4]R&D Centre, National Agro Foundation, Chennai, Tamil Nadu, India

[2]Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

ABSTRACT

Colour was always an important attribute of food, as it was an indicator of quality and also a strong determining factor for acceptance of food. Natural colourants as substitutes for synthetic dyes in the food and pharmaceutical industry are of interest due to safety concern. This research mainly focused on the feasibility of utilizing the fruit of Opuntia ficus-indica (prickly pear cactus) of the Cactaceae family as a natural colourant in food. The liquid colourant was formulated by extracting, homogenizing and concentrating the fruit pulp at 40°C with 0.1 % sodium benzoate. It was then assessed for physio-chemical characteristics, phytochemicals (antioxidant, tannin, total phenol content, total flavonoids), storage stability and nutritional parameters. The study revealed that the cactus pear has a high source of betalain (484.82mg/100g), with the liquid colourant having a pH range between 3 to 9 and heat stability till 80°C. As per the microbial study, the colourant was free from pathogens with negative results for E.coli and Salmonella and has a shelf-life of 30 days under refrigeration. The liquid colourant applied to food product was screened for the colour value, texture and sensorial acceptability. Sensory analysis for acceptability of products indicated high preference for the coloured food product.

KEYWORDS: Prickly Pear Cactus, Natural Colourant, Betalain, Storage Stability, Food Products

Original Article

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1.INTRODUCTION

Colour is important in products including food as a mean to identify, judgeits quality and for the basic aesthetic value. Largely, enjoyment of foods depends on the eye appeal, colour being the first attribute perceived by the senses. Besides flavour and colour, perceptions appear to be closely linked. The general objective of adding colour to foods was to make itappealing and recognizable (Sampathu et al., 1981). Addition of colourants to foods to make it attractive is not recent. Extracts from spices and vegetables were used as early as 1500 B.C. in India and China. Colourants from naturally occurring minerals, plants and animals were prepared along with spices, which played a prominent part in the development of early civilization.

Worldwide, there is an increasing inclination towards natural colourants rather than synthetic compounds in most consumer products including food items. Many factors, including regulations that either completely or selectively ban artificial colours in food items and personal awareness to consume safe natural products could influence the need for natural food colourants. This calls for more research in the field of natural food colours to meet the global demand, wherein researchers are establishing standardized procedures for extraction to overcome the limitations of natural food colours. From the ancient times, the colours of natural vegetable or animal origin such as saffron, turmeric, annatto, chlorophyll, and cochineal have been used by humankind (Santhanakrishnan, 1981).

There is an increased interest and demand for natural colours in foods, though natural colours have some limitations when compared to artificial colours, but there are also instances where natural food colours are able to perform whereas the synthetics fail, the classic example being the colouring of cheddar cheese with annatto. Application of bio food colours is increasing because they are safe to use in foods apart from their nutritional value and their stability in the presence of vitamin 'C' (Freund et al., 1988). The natural colourant sources include plant and animal origin. The plant based colorants are derived from roots, leaves, flowers, barks, fruits and seeds of plants. There are about 500 sources of extractable plants (Bhat, 1953). Based on chemical characteristics, the natural colourants can be classified as carotenoids, chlorophylls, anthocyanins, betalains, flavones etc.

Among higher plants, the occurrence of betalains is restricted to the Caryophyllales, which produce red, yellow, pink and orange colours. Till date, more than 50 structures of naturally occurring betalains have been identified, which include two classes of compounds such as betacyanins which are red violet in colour and betaxanthins which are yellow in colour. Among the numerous natural sources of betalains, red beet and prickly pear are the only foods containing these classes of compounds. Unlike anthocyanins, they are less sensitive to changes in pH and are used in desserts, beverages in India. Its aqueous concentrated extracts can be spray dried and is used as a food colourant (Freund et al., 1988). One of the best sources of betalains is the prickly pear, *Opuntia ficus-indica*. Although the cactus pear species originated from arid and semi-arid areas, it is now cultivated worldwide, specifically O. *ficus-indica* for its fruit (Inglese et al., 2002). Under these circumstances, this study was designed with the aim to develop a natural food colourant from the fruit of the prickly pear cactus (*Opuntia ficus-indica*) and explore its applicationin food products.

2.METHODOLOGY

Fresh cactus fruit was procured from villages in Sivaganga District, Tamil Nadu and food grade chemicals were utilised for all the analysis. Fresh ripe fruit were deskinned, deseeded with the pulp concentrated at 40°c and homogenised. Sodium benzoate of 0.1% was added to increase the shelf life and stored in food grade bottles under refrigeration.

2.1 Physico-chemical and proximate analysis

The colourant thus obtained was tested for various physical, chemical and proximate analyses as per the methods given in Table 1.

