



## Effect of *Celosia cristata* extract on the quality of flavoured milk during storage

RUKHSAAR SAYEED, MONIKA THAKUR\* and ADIL GANI

Amity Institute of Food Technology, Amity University, Noida, Uttar Pradesh 201 301, India, and  
University of Kashmir, Hazratbal, Srinagar, Jammu and Kashmir 190 006, India

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### ABSTRACT

An experiment was conducted to study the effect of addition of *Celosia cristata* extract on the storage stability of rose flavoured milk at Amity Institute of Food Technology, Amity University Uttar Pradesh in 2019. Different levels (0.25–1%) of *C. cristata* extract rich in betalains were used as natural colorant in rose flavoured milk. The product was analysed for shelf life during which it was stored under refrigerated storage for 12 days and analysed for chemical, microbiological and sensory tests at regular intervals. During storage, pH declined while the total soluble solids (TSS) increased significantly. A significant reduction in the colour, flavour and mouth feel scores was observed after 8 days of storage. Total plate count (TPC) increased in both control and CCE incorporated drinks with storage period. The increase in TPC was more in control as compared to CCE incorporated drink. Though the sensory scores also declined with storage, the CCE incorporated drink obtained good acceptability scores even after 10 days of storage at refrigeration temperature.

**Key words:** Antimicrobial, Basil seeds, *Celosia cristata*, Flavoured milk, Sensory quality

The demand for healthy foods and beverages has increased among the consumers over the last few decades (Ozen *et al.* 2012). Flavoured milk is becoming an integral part of market milk industry. The combination of routine food with medicinal herbs can be an excellent source for development of functional foods. There are a number of Indian herbs and spices which show compatibility with dairy products and their addition to milk would add variety, increase consumption, provide health benefits and improve storage quality of dairy products. In this study, a combination of cocks comb flower (*Celosia cristata* L.) extract and basil seeds (*Ocimum basilicum* L.) has been used to develop a functional milk drink. Basil seeds have therapeutic potential and have been used as bulking and stabilizing agent in the drink.

*Celosia cristata* L. commonly known as cockscomb belongs to the order Caryophyllales and family Amaranthaceae. The plant is known for its vibrant coloured inflorescence and, thus, is used as an ornamental plant. Besides developing landscape plenty of medicinal qualities are also known from the plant with promising activity. The plant owes these activities to the presence of bioactive compounds such as phenolic compounds, tannins, flavonoids, sterols, kaempferol, carbohydrates, saponin,

sterols, amino acids and quercetin (Woo *et al.* 2011, Rubini *et al.* 2012, Surse *et al.* 2014, Islam *et al.* 2016). The extract of red variety of cockscomb flower is used by local inhabitants of the Himalayan belt for coloring food. The deep-red color of *C. cristata* flowers is due to the presence of betalains. Betalains contain several desirable biological activities, including antioxidant, anti-inflammatory, hepatoprotective, and antitumor properties (Escribano *et al.* 1998, Kapadia *et al.* 2003, Winkler *et al.* 2005). Thus, herbal extract obtained from *C. cristata* can be used as natural coloring pigment with specified therapeutic properties and may open new opportunities in dairy and food industry to develop natural colour based functional dairy and food products. Therefore, the present investigation was aimed to develop a milk drink incorporated with *C. cristata* extract and study the effect of the extract on the shelf-life of the developed product.

### MATERIALS AND METHODS

*Extract preparation:* The *C. cristata* flowers were separated from the stalks and the leaves were removed. These were then threshed to remove the seeds and dirt. The *C. cristata* flowers were shade dried. The dried flowers were subjected to pulverization to obtain finely ground powder. Powdered sample in solvent (70% ethanol) was treated in a microwave for 45 s. The extract was filtered through Whatman No. 1 filter paper and centrifuged at 12000 rpm for 15 min. The supernatant was concentrated under vacuum in rotary vacuum evaporator (HAHNSHIN, South Korea)

\*Corresponding author e-mail: mthakur1@amity.edu

at 35°C until all the solvent was recovered at the end. The resulting extract was stored at 4°C until use. The extract developed from the flower was viscous and thick. It was then solubilized in purified water to obtain a desired TSS 9.0., after which it was used for analytical purposes.

