iJPPHT
e ISSN-2231-6426
Volume 8 | Issue 2 | December, 2017 | 123-130
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International Journal of Processing and Post Harvest Technology

RESEARCH PAPER
DOI: 10.15740/HAS/IJPPHT/8.2/123-130

Extraction and standardization of betalain from *C. argentea* var. *cristata*

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■ **Research chronicle : Received :** 07.09.2017; **Revised :** 11.11.2017; **Accepted :** 25.11.2017

SUMMARY :

In the current work, studies were carried out to extraction and standardization of the betalains from *Celosia argentea* var. *cristata* (CAC) inflorescence for possible use as a source of natural colour for the food industry. Pigment extraction was carried out using water as the extractant. Results showed that maximum betacyanins were extracted at a pH of 5 using citrate buffer, at a sample: solvent ratio of 1:3, the temperature of 20°C for 90 minutes.The extracted pigments were found to stable at 4°C upto 15 days.

KEY WORDS : Betalain, Pigment, Natural colour

How to cite this paper : Patil, Rahul Ramrao and Ranganathan, T. V. (2017). Extraction and standardization of betalain from *C. argentea* var. *cristata*. *Internat. J. Proc. & Post Harvest Technol.*, **8** (2) : 123-130. **DOI: 10.15740/HAS/IJPPHT/8.2/123-130.**

The *Celosia argentea* var. *cristata* (CAC) also known as common Cockscomb found in Africa and South East Asiais used as a vegetable and for traditional medicine in China (Cai *et al.*, 1998). The average yield of this crop is 7.60 ton per hector (Ekunwe *et al.*, 2011). There is inadequate data available for the production of Celosia species, it has a total cost of $ 6.28 million for all sales bedding and garden plant production (Zuck, 2015). Betalains are natural pigments, characterized as N-heterocyclic (Stintzing and Carle, 2008b). These arewater-soluble, with betalamic acid as the precursor for biosynthesis. Betalains can be classified as yellow-orange (betaxanthins) pigments and red-violet (betacyanins), depending on whether the conjugation is with cyclo-Dopa and amino acids or amines (Azeredo 2009; Khan and Giridhar, 2015 and Khan, 2016). Betalains are restricted to Caryophyllales and a greater interest in their studies is due to their less biological distribution (Harivaindaran *et al.*, 2008). Betalains are reported to possess antioxidant and radical scavenging activity (Strack *et al*., 2003). The betalain pigments in the plants of 37 species and 8 genera of Amaranthaceae family characterized to16 red-violet betacyanin and 3 yellow betaxanthins (Cai *et al.*, 2005). Currently available common sources for betalain are red beet, amaranth, yellow beet, swiss chard and cactus pear (Azeredo, 2009).

Betalains from red beets give earthy flavour due to theaction of pyrazines and geosmin derivatives. Their elevated nitrate concentration and subsequent nitrosamine formation restrict their use in foods. Hence, it is of utmost importance to explore new and promising sources of betalain (Harivaindaran *et al.*, 2008).

In the present work, attempts were made to explore the possibility of *Celosia argentea var. cristata* (CAC) inflorescences as an alternative to beet pigments. The betalain pigments were extracted and studied for their stability in optimized conditions.

EXPERIMENTAL METHODS

Materials :

Red-purple colored flowers of *Celosia argentea* var. *cristata* purchased from local flower market of Coimbatore. The fresh inflorescences were separated from stemsand stored in plastic bags at a temperature of 5°C. Citric acid, sodium citrate, ethanol 1N HCL and 5N NaOH. The absorbance of all sample was measured at 538nm using Perkin Elmer Lambda 25 UV spectrophotometer.

Method of extraction :

The method followed was modified (Harivaindaran *et al.,* 2008). Accordingly, 10g CAC inflorescences were macerated using a mortar and pestle. It was then extracted with different solvents (a. water, b. water: ethanol, c. ethanol) at the sample: solvent ratio of 1:3 and kept in an incubator shaker at 10°C for 30 min. The extract so obtained was quickly cooled to a temperature of 4±1 ºC. It was then centrifuged at 1096 g for 10 min and filtered using Whatman no. 1 filter paper to obtain a clear extract. The absorbance of all samples was measured at 538 nm using a UV-Spectrophotometer to determine total betacyanin concentration while pH was measured using a pH meter. The most suitable extractant was selected.