Table 1: Methods for the analysis of physico-chemical and proximate properties of liquid colorants obtained from *Opuntia ficus-indica*

Parameter	References
pH	Beckman, Model 3500
Brix	Wardowski et al., 1979
Total Sugar	AOAC (1980)
Total Solids	Sawaya et al., 1983
Moisture content	Bonner,1981
Ash content	AOAC (1980)
Fat content	Hernández-Urbiola et al., 2011
Crude fibre	Retamal et al., 1987
Protein	Uchoa et al., 1998
Carbohydrate	Salim et al., 2009
Total energy	Chiteva and Wairagu, 2013

2.2 Colour Stability

The content of extracted natural food colour betalain content and its colour stability were assessed by the method detailed by Butera et al., 2002. Further, the effect of different pH ranges (1 to 13) and heat (40 to 100°C) on the colour stability was studied. The different pH ranges were adjusted by 0.1M HCl/0.1M NaOH. To check the stability of the natural colourant at different ranges of temperature, samples were taken and placed in a water bath for about 15min at respective temperature. The stability of betalain content was determined in UV-VIS spectrometer at 535nm (Lee et al., 2005).

2.3 Analysis of Storage Stability

The extracted natural liquid colourant was studied for stability towards temperature (at room and refrigerated temperature) and light (using transparent and amber coloured bottles).

2.4 Microbial Analysis

Microbial analysis such as the total plate count (IS 5401, 2002), Coliform count (IS 5401, 2002) and Salmonella (IS 5887, 1999) were conducted to study the growth of microorganisms and analyse whether the colourant can be used in food.

2.5 Phytochemical Analysis

The phytochemical analysis such as antioxidants, tannins, flavonoids and total phenolic content were carried out as per the standard procedure given in the Table 2.

Table 2: Methods of phytochemical analysis of liquid colorants obtained from *Opuntia ficus-indica*

Parameter	Method & References
Antioxidants	Brand-Williams et al., 1995
Tannins	The modified vanillin assay (Price et al., 1978)
Flavonoids	Aluminium chloride method (Zhishen et al., 1999)
Total phenolic content	Folin–Ciocalteau assay (Singleton and Rossi, 1995)

3.RESULT AND DISCUSSIONS

The study assessed various aspects of the natural colourant derived from the fruit of *Opuntia sp.* to indicate the stability and feasibility for its addition in food products.

3.1 Analysis of Physical and Chemical Properties

Once the natural colorant betalainwas derived from *Opuntia* sp., it was analysed for various physico-chemical parameters and the results are presented in the Table 3. As per the results, the pH of the liquid colourant from the fruit pulp was 5.2, however on addition of sodium benzoate, it declined to 4.5, indicating its acidic nature and potential good shelf life. On acid titration, the lower acidity of liquid colourant indicated the freshness and low acidity of the natural liquid colourant signifying long shelf life**.** The sugar content of the natural liquid colourant was 5, expressed in refractometer as degrees brix value. The developed natural liquid colourant contained 3.1% amount of sugar content, which is also within an acceptable range to incorporate in different food products.

Table 3: Physico-chemical properties of liquid colorants obtained from *Opuntia ficus-indica*

Parameter	Values
pH	5.2 4.5 after sodium benzoate addition
Brix	5
Total Sugar %	3.1
Total Solids %	7.24

3.2 Proximate Analysis

The proximate analysis of the colourant derived from *Opuntia* included, the total solids, moisture content, ash, fat, crude fibre, protein, carbohydrate and is presented in Table 4. The amount of solid content present in the sample was 7.24%, which would help to incorporate the liquid colourant better in different food products.

Drying, storage, marketing, and roasting are four important aspects, where moisture plays an important role. The moisture content influences the physical properties and product quality of nearly all substances and materials at every stages of processing (Sawaya et al., 1983). The moisture content of the fruit pulpextract was 93.9%. Moisture content is important for sensory perception of food and any change will affect its flavour and texture as well as the physical and chemical properties. The presence of free moisture is directly related to water activity. This enables the food interact with microbes and its environment. Analysis of ash content helps to determine the amount and type of minerals present in food which can determine the physiochemical properties of foods. The ash content of the sample was 0.8 %, with the pulp rich in potassium, fair in calcium, magnesium and phosphorous and poor in sodium and iron content.

Dried, ground sample was extracted with diethyl ether which dissolves fats, oils, pigments and other fat soluble to assess the fat content. The fat content present in the sample was 0.12%. Crude fibre is determined gravimetrically after chemical digestion and solubilisation of other materials present in the sample. The sample is defatted and successively treated with specified concentrations of sulphuric acid and sodium hydroxide to extract the fibre. As per the analysis, the sample contained less crude fibre 0.46%.The amount of protein, carbohydrate, energy presentin the sample was 0.19 %, 4.93 % and 19.96 Kcal respectively.