**Preparation of *C. cristata* incorporated milk:** Toned milk (3% fat and 8.5% SNF) was filtered and pasteurized. Granular sugar and imbibed basil seeds were added to the milk @7% and 1% respectively. Pasteurized (HTST) *C. cristata* extract (CCE) at different levels (0.25, 0.5, 0.75 and 1.0%) was added to the milk and mixed thoroughly, followed by addition of rose essence (0.2%). The milk was packed in sterilized glass bottles, sealed and stored at refrigeration temperature ( $5.0 \pm 0.1^\circ\text{C}$ ) for determining the shelf life of the product. The control was prepared without the addition of CCE.

**Shelf life study of the product:** The product was analyzed for shelf life study during which it was stored for a period of 12 days and analyzed for chemical, microbiological and sensory tests at regular intervals. All the studies were conducted in the year 2019 at Amity University, Noida, Uttar Pradesh, India. The details of these analyses are mentioned below.

#### Chemical analysis

**Total Soluble Solids:** The total soluble solids (TSS) content was determined by digital Refractometer (RX-7000a, Atago India Instruments Pvt. Ltd., India). The refractometer was calibrated with distilled water before measuring TSS of the sample. TSS was measured by placing a drop of milk beverage on the prism of the refractometer. An average of 6 readings was recorded and expressed as °Brix.

**pH:** The pH of milk was determined by using a Systronic digital pH meter. The pH meter was calibrated with commercial buffer solutions before measurement. pH electrode was dipped in about 10 ml sample held at room temperature and pH was recorded after stabilization.

**Microbiological analysis:** The samples were analysed microbiologically for total plate count (TPC). Total plate count was determined on plate count agar (PCA) (Hi Media Pvt. Ltd., Mumbai) by standard protocol of IS-5402 (2012). Various dilutions ( $10^0$  to  $10^{-4}$ ) of the sample were prepared with sterile 0.85% NaCl 1 ml from each dilution, in duplicates, these were poured on the sterile Petri plates. PCA at  $44\text{--}47^\circ\text{C}$  was poured on top of the sample and carefully mixed with the inoculum by rotating the Petridish. The mixture was allowed to solidify by leaving the Petri dishes standing on a cool horizontal surface. After complete solidification, the prepared dishes were placed in the incubator at  $30 \pm 1^\circ\text{C}$  for  $72 \pm 3$  h. After the specified incubation period the colonies on the plates were counted, using the colony counting equipment. Colony forming units per ml of sample were calculated by taking average number of colonies which was multiplied by reciprocal of dilution factor and expressed as cfu/ml of the sample.

**Sensory analysis:** The milk drink was evaluated for different sensory parameters like color, flavour, mouth

feel, and overall acceptability using 9-point hedonic scale by 10 expert judges using standard score card with scores ranging from liked extremely (9) to disliked extremely (1) (Amerine *et al.* 1965).

**Statistical Analysis:** Data obtained during the course of investigation were subjected to statistical analysis to test the effect of CCE on chemical, microbial and sensory quality of the product. Data was reported as mean  $\pm$  standard deviation. Statistical analysis of sensory scores and comparison of data were done using Duncan's multiple range tests at 5% significance level and Statistical package SPSS 10.1 (USA).

## RESULTS AND DISCUSSION

**Selection of optimum CCE % for addition in flavoured milk based on sensory scores:** The results obtained in the sensory analysis are presented in Table 1. Different levels of *Celosia cristata* extract (CCE) were added to the milk. These were subjected to sensory analysis in order to select the optimum level of CCE required to produce a drink with pleasing colour, appearance and thus, overall acceptability. The pink colour was attributed to the betalain pigment present in CCE. It is well evident from the results that colour and appearance scores of flavoured milk increased as the CCE level increased, but only up to 0.75%. Score increased from 3.8 for control to 8.2 for CCE incorporated milk, when 0.75% CCE was added. The concentration was standardized on the basis of 9-point Hedonic scale. No previous researches were available on the incorporation of *C. cristata* betalains in drinks so far.