Determination of total betacyanin content in samples:

The absorbance readings obtained at 538 nm was used to calculate the total betalain concentration for each sample using the following formula (Stintzing and Carle 2008a).

$$\text{Betacyanin content in (mg / 100 g)} = \frac{\text{Abs 538 x MW x DF x V x 100}}{\varepsilon \text{ x } l \text{ x w}}$$

where,

A538 – Absorbance at 538 nm.

MW- Molecular weight 726g mol^{-1} and 339g mol^{-1} for betacyanin and betaxanthins, respectively.

V- Volume of total extract in (ml)

DF- Dilution factor

ε– Absorptivity (molar extinction co-efficient) 56,600 L/mol*cm and 48000 L/mol*cm in waterfor betacyanin and betaxanthins, respectively

l- Path length 1 cm

w- The fresh weight of extracting material.

Betacyanin retention in per cent:

Betacyanin retention in per cent was calculated by using following formula:

$$\text{Betacyanin retention (\%)} = \frac{A}{A_0} \text{x100}$$

where,

A- Concentration of betacyanin at any day

A_0- Concentration of betacyanin at initial day.

Determination of optimal solvent for extraction:

Extractions were carried out with the following solvents – a) water, b) ethanol and c) water + ethanol (1:1). The solvent showing the maximum extraction of pigment was subsequently used forfurther optimization studies.

Determination of raw material to solvent ratio :

Extractions were carried out with the following raw material to the solvent ratio - 1:3, 1:4, 1:5, 1:6 and 1:7. The best result was subsequently used for optimization of temperature.

Determination of optimal temperature:

Extractions were carried out at the following temperatures -10ºC, 20ºC, 30ºC, 40ºC and 50ºC. The best result was subsequently used for optimization of time.

Determination of optimal time :

Extractions were carried out for the following time periods (in minutes) -30, 60, 90, 120 and 150. The best result was subsequently used in the optimization of pH.

Determination of optimal pH :

Extractions were carried out with citrate buffers of pH 3, 4, 5, 6 and 7. The best result was taken as optimum

pH for pigment extraction.

Storage stability of pigment extracts :

To study the stability of extracted betacyanins, samples prepared with a raw material to solvent ratio of 1:3 was kept at 20ºC (S20) in an incubator shaker for 90 min. Simultaneously a sample (control) run was conducted at room temperature 33±1ºC for 90 min. Both the extracts were filled in test tubes of 10 ml capacity and tightly plugged. These were then kept for storage stability test inan incubator shaker with 660lm (luminous flux) at 30 ºC and 4ºC refrigeration temperature. Simultaneously, to study the effect of light and dark on degradation, samples were covered with aluminum foil to prevent light exposure for dark storage. Betacyanin content of all the samples was checked every day till a storage period of 15 days.

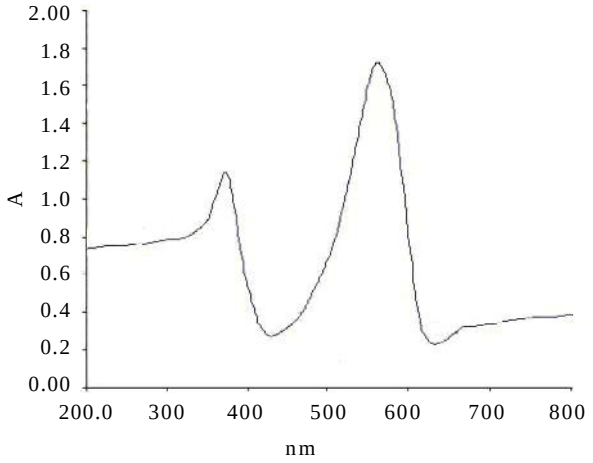


Fig. 1: Absorption spectrum of freshly extracted betalain .... *Celosia argentea* var. *cristata*

Statistical analysis:

All analyses were done in triplicates. The data were processed with one-way ANOVA (Analyses of Variance) in SPSS Version 20 for Windows. Posthoc-Tukey test was performed for significant differences between the mean values for treatments ($p<0.05$).