Table 4: Proximate properties of liquid colorants obtained from *Opuntia ficus-indica*

Parameter	Fruit pulp ± SD
Moisture content %	93.9 ± 025
Ash content %	0.8 ± 0.15
Fat content %	0.12 ± 0.02
Crude fibre %	0.46 ± 0.06
Protein %	0.19 ± 0.07
Carbohydrate %	4.93± 0.04
Total energy Kcal	19.96± 0.08

3.3 Colour Stability

The total betalain content of the liquid colourant extracted with aqueous media was 484.21 mg/100g. Similar results in *Opuntia stricta* were reported by Castellar et al., (2003). The pH has a great influence on the stability of betalain pigment (Table 5). The absorbance of betalain was stable between pH 3 to pH 8 but declined thereafter (Fig. 1). Betalain is stable in mildly acidic solutions in which the pH level ranges from 4.0 to 6.0 (Von Elbe et al., 1974).

Figure 1: Effect of pH on colour stability

Temperature affects the stability of betalain, as the study showed speedy destruction of betalain at higher temperatures (Table 6). Betalain degrades at high temperature (100°C). However, the effect of low temperatures (≤40°C) on the stability of betalain is significant (Fig. 2). Similar results were reported by Jenshi Roobha et al., (2011) in *Musa acuminata* bract. Hence, it is suggested that high temperature and long heating should be avoided in the processing, storage and usage of betalain extracted from cactus fruit. However, when applied for food products that were baked and steamed, the colour retains its property, even when it was mixed with other substrates even at high temperature. Thus, cactus fruit pulp is thermally in acceptable range, with the betalain content of the liquid colourant stable till 80°C.

Figure 2: Effect of heat on colour stability

Table 5: Effect of pH on colour stability

pH range	Betalain content (mg/100g)
pH 1	40.4
pH 2	294.0
pH 3	436.6
pH 4	478.0
pH 5	484.9
pH 6	437.5
pH 7	425.6
pH 8	423.1
pH 9	402.3

pH 10	255.8
pH 11	125.8
pH 12	59.8

Table 6: Effect of heat on colour stability

Temperature	Betalain content (mg/100g)
40°C	462.4
50°C	437.2
60°C	395.4
70°C	375.0
80°C	183.4
90°C	138.7
100°C	98.9

3.4 Storage Stability

The extracted bio food colour was studied for the storage stability of various physico chemical characteristics (Total Betalain content) towards temperature (room temperature and refrigerated) and light (transparent glass bottles and amber coloured glass bottles).

The effect of room temperature and refrigerated temperature on the physio chemical characteristics of the bio food colour extracted from the Prickly Pear was analysed (Table 7).The optical density value for the concentration of bio food colour was (0.5920) on the 0^{th} day which decreased to (0.3507) on the 30^{th} day. At refrigerated temperature, the concentration on the 0^{th} day was (0.5920) and (0.5235) on the 30^{th} day. Similar results were reported by Kyujo suyama et al., (1982), that as the temperature increases above the room temperature, the intensity of colour diminishes and no shift of absorption maximum was observed (Driver et al., 1979).

Table 7: Effect of temperature on storage stability

Physio chemical characteristics	Room temperature		Refrigerated temperature	
	0^{th} day	30^{th}day	0^{th} day	30^{th}day
Concentration (O.D value)	0.5920	0.3507	0.5920	0.5235
Total betalain content (mg/100g)	542.66	321.47	542.66	479.87

The effect of light on the physico chemical characteristics of the bio food colour extracted from the fruitwas also analysed by storing in transparent and amber coloured glass bottles (Table 8). The optical densityvalue for the concentration of bio food colour (0.5920) on the 0^{th} day reduced to (0.3743) on the 30^{th} day, when stored in transparent glass bottles, whereas it reduced to (0.5108) on the 30^{th} day when stored in amber coloured glass bottles.

Table 8: Effect of light on storage stability

Physio chemical characteristics	Transparent glass bottle		Amber colour glass bottle	
	0^{th} day	30^{th} day	0^{th} day	30^{th} day
Concentration (O.D value)	0.5920	0.3743	0.5920	0.5108
Total betalain content(mg/100g)	542.66	343.10	542.66	468.23

The exposure of betalain in solution to light significantly affected its stability. A reduction was also noted in the betalain content (Attoe and Von Elbe, 1981). Kyujo suyama et al., (1982) studied the sensitivity of red pigments from

Perillaocymoides towards light by exposing to the day light. Results demonstrated the sensitivity of red colour to light and the need to protect products against exposure to day light**.**

