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Stability of anthocyanins as pre-extrusion colouring of rice extrudates

Arvind V. Durge, Shatabhisa Sarkar, Rekha S. Singhal *

Food Engineering and Technology Department, Institute of Chemical Technology, Matunga, Mumbai 400 019, India

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ABSTRACT

The suitability of anthocyanin as a pre-extrusion colour nutraceutical of rice flour was evaluated as a function of extrusion parameters viz. moisture content, screw speed and temperature of extrusion. The retention of anthocyanin increased with an increase in the moisture content of feed material and screw speed, but decreased with an increase in the die temperature. The effect of citric acid and sodium bicarbonate on colour stability of extrudates during processing, and that of light and packaging materials during storage at ambient conditions (30 ± 2 °C) were evaluated. Addition of 1% citric acid increased the retention of anthocyanin up to 18.2% which could reduce the requirement of pre-extrusion colouring by almost 25%. Metallized polyethylene was found to be a better packaging material than low density polyethylene and protected the extrudates from light as well as losses during storage.

1. Introduction

Colour in food system plays a vital role in consumer acceptance of any food product. With increasing public concern on the safety of synthetic colourants, natural colours are assuming greater prominence. Anthocyanins (E163) are flavonoid phenolic colouring compounds, widely distributed among fruits, berries and flowers (Wissgott & Bortlik, 1996). Antioxidant activity of anthocyanins plays a key role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes (Konczak & Zhang, 2004). There are several studies focusing on the effect of anthocyanins in cancer treatments (Lule & Xia, 2005), human nutrition (Stintzing & Carle, 2004), and its biological activity (Kong, Chia, Goh, Chia, & Brouillard, 2003). These attractive natural colouring pigments are water-soluble and this property facilitates their incorporation into numerous aqueous food systems. However, usage of colourants in foods and the level of incorporation are administered by the regulatory requirements of the individual countries. Maximum limits on levels of addition of synthetic colours are imposed by many countries, but the use of anthocyanin often has no numerical limit, and maximum dose is simply that which is sufficient to give the desired colour strength (Henry, 1992). However, colour stability of anthocyanins depends on a number of factors such as the structure and concentration of the anthocyanin, pH, temperature, oxygen and presence of complexing agents such as phenols and metals (Janna, Khairul, & Maziah, 2007).

Extrusion cooking is versatile and time efficient process in food processing. It involves continuous cooking, mixing and forming process. The processing conditions used in extrusion cooking (high temperature and low water content) result in numerous chemical and structural changes in food like starch gelatinization, protein denaturation, vitamin and pigment degradation (Harper, 1981). Generally, the extruded products are not coloured, and are also flavoured post-extrusion. However the use of natural colours prior to extrusion would not only add to visual appeal and acceptability, but could also form a suitable matrix for delivery of nutraceutical phytochemicals to consumers. This necessitates evaluation of stability of these phytochemicals.

Reports on loss of indigenous anthocyanins (55%) in blue and red pigmented maize during extrusion processing (Mora-Rochin et al., 2010), and that present in cranberry pomace added to corn starch (White, Howard, & Prior, 2010), or grape pomace (Khanal, Howard, & Prior, 2009) and use of ascorbic acid to reduce the loss of anthocyanins during extrusion (Chaovanalikit, Dougherty, Camire, & Briggs, 2003) are available.

To the best of our knowledge, stability of added anthocyanins as pre-extrusion colouring has not yet been reported in literature. The present work reports on optimising the quantity and the stability of anthocyanin for pre-extrusion colouring as a function of extrusion variables. Further, the effect of citric acid and sodium bicarbonate, and evaluation of packaging material for coloured extrudates were also undertaken.

2. Materials and methods

2.1. Materials

Rice (*Oryza sativa*) was procured from local market of Matunga, Mumbai. Rice was ground to flour to pass through 840 μm and flour was packed in polyethylene bags. Anthocyanin extracted from red

* Corresponding author. Tel.: +91 22 33611111; fax: +91 22 24145614.
E-mail address: rs.singhal@ictmumbai.edu.in (R.S. Singhal).

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carrot in the powder form (98% purity, pH stability 4.0 to 6.0) was procured from Indian Trading Company, Mumbai, kept in a refrigerator (10 ± 2 °C) until further use. Packaging materials were procured from Shako Flexipack Pvt. Ltd., Sion Mumbai. Citric acid and sodium bicarbonate (food grade) were procured from SD Fine Chemicals, Mumbai, India. All chemicals used for analysis were of AR grade.

2.2. Methods

2.2.1. Preparation of sample

Moisture content (MC) of rice flour was determined by (AACC, 1976) and calculated amount of distilled water was added to the flour and mixed thoroughly to assure uniform distribution to obtain desired MC. The mixture was then packed in air tight jars and allowed to equilibrate overnight prior to extrusion.

2.2.2. Optimization of anthocyanin content in extruded rice flour

Colour percentage was varied from 1% to 3% (w/w) and extrusion parameters were kept constant at feed moisture content (MC) of 14%, die temperature of 160 °C, a screw speed of 150 rpm, and a feed rate of 70 g/min. Extrusion cooking of conditioned flour was carried out using Brabender single screw extruder (Model No. 8 235 00, Germany), with 20:1 barrel length to diameter ratio and a screw with compression ratio of 2:1. The extruder was fitted with a die nozzle of 5 mm diameter. Sensory evaluation of the rice extrudates was optimised for the content of pre-extrusion addition of anthocyanin. Sensory evaluation was done on a hedonic scale of 1–9 using 10-member panel as follows: 9—like extremely, 8—like very, 7—like moderately much, 6—like slightly, 5—neither like nor dislike, 4—dislike slightly, 3—dislike moderately, 2—dislike very much, and 1—dislike (Camire, King, & Bittner, 1991).