EXPERIMENTAL FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Determination of optimal solvent:

As observed in Fig. 2 (a) it was found that when water used as asolvent, betacyanin extraction was the highest (192mg/100g). The betacyanin content reduced to 56.25 per cent and 28.13 per cent when water-ethanol and ethanol were used as the extractants respectively. These results confirm the water-soluble nature of betacyanins as reported by (Azeredo, 2009) and different from the betacyanins of red dragon and Hylocereus cacti (Dam *et al.*, 2012). This is in line with the reports about the family of amaranths, where betacyanin content is reported to range between 46 to 199 mg/100g and 15.4 to 46.9 mg/g, for fresh weight and dry extracts, respectively (Cai *et al*., 2005).

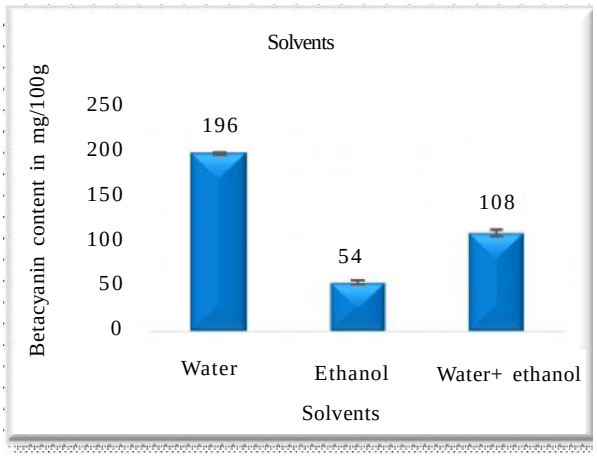
Determination of optimal material to solvent ratio:

It was observed that material to solvent ratios of 1:3 and 1:4 gave the highest and optimum betacyanin extraction (199 and 198mg/100g, respectively) and there was no significant difference between two ratio Fig. 2

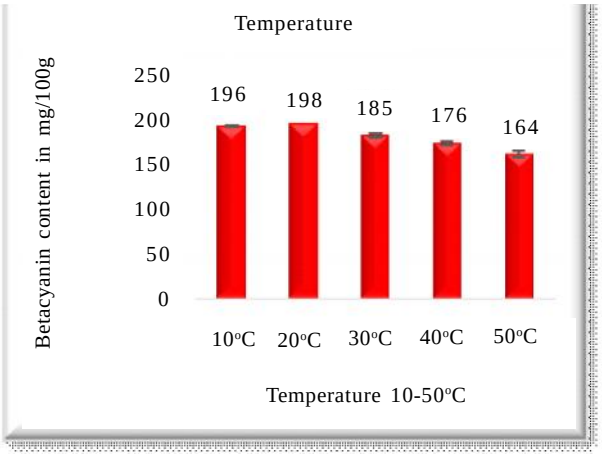
Table 1 : The extraction of betacyanin from *Celosia argentea* var. *cristata*

Solvents	Water	Ethanol	Water + ethanol		
Betacyanin content	196.00±1.5	54.00±2.1	108.00±3.6		
Solvent to sample ratio	1:3	1:4	1:5	1:6	1:7
Betacyanin content	199.00±1.0*	198.00±2.6*	191.00±1.0	187.00±2.1	187.00±0.6
Temperature	10ºC	20ºC	30ºC	40ºC	50ºC
Betacyanin content	196.00±0.5*	198.00±0.5*	185.00±2.6	176.00±2.6	164.00±3.6
Time	30 min	60 min	90 min	120 min	150 min
Betacyanin content	153.00±1.0	175.00±2.5	199.00±1.0*	192.00±2.1	196.00±1.5*
pH	3 pH	4 pH	5 pH	6 pH	7 pH
Betacyanin content	176.00±3.0	181.00±1.5*	198.00±1.2*	194.00±3.5*	184.00±1.0*

Data are expressed in mean triplicates ± standard deviation. All the values are expressed in mg/100g
*represents significance at $p\leq0.05$ along the lines are not significant with ($p>0.05$ Tukey test)



(a)