There was no further improvement in the colour and appearance when 1.0% CCE was added and the scores reduced to 7.2. The colour addition had a cascading effect on all other attributes. Flavour scores of CCE incorporated milk also increased slightly from 6.80–7.20. This may be attributed to the pinkish colour imparted by the addition of 0.75% CCE to the rose flavoured drink. The scores for mouth feel of CCE incorporated milk increased from

Table 1 Effect of addition of CCE on the sensory scores of flavoured milk

CCE %	Colour and appearance	Flavour	Mouth feel	Overall acceptability
0 (Control)	3.8 $\pm$ 0.40 <sup>ap</sup>	6.8 $\pm$ 0.40 <sup>ar</sup>	7.4 $\pm$ 0.48 <sup>ar</sup>	5.8 $\pm$ 0.40 <sup>aq</sup>
0.25	4.2 $\pm$ 0.83 <sup>abp</sup>	7.2 $\pm$ 0.44 <sup>ar</sup>	7.4 $\pm$ 0.54 <sup>ar</sup>	6.2 $\pm$ 0.44 <sup>aq</sup>
0.50	5.2 $\pm$ 0.83 <sup>bp</sup>	7.2 $\pm$ 0.44 <sup>ar</sup>	7.6 $\pm$ 0.54 <sup>ar</sup>	6.4 $\pm$ 0.54 <sup>aq</sup>
0.75	8.2 $\pm$ 0.83 <sup>cp</sup>	7.4 $\pm$ 0.54 <sup>ap</sup>	7.6 $\pm$ 0.54 <sup>ap</sup>	7.6 $\pm$ 0.54 <sup>bp</sup>
1.0	7.2 $\pm$ 0.83 <sup>cp</sup>	7.2 $\pm$ 0.44 <sup>ap</sup>	7.4 $\pm$ 0.54 <sup>ap</sup>	7.6 $\pm$ 0.54 <sup>bp</sup>

Values expressed are mean  $\pm$  standard deviation; Different superscripts show significant differences in the rows and within the column.

Table 2 Effect of storage on TSS, pH and microbial count of flavoured milk at refrigeration temperature

Parameter	Storage days													
	0		2		4		6		8		10		12	
	C	CEM	C	CEM	C	CEM	CM	CEM	C	CEM	C	CEM	C	CEM
TSS	14.0 ± 0.11	14.5 ± 0.40	14.2 ± 0.32	14.5 ± 0.17	14.2 ± 0.76	14.6 ± 0.42	14.5 ± 0.33	14.7 ± 0.65	14.5 ± 0.43	14.7 ± 0.77	14.6 ± 0.56	14.8 ± 0.86	14.6 ± 0.55	14.9 ± 0.85
pH	6.76 ± 0.52	6.74 ± 0.02	6.70 ± 0.75	6.72 ± 0.19	6.64 ± 0.07	6.68 ± 0.54	6.60 ± 0.09	6.63 ± 0.29	6.55 ± 0.66	6.60 ± 0.59	6.42 ± 0.40	6.58 ± 0.31	6.30 ± 0.72	6.53 ± 0.44
TPC (cfu/ml)	151	14	200	20	450	37	640	52	740	94	Tntc	150	Tntc	260

C, control milk; CEM, *Celosia* extract added milk; Tntc, too numerous to count; Values expressed are mean ±standard deviation.

7.40–7.60, which is not a significant difference. Overall scores also increased from 5.80 for control to 7.60 for CCE incorporated milk, by the addition of 0.75% CCE. Though use of 1.0% CCE imparted pink colour, there was no further improvement in the sensory quality of flavoured milk. So, 0.75% CCE was chosen for further studies.

*Effect of storage on TSS of flavoured milk:* The results of the effect of storage period on TSS of flavoured milk are presented in Table 2. During storage the TSS increased from 14.0°Brix to 14.8°Brix in case of control and from 14.5°Brix to 14.9°Brix in CCE incorporated milk. The rate of increase was more in control as compared to CCE drink. The slight increase in TSS levels (°Brix) may be due to the hydrolysis of components during storage.

*Effect of storage on pH of flavoured milk:* The pH of the samples at different period of storage is presented in the Table 2. The pH of flavoured milk decreased during storage period. This may be due to degradation of lactose into acids. The initial average values of pH in control samples and CCE milk were 6.76 and 6.74, respectively. The corresponding values of pH of respective samples at the end of 12<sup>th</sup> day of storage period were 6.30 and 6.53, respectively. There was higher decline in pH control as compared to CCE incorporated drink.

*Changes in microbial counts of flavoured drink during storage:* The factors that influence the stability of pasteurized milk include the quality of raw material, pasteurization time, resistant microorganisms to pasteurization temperatures particularly psychrotrophics, post pasteurization contaminants

and storage temperature (Cromie 1991). According to BIS the Total plate count (TPC) of pasteurized milk in its final container should not exceed 30,000 cfu/ml. TPC of both the drinks increased with the progress in storage duration (Table 2). This was due to the germination of psychotropic spores during storage. However, the increase was higher in control than in CCE incorporated drink.

