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RESEARCH ARTICLE

Extraction of natural food color from *Celosia cristata* using orbital shaking apparatus

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ABSTRACT

As there is a growing demand for eco-friendly and non-toxic food colorants, the colors obtained from plant sources have proven to be an alternative to the synthetic food colors. In the present study, natural pigment and color was extracted from the flowers of *Celosia cristata* using orbital shaking apparatus. Three different temperatures (20, 40, and 60 °C) and three different solvents (distilled water, 30% ethanol, 50% ethanol) were used for the extraction. Optimum extraction conditions for maximizing the total betalin content (17.67 mg/100g), antioxidant activity (38.54%) and colorant yield (23.98%) were temperature of 40 °C using distilled water for an extraction period of 2 hours. Betalin content decreased at higher temperature (60 °C) of extraction that depicts the thermal degradation of the betalin content. The solid liquid ratio taken for extraction was 1:10 (w/v).

Keywords: Betalin, cockscomb, orbital shaking apparatus, temperature, solvent

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INTRODUCTION

The most important quality parameter of any food product that determines its acceptance by consumers is the presence of pigments. Nowadays a number of plant pigments in different forms are being used in the food industry in manufacture of food products (Boo et al., 2011). In addition, color is considered as an important criterion in determining the quality as well as freshness of any food. The synthetic food colorants are known to pose some ill effects on human health. Moreover, the use of synthetic food colorants is restricted by legal regulations of both international and national authorities (Masar et al.,1996). Hence, there is a growing demand for the use of natural and non-toxic colorants particularly for health sensitive applications like food products. The application of low-cost technologies to obtain molecules to be used as food additives or nutraceutical products is an appropriate strategy for the exploitation of some under-utilized plants (Maran et al., 2014).

Celosia cristata, belongs to family Amaranthaceae and is commonly known as cockscomb. It is also known as Red Cockscomb, Foxtail Amaranth, Crested *Celosia* and Fire-flame Bush. The tight, velvety texture of the Cockscomb flowers look like brain tissue

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to some people. Ayurveda in India and even Chinese herbal therapeutics mention the plant with potent medicament value as it is used in treatment of various diseases. This plant is found to have several activities like antioxidant, antiviral, anti helmintic actions. Color of flowers of this plant varies from yellow, maroon to dark red (Taha and Wafa, 2012). The color of flowers is due to the presence of betalins. Betalins possess a high antirdical effect and antioxidant activity, representing a new class of dietary antioxidants. Recent studies suggest that the colored vegetative tissues of plants in the family amaranthaceae contain various betalin pigments and are often produced in high biomass, and therefore, attract interest as potential alternatives to the well-known betalins from beet roots. Various studies demonstrated high potential for Amaranthacaea pigments for use as natural food colorants (Cai et al. 2001).

MATERIALS AND METHODS

Preparation of dried flower powder

Flowers of *Celosia cristata* were procured from the local market of Kashmir and then the flowers were sorted, cleaned and dried at about 60 °C for 3 to 4 hours. The dried flowers were ground and stored in HDPE bags till use.

Extraction of color by orbital shaking apparatus

Extraction by orbital shaker was performed according to the method given by (Parekh and Chanda, 2007) with slight modifications. Extraction was conducted by adding 5g of sample and 100 mL of solvent into a conical flask, to prevent solvent evaporation the flask was covered properly. Flasks were subjected to continuous shaking at a constant speed of 120 rpm in a temperature controlled orbit-shaker at 20 °C, 40 °C and 60 °C to facilitate the diffusion of betalins into the solvent. The mixture obtained was then filtered through whatman filter paper no.4 and then concentrated in rotary vacuum evaporator at 40 °C. The extract was further dried in hot air oven to estimate the yield of the colorant obtained.

Calculation of betalins (Cai et al., 1998)

$$\text{Pigment content (mg/g)} = \frac{\text{Ai.Fd.Mw}\times\text{V}\times100}{10\times\varepsilon\times\text{L}\times\text{md}}$$

Where, A_i is the absorption at given wavelength, M_w is molecular weight of the pigment, F_d is dilution factor, V is the extract volume (ml), m_d is weight of sample, ε is molecular extinction coefficient of the pigment and L is path length. All the experiments were done in triplicates.

Total yield of colorant powder (Cai et al., 1998)

Total yield was calculated according to the formula given below:

$$\%\ \text{Yield} = \frac{\text{weight of color obtained}}{\text{weight of initial sample}}\times100$$

Calculation of antioxidant activity of betalin extracts (Lee et al., 2003)

The antioxidant activity of the extracted betalin samples was determined by DPPH method. Extracts each of 0.1ml were vortexed for 30s with 3.9 ml of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also be analyzed. The DPPH solution with no added extract was analyzed as control. Scavenging activity was calculated as follows:

$$\textbf{DPPH radical scavenging activity \%} = \frac{(\text{A control} - \text{A sample})}{(\text{A control})} \times 100$$

A control = absorbance of control, A sample = absorbance of sample and A is the absorbance at 515 nm.

Statistical Analysis

The results obtained were reported as mean ± standard deviation (SD) for triplicate determinations and further the data was subjected to two way analysis of variance (ANOVA) using Tukey's test to establish significant effect of different extraction temperatures and solvents used on the parameters studied at a level of significance of $p \leq 0.05$. The statistical analysis was conducted by using SPSS.16.0.