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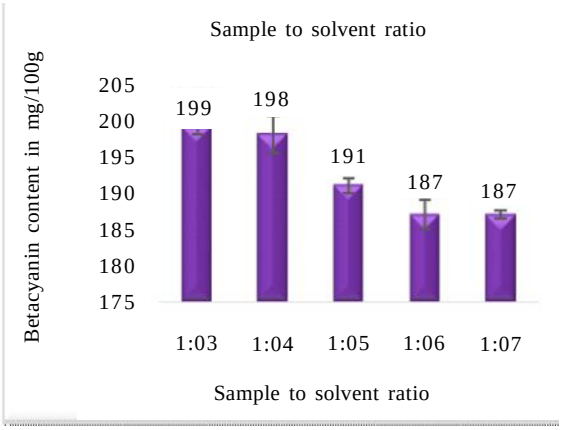

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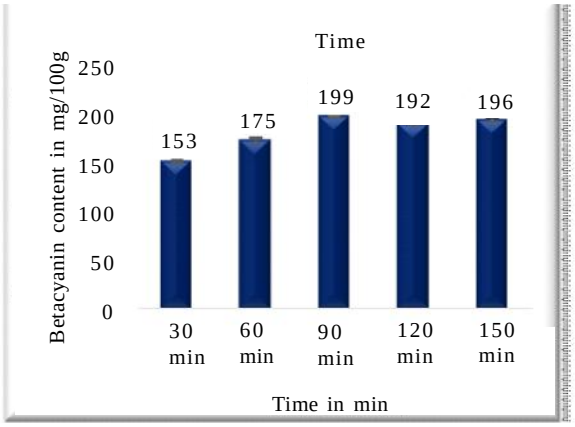
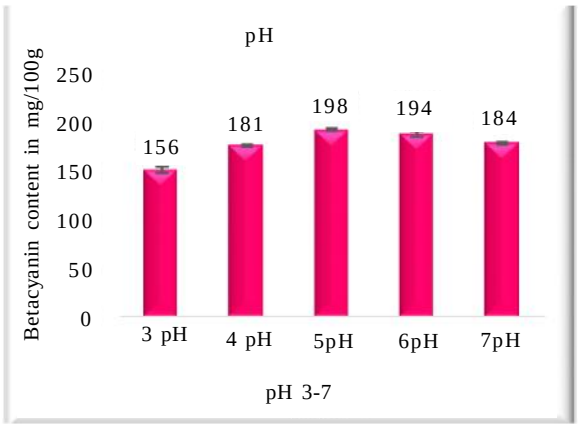


(d)



(e)

Fig. 2: The extraction of betacyanin from *Celosia argentea* var. *cristata*, (a). Effect of solvent; (b). Effect of ratio of raw material and solvent; (c). Effect of extraction temperature (°C); (d). Effect of extraction time in (min); (e). Effect of citrate buffer pH, statistical results confirmed differences between mean values significantly different (P<0.05)

(b). Any further increase led to a decreased extraction. This can be explainedby the fact that an increased solvent (water), leads to increasein the degradation of betacyanins, by aldimine bond cleavage (Azeredo, 2009). At lower concentrations than the optimum, a lesser extraction might be due to the saturation of the solvent by the betalain compounds (Dam *et al.*, 2012).

Determination of optimal temperature for extraction:

Extraction at the temperatures between 10 and 20ºC gave highest betacyanin content 198mg/100g and there was no significant difference observed Fig. 2(c). This implies that betacyanin can be extracted at temperatures less than or upto 20ºC. An increase in the temperature of extraction beyond this results in the degradation of the pigment tomonochrome cyclo-Dopa-5-O-glycoside and bright yellow betalamic acid (Azeredo, 2009).

Determination of optimal time for extraction:

Maximum extraction of betacyanins was observed when the samples were subjected to an extraction period of 90 minutes (199 mg/100g). Extraction less than 90 minutes showed less betacyanin content in extract Fig. 2 (d), due to lesser contact time between the solvent and the solid. When the extraction time exceeded 90 minutes, areduction in the extracted pigment was observed. This might be due to the degradation of betacyanins as mentioned by (Harivaindaran *et al.*, 2008).