3.5 Microbial Analysis

Microbial analysis of the colourant was undertaken. The result of the total plate count was noted after 24 hours of incubation. The isolated colonies were counted in different dilutions such as 4, 5, 6 and 7 by using colony counter. The colonies growth decreased with increased dilution. The samples were tested for *Salmonella* and *Coliform* to assess their suitability for application in food products. If the sample turns yellow, it is positive and an indication of presence of microbes and hence, unacceptable for food applications. The result was viewed after 24 hours after incubation. The sample had no colour change and tested negative for *Salmonella* and *Coliform*. According to similar study conducted by Ali and Mohamedy, 2011, indicated that the prickly pear dye was highly effective antimicrobial against all tested microorganisms such as *Bacillus subtilus* and *E. coli.*

3.6 Phytochemical Analysis

The total tannin and flavonoid content present in the sample were measured at 500 nm and it was 0.34 mg/ml CE whereas the flavonoid content was 45.01 mg/ml QE. The total phenol content present in the extract was 0.018 mg/ml GAE and it was measured at 765nm. The sample were analysed to determine antioxidant nature. DPPH is a stable free radical, the colour changes from violet to yellow upon reduction by the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Dehpour et al., 2009). The method modified by Brand Williams (1995) was employed to investigate the free radical scavenging activity. DPPH scavenging potential of different *Ixora coccinea* aqueous extracts was measured based on the scavenging ability of stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radicals. In the same way, antioxidant determination is carried out in aqueous extract of cactus fruit pulp. The value obtained for antioxidant in aqueous solution of cactus fruit pulp is 26.17 % of scavenging activity.

3.7 Sensory Evaluation

Products like jelly, squash, ice-cream, cake and cookies were incorporated with the natural liquid *colourant* for sensory evaluation based on paired comparison test. Stintzing et al., (2001) reported that cactus fruit extract can be used as a natural food colourant. To evaluate the food products, selected panellists were given different sets of the samples, coded as CP1A, CP1B, CP2A, CP2B, CP3A, CP3B, CP4A, CP4B and CP5A, CP5B (jelly, squash, ice-cream, cookies and cakes respectively as given in Fig.3 , where A was the control with colourless sample and B, colour incorporated products. Significantly high sensory score were observed in colour incorporated samples (B), perhaps due to themore appealing and attractive pink colour of the cactus fruit (Fig. 4). Similarly, betalain derived from red beetenhanced the palatability and perceived nutrition of the products, which, although of higher cost and tend to approve the use of natural ingredients in processed foodstuff (Kakali Roy et al., 2004).

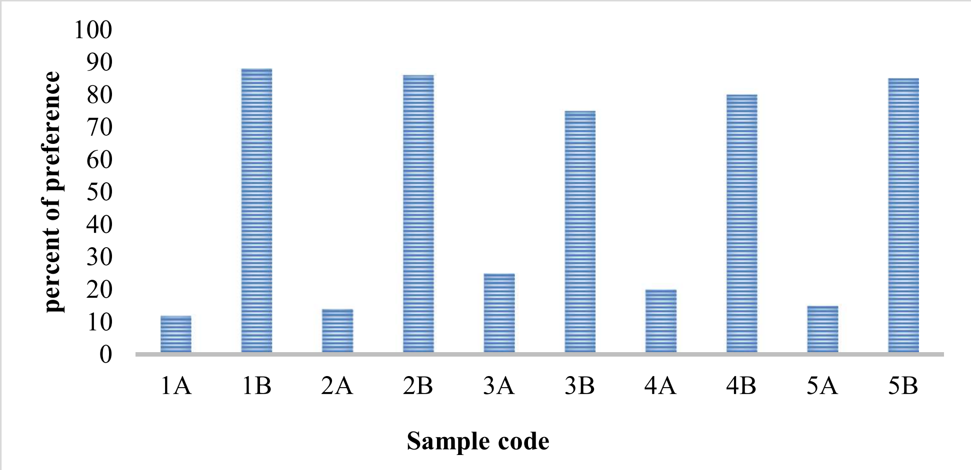

Figure 4: Sensory graph of control and colourant added food products

CONCLUSIONS

The study was undertaken to extract natural food colours from the Prickly Pear (*Opuntia ficus-indica*) with aqueous media and assessing the extracted natural liquid colorant for physicochemical phytochemical, microbial and storage stability characteristics. The colourant was used in food products like jelly, ice-cream, squash, cookies, cake to assess the acceptance. The pH of the developed liquid colorant was 5.2, which could be further reduced to about 4.5 on addition of 0.1% sodium benzoate indicating good self-feasibility. Sensory evaluation, absence of pathogenic and non-pathogenic bacteri1a, indicated that the natural liquid colourant extracted from *Opuntia* is a good source of appreciable colour which is safe and can be easily incorporated in different food products to replace the synthetic colourant in the food industry. As betalain has a high source of natural colourant, the pulp can be used for development of nutraceuticals for therapeutic purpose with good source of antioxidant activity and nutritional value. Further studies can focus on creating powder form and commercialization which can have wider appeal due to its easy and safe form for food and pharmaceutical industries.

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