2.2.3. Optimization of extrusion processing parameters

The extrusion processing conditions were carried out by varying the MC from 14% to 18%, screw speed from 150 rpm to 170 rpm, and die temperature from 140 °C to 180 °C. The extrudates were sealed in polyethylene bags and stored in desiccators until further analysis.

2.2.4. Analysis of the coloured extrudates

2.2.4.1. Colour measurement (Hunter L, a* and b* values).* The colour of extrudates was measured using Hunter Lab Colourimeter (Model DP-9000 D25A), (Hunter associates laboratory, Reston, VA, USA) in terms of Hunter L^* value (lightness, ranging 0–100 indicating black to white), a^* value ($+a^*$ value indicates redness and $-a^*$ value indicates greenness) and b^* value ($+b^*$ value indicates yellowness and $-b^*$ value indicates blueness) (Camire, Chaovanalikit, Dougherty, & Briggs, 2002). The samples were ground and passed through a 40 micron sieve prior to analysis.

2.2.4.2. Retention of anthocyanin colour. The retention of anthocyanin was done by extraction with methanolic HCl by the method of Delgado-Vargas, Jimenez, Paredes-Lopez, and Francis (2000) with modifications. The ground coloured extrudates (5 g) was extracted in 25 ml of solvent (1% acidified methanol: water in 1:1 ratio) and kept on rotary shaker (200 rpm) for 24 h followed by centrifugation at 8000 *g*. The supernatant so obtained was filtered and the clear supernatant which was analysed at 537 nm in a Helios (α) UV–vis spectrophotometer (Thermoelectron, Erlangen, Germany) for quantification. Quantification was carried on the basis of a standard plot obtained using 0.0–1.0 mg/ml anthocyanin that was procured from the Indian Trading Company, Mumbai. All analyses including the standard graph was carried out in triplicates.

2.2.4.3. Organoleptic evaluation of anthocyanin coloured extrudate. Acceptability of extrudates was determined by sensory evaluation using 10-member panel on nine point hedonic scale as described above.

2.3. Effect of additives, packaging material and light on colour of retention in anthocyanin-coloured extrudates

Effects of addition of citric acid and sodium bicarbonates on colour stability of extrudates of final sample with desirable properties were carried out. Both the additives were used at 1% and 2% w/w basis. Further, on the basis of the results obtained, the content of anthocyanin added prior to extrusion in flour supplemented with 1% citric acid was carried out and analysed for retention and organoleptic quality. Anthocyanin coloured extrudates with desirable properties were packed in LDPE and metallized polyethylene, stored at ambient temperature (30 ± 2 °C), and evaluated for percent retention of colour during a 45 days storage study. Similarly packed samples were stored under light intensity of 500 lx (lux), and studied for % retention of colour during 28 days storage. Further, a semi-log plot of percentage retention of percent retention of colour vs. days (Cai, Sun, & Corke, 1998) was done to obtain the rate constant (k) as the slope of the graph. Half-life ($t_{1/2}$), the time required for the anthocyanin was calculated from the rate constant as 0.693/k.

2.4. Statistical analysis

All the results were statistically analysed by Tukey's test (*t*-test) at a probability level of 5% ($p<0.05$) using the statistical programme known as NCSS-PASS software.

3. Results and discussion

3.1. Optimization of content of anthocyanin for pre-extrusion of rice extrudates

Anthocyanin in the powder form was added at 1, 2 and 3% (w/w) to rice flour after equilibration to 14% MC and extruded at a die temperature of 160 °C and screw speed of 150 rpm. All the extrudates had moisture content below 7%. Hence they were evaluated for their sensory quality using a 10-member panel without any further drying. Rodríguez-Miranda et al. (2011) found the moisture content in extruded snacks prepared from flour blends of taro and nonnixtamalized maize to be lower than 10% which prevented spoilage of extruded products. The extrudates containing 1, 2 and 3% anthocyanin showed a sensory rating of 6.50 ± 0.37, 8.45 ± 0.22, and 7.25 ± 0.17, respectively, suggesting that all the products were acceptable organoleptically . The extruded sample with 2% anthocyanin was most acceptable in terms of appearance, colour and overall acceptability, and therefore used for further studies.

3.2. Optimization of extrusion processing parameter on anthocyanin retention

3.2.1. Colour measurement

The visual appeal of colour makes it an extremely important quality parameter and is directly related to the acceptability of food products. The colour characteristics of anthocyanin coloured extrudates were measured in terms of L^*, a^* and b^* values. The L^* value of extrudates increased with an increase in temperature from 140 to 180 °C, and decreased with an increase in MC of feed material from 14 to 18% (Table 1). The a^* value of anthocyanin coloured extrudates increased with an increase in MC and screw speed, and decreased with an increase in temperature (Table 1). The increased a^* value at higher MC may be due to the entrapment of the pigments in denser matrix of the product. The residence time of raw material decreased with an increase in screw speed which resulted in minimum thermal treatment and thereby higher retention of colour. A decrease in the redness of extrudates with