Determination of optimal pH for extraction:

The result showed in Fig. 2(e) indicate that betacyanin are extracted optimally at a pH of 5 (198mg/100g). The results obtained agrees well that reported by other authors Azeredo (2009); Khan (2016) and Dam *et al.* (2012).

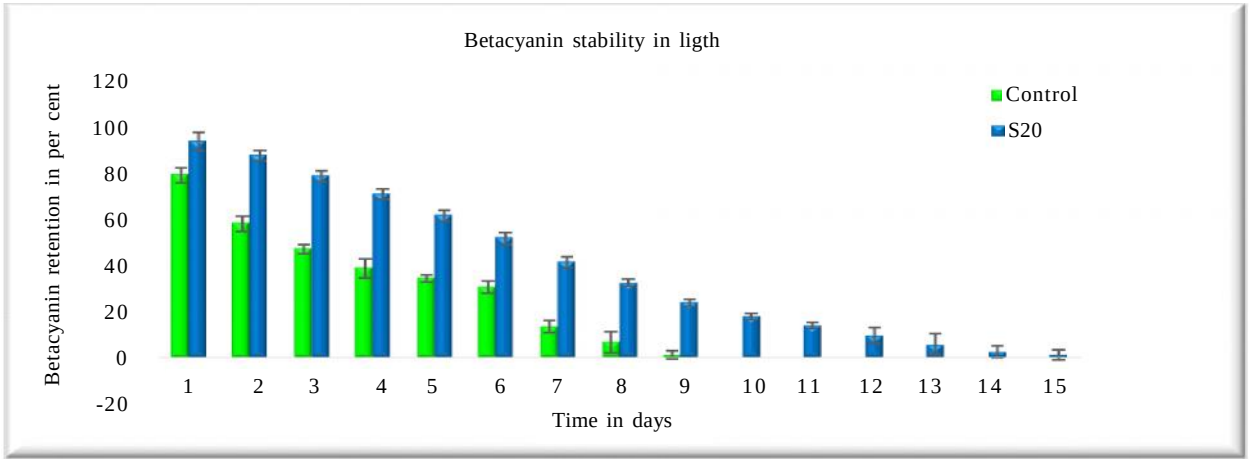
Determination of storage stability of pigment extract:

It was observed that all samples stored at 30ºC showed a high percentage of degradation. While samples extracted at 33±1ºC (control) showed just 1.23 per cent retention after 9 days in light, as compared to 24 per cent for those kept in dark. By the 15th day, the samples extracted at 20ºC (S20) got completely degraded Fig. 3 (a). Under similar conditions (9 days), samples stored in dark, could retain 24 and 41.7 per cent for extracted sample at 33±1ºC (control) and 20ºC (S20), respectively. It was observed that samples stored in dark could retain 2.5 per cent (S20) of the extracted betalains till 15 days Fig. 3 (b). In contrast, samples stored at low-temperature 4ºC (refrigeration) could retain upto 91 per cent and 92

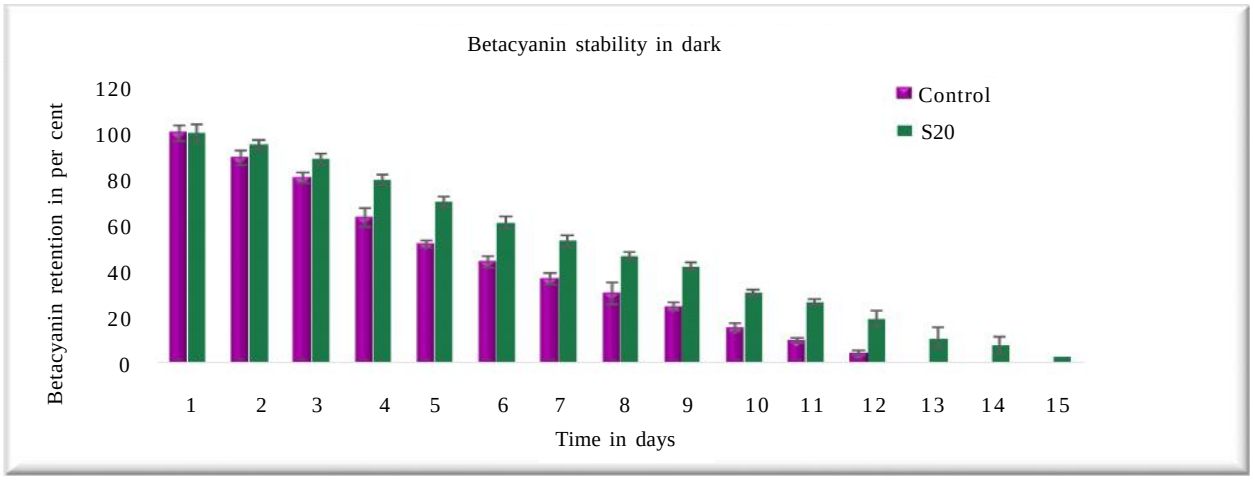
Table 2 : The betacyanin stability and colour retention from *Celosia argentea* var. *cristata*