RESULTS AND DISCUSSION

Effect of solvent and temperature on total betalin (mg/g) content of the extract

As per the results shown in table 1, betalin content was found to be significantly ($p \leq 0.05$) higher at temperature of 40 °C. the total betalin content decreased significantly ($p \leq 0.05$) when the temperature of extraction was increased to 60 °C, this indicates that degradation of betalins occurs at higher temperature. Same results were obtained by (Attia et al., 2013), they found that prolonged exposure of pigment at moderate temperature ranging between 40 °C and 50 °C, showed no degradation and hence betalins were stable whereas at above 50 °C, the degradation of betalins increased gradually by increasing temperature. The higher degradation of betalins was observed at temperatures above 70 ℃. The results obtained suggest that the betalin extraction was maximum in distilled water followed by 30% ethanol and was minimum in 50% ethanol, Similar results were obtained by (Sturzoiu et al., 2011).

Table 1: Betalin content of extract obtained from *Celosia cristata* at different temperatures using different solvents

Solvent	**Temperature**		
	20 °C	**40 °C**	**60 °C**
Distilled water	12.18^{aC}±0.02	17.67^{aA}±0.58	14.67^{aB}±0.53
30% Ethanol	8.97^{bC}±0.04	13.41^{bA}±0.42	10.81^{bB}±0.51
50% Ethanol	8.28^{cC}±0.08	11.72^{cA}±0.07	9.71^{cB}±0.24

Values are the mean ± standard deviation of at least 3 determinations. Different letters (a-c) within a column denote significant differences ($p \leq 0.05$) between different solvents and different letters(A-C) within a row denote significant differences ($p \leq 0.05$) between different temperatures.

Effect of solvent and temperature on antioxidant activity (%) of extract

In this present study it was found that higher the betalin content of the extract, higher is the antioxidant activity. Betalins are water soluble plant pigments, so water as a solvent showed significantly ($p \leq 0.05$) higher antioxidant activity than aqueous ethanol, similar results were found by (Attia et al., 2013). According to a study conducted by (Fathordoobady et al., 2016), various solvents were evaluated in the extraction of betalins from the *Hylocereus polyrhizus* aqueous extraction was the most efficient, achieving a greater concentration of the pigments and antioxidant activity. The extractions done with solutions with low concentrations (10 and 30% v/v) of ethanol in water also resulted in a high concentration of pigments, but still lower than that obtained in the distilled water.

Table 2: Antioxidant activity of extract obtained from *Celosia cristata* at different temperatures using different solvents.

Solvent	Temperature		
	20 °C	40 °C	60 °C
Distilled water	31.25^{aC}±0.02	38.54^{aA}±0.42	34.34^{aB}±0.56
30% ethanol	24.25^{bC}±0.04	31.32^{bA}±1.27	27.79^{bB}±0.19
50% ethanol	20.37^{cC}±0.03	30.75^{bA}±0.04	23.50^{cB}±0.31

Values are the mean ± standard deviation of at least 3 determinations. Different letters (a-c) within a column denote significant differences ($p \leq 0.05$) between different solvents and different letters(A-C) within a row denote significant differences ($p \leq 0.05$) between different temperatures.

Effect of solvent and temperature on antioxidant total yield (%) of colorant obtained

As per the results given in table 3, it was observed that the yield of colorant obtained increased significantly ($p \leq 0.05$) when the temperature of extraction increased from 20 to 40 °C. The increase in temperature upto 40 °C results in softening of plant tissues which in turn increases the penetration of solvent inside the material. The higher solvent penetration enhances the solubility as well as diffusivity of the colorant in the solvent as a result the extraction yield is increased (Yong et al., 2006). However, higher temperatures like 60 °C result in lower extraction yield due to the effect of higher temperatures on the destruction of plant plant pigments. The results obtained were in accordance with those obtained by (Maran et al., 2014) while studying the effect of different time and temperature combinations on the extraction of extraction of natural anthocyanin and colors from pulp of jamun fruit.

Table 3: Total yield (%) of colorant obtained from *Celosia cristata* at different temperatures using different solvents.

Solvent	Temperature		
	20 °C	40 °C	60 °C
Distilled water	16.41^{aC}±0.42	23.98^{aA}±0.64	19.24^{aB}±0.23
30% Ethanol	12.14^{bC}±0.69	19.63^{bA}±0.29	14.76^{bB}±0.29
50% Ethanol	10.38^{cC}±0.36	17.44^{cA}±0.41	13.15^{cB}±0.65

Values are the mean ± standard deviation of at least 3 determinations. Different letters (a-c) within a column denote significant differences ($p \leq 0.05$) between different solvents and different letters(A-C) within a row denote significant differences ($p \leq 0.05$) between different temperatures.

CONCLUSION

In this study, natural color was extracted from *Celosia cristata* by using orbital shaking apparatus. Experimentation conducted showed that temperature of extraction and type of solvent used markedly influence the extraction of colorant from the flowers of *Celosia cristata.* The study also revealed that higher temperatures affect the yield of pigments obtained. Results obtained suggest that water is the best solvent for extraction of betalin pigments. Moreover, the use of water as a solvent for extraction can contribute to low operating cost, eco-friendliness, low energy consumption, non-flammability and non-toxicity.

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