The TPC in the control drink was recorded to be 151 cfu/ml but the same value was recorded to be 14 cfu/ml for the CCE incorporated drink. On the 10<sup>th</sup> day of storage the TPC for the control sample was too numerous to count (TNTC). This microbial count increased to 260 cfu/ml after 12 days of storage. The shelf life of normal pasteurized milk is 5–7 days under refrigerated storage. This was increased to 12 days with addition of CCE. The less microbial load of CCE incorporated milk drink could be due to the anti-microbial properties exhibited by the extract. This could be concluded from the study done by Yun and co-workers who evaluated the antimicrobial properties in different *Celosia cristata* L. extracts against microorganisms, viz. *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* and found out that *C. cristata* extracts had the ability to inhibit bacterial growth (Yun *et al.* 2008).

*Changes in sensory scores of the flavoured milk during storage:* The data of sensory scores are shown in Table 3. It can be seen from the results that the sensory score for both drinks i.e. control and CCE incorporated milk scores decreased as the storage progressed. The control was not

Table 3 Effect of storage on sensory score of CCE added flavoured milk at refrigeration temperature

Parameter	Storage days													
	0		2		4		6		8		10		12	
	C	CEM	C	CEM	C	CEM	CM	CEM	C	CEM	C	CEM	C	CEM
TSS	14.0 ± 0.11	14.5 ± 0.40	14.2 ± 0.32	14.5 ± 0.17	14.2 ± 0.76	14.6 ± 0.42	14.5 ± 0.33	14.7 ± 0.65	14.5 ± 0.43	14.7 ± 0.77	14.6 ± 0.56	14.8 ± 0.86	14.6 ± 0.55	14.9 ± 0.85
pH	6.76 ± 0.52	6.74 ± 0.02	6.70 ± 0.75	6.72 ± 0.19	6.64 ± 0.07	6.68 ± 0.54	6.60 ± 0.09	6.63 ± 0.29	6.55 ± 0.66	6.60 ± 0.59	6.42 ± 0.40	6.58 ± 0.31	6.30 ± 0.72	6.53 ± 0.44
TPC (cfu/ml)	151	14	200	20	450	37	640	52	740	94	Tntc	150	Tntc	260

C, control milk; CEM, *Celosia* extract added milk; Values expressed are mean ±standard deviation; Different superscripts show significant differences in the rows and within the column.

evaluated for sensory attributes after 8 days of storage because of its high microbial count after 8<sup>th</sup> day. The control received a colour score (4.80) which is much less than the score of 8.4 for CCE incorporated sample. The colour score for control drink decreased to 3.8 on the 8<sup>th</sup> day of storage and to 7.20 for CCE milk on the 12<sup>th</sup> day of storage. This suggests that the CCE colour remained stable during the storage period. Flavour scores decreased during storage. The score on day 0 for control and CCE incorporated drink was 7.40 and 7.60 which decreased to 5.40 and 6.20, respectively. This may be because of development of acidic flavour caused by decrease in pH. The scores for mouth feel during storage varied from 8.40–7.20 at the end of storage, which was significantly less than the zero-day score. The mouth feel scores for control also reduced significantly during storage. The overall acceptability scores also reduced significantly. But the reduction was more prominent in control than in CCE milk and both the drinks were adjudged unacceptable due to perceived fermented/acidic flavour.

It can be concluded that the CCE addition gave good colour and appearance to the flavoured milk as well as there was no adverse effect on body and texture, flavour and overall acceptability of flavoured milk. It was found from the sensory analysis that the colour of the flavoured drink showed stability during the period of storage of the flavoured milk. The flavoured milk incorporated with CCE was analysed for pH, TSS, microbial and sensory analysis for a period of 12 days. During refrigeration the beverages exhibited increasing trend in TSS and decreasing trend was observed in pH. The microbial analysis showed non-significant change in total plate count during refrigeration, as compared to the control which showed high total plate count on the 10<sup>th</sup> day. The studies revealed that CCE based milk beverage prepared with 0.75% extract scored maximum for sensorial quality attributes such as colour, flavour and overall acceptability. Thus, CCE can be used as a substitute for synthetic colours in milk beverages without any detrimental effects on the quality of the product as perceived by the consumer.

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