Storage condition	Dark		Light		4°C (Refrigeration temperature)	
Sr. No.	Control*	S20*	Control*	S20*	Control	S20
1.	99.49±2.0	99.50±3.5	79.37±3.5	94.16±3.9	98.96±3.5	98.48±3.9
2.	88.83±3.5	94.47±3.9	58.20±3.2	87.88±2.2	98.44±3.2	97.98±2.2
3.	80.20±3.2	88.44±2.2	47.17±2.2	79.00±2.4	97.92±2.2	97.47±2.4
4.	62.94±2.2	79.40±2.4	38.78±4.2	71.15±2.2	97.40±4.2	96.97±2.2
5.	51.27±4.2	69.85±2.2	34.56±1.6	61.72±2.4	96.88±1.6	96.46±2.4
6.	43.65±1.6	60.80±2.4	30.75±2.6	51.97±2.7	95.83±2.6	95.96±2.7
7.	36.55±2.6	52.76±2.7	13.55±2.5	41.55±2.5	93.75±2.5	95.45±2.5
8.	29.95±2.5	46.23±2.5	6.66±4.6	32.49±1.6	94.27±4.6	94.95±1.6
9.	24.37±4.6	41.71±1.6	1.23±1.8	23.96±1.7	93.75±2.5	94.44±1.7
10.	15.23±1.8	30.15±1.7		17.87±1.5	93.23±2.5	93.94±1.5
11.	9.64±1.7	26.13±1.5		13.83±1.3	92.71±2.3	93.43±1.3
12.	4.06±1.2	19.10±1.3		9.44±3.5	92.19±2.7	92.93±3.5
13.	0.51±1.0	10.55±3.5		5.66±4.6	92.19±3.8	92.93±4.6
14.		7.54±4.6		2.42±2.5	91.67±3.5	92.93±2.5
15.		2.51±3.6		1.06±2.2	91.67±3.6	92.42±2.2

Data are expressed in mean triplicates ± standard deviation. All the values are expressed in percentage
*represents significance at $p \leq 0.05$ along the lines are not significant



(a)



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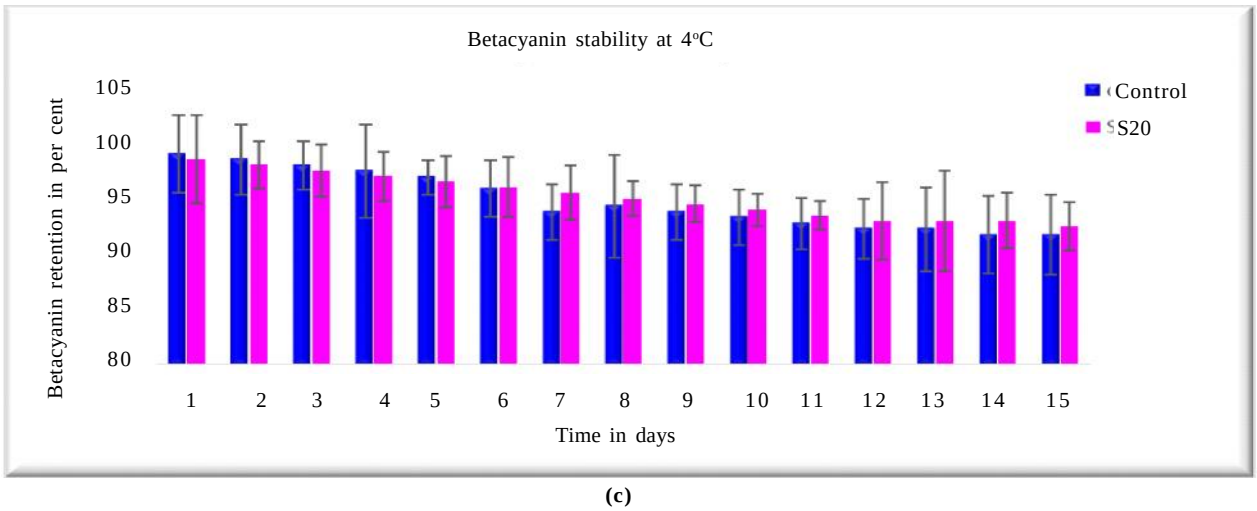

(c)

Fig. 3: The betalain stability and colour retention from *Celosia argentea* var. *cristata*, (a) Betalain stability in light; (b). Betalain stability in dark; statistical difference between storage time with respect to light and dark shows (P>0.05).(c)Betalain stability at 4ºC refrigeration temperature statistical difference between storage time with respect to refrigeration temperature shows (P<0.05)

per cent of the betacyanin content, till 15 days, for samples extracted at 33±1ºC (control) and 20ºC (S20), respectively Fig. 3 (c). This observation clearly shows the importance of light and temperature on the stability of CAC betalains. This results are in line with the observations made by other authors (Woo *et al.*, 2011).

In the present work, optimization of extraction of betalainfrom CAC was carried out. Factors optimized included, type of solvents, the sample to solvent ratio, temperature, time and pH. Studies were also conducted to determine the storage stability of extracted betalain in light, dark and refrigeration temperature. Results showed that the highest yield of betacyanin content was with a citrate buffer of pH 5, the sample to solvent ratio 1:3 and at temperatures between 10 and 20ºC temperature, for 90 minutes. Thus, a lower temperature and a prolonged time of extraction resulted in a better extraction of betacyanins from CAC. On increasing, the temperature, a reduction in the extraction of betacyanin was observed. A maximum reduction of 82 per cent was observed at an extraction temperature of 50ºC when compared with 20ºC. This might be ascribed to the degradation of betacyanins at higher temperatures as mentioned by (Herbach *et al.*, 2006). CAC extract showed a maximum peak at 470 nm for betaxanthins and 538 nm for betacyanin, with a concentration of 198 mg/100g and 97.9 mg/100mg at the corresponding wavelengths, respectively. This was in good agreement with the earlier reports on Celosia species by (Cai *et al.*, 2002 and Cai *et al.*, 1998). Stability of extracted pigment was studied, which shows that betacyanin content declined over a period 15 days upon exposure to light and dark. Earlier studies also reported alight sensitivity of betalains by light absorption in the ultraviolet range and visible light which leads to degrading betalains (Harivaindaran *et al.*, 2008). The degradation of the betalains was observed in both samples exposed and protected from light, as in the case of other sources. This is reported to be due to the C-11 position is the most prone site for nucleophilic attack by water (Woo *et al.*, 2011).

Conclusion:

Celosia argentea var. *cristata* (CAC) inflorescences could be seen as a potential and promising source of betacyanins to be developed as anatural colorant. From this study, it was observed that samples extracted between 10 and 20ºC with citrate buffer pH 5 and for 90 minutes gave the maximum betacyanins concentration of 199mg/100 g. This sample could be stored for 15 days, under refrigerated conditions. Extractions at temperatures exceeding 30ºC resulted in a loss of the pigments, thus, suggesting that colour losses during processing are inevitable. The betacyanin concentration was found to be higher than that of sources like opuntia. Stability studies indicate that the temperature of storage plays an important role in the stabilization of betacyanin. Results indicate that CAC inflorescences could be a potential source of betacyanin.

Acknowledgement:

The author would like to thank UGC for granting Rajiv Gandhi National Fellowship (RGNF) for this research work, and also to the Research Supervisor Dr. T. V. Ranganathan for his guidance and support to the work.

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