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Extraction, dealcoholization and concentration of anthocyanin from red radish

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ABSTRACT

The extraction procedure is of great importance for the extraction of natural colorants. In the present study, an alternate process is reported for anthocyanin from the peels of red radish (*Raphanus sativus* L.). Different extracting mediums are used and the mixture of 50% ethanol and acidified water resulted in maximum anthocyanin content (37.26 mg/100 ml) with better chroma (69.03) and hue angle (44.54). Membrane pertraction is used for the first time to dealcoholize and concentrate the anthocyanin extract (from 37.26 mg/100 ml to 62.58 mg/100 ml). The extract is further concentrated using osmotic membrane distillation (485 mg/100 ml) at ambient temperature and atmospheric pressure.

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1. Introduction

Color is one of most important properties of foods and beverages and is a basis for their identification and acceptability. Normally, food color is due to naturally occurring pigments, but synthetic colorants are often added to confer the desired color to the final product. Although synthetic colors had been favored over the past 100 years, their use has been reduced in the past four decades as synthetic food colorants are being recognized to be carcinogenic and harmful to the consumers. Processors have recently turned to naturally derived colorants as a viable alternative, responding to an increasing consumer demand for natural products. However, availability of natural pigment sources, extraction, and concentration processes and stability of colorants must be taken into account in the production of such ingredients.

Anthocyanins are extracted from a wide variety of sources such as fruits (grapes, red raspberry, cranberries) [1–3], vegetables (red cabbage, red radish, sweet potato) [4–6] and petals of some flowers [7,8]. Anthocyanins are considered as potential replacements for synthetic counterparts because of their bright attractive colors, water solubility that allows their incorporation into aqueous food systems and a number of health benefits such as improved visual acuity, anticancer and antiviral activities [9–11]. However, anthocyanin containing products are susceptible to color deterioration during processing and storage. The main limitations of most of the anthocyanin pigment for commercial applications is its chemical instability and low tenctorial strength. In the present work, anthocyanin from red radish is selected based on its stability compared to other anthocyanin colorant due to the presence of acylated pelargonidin derivatives. These are characterized as pelargonidin-3-sophoroside-5-glucoside (pg-3-soph-5-glu, or raphanusin) derivatives [12,13]. Also, red radish is a potential alternative source for the peroxidase enzyme [14] while the peel (epidermal tissue, normally considered as waste material) can be utilized for the recovery of anthocyanin.

The extraction procedure mainly depends on the nature of the color, source of the material and type of extracting solvents. An efficient extraction procedure should maximize anthocyanin recovery with minimal amount of adjuncts and minimal degradation or alteration of its natural state. Several extraction procedures employed for the extraction of anthocyanin are efficient, but the extracts were not safe for human consumption due to potential toxic effects from the residual solvents. The solvents such as ethanol, methanol, and acetone are generally used for the extraction of natural colors. However, some of these solvents are toxic to health (such as methanol/acetone). Further, the presence of even relatively safer solvents (such as alcohol) in the natural color extract may limit its application as food colorant. In order to know the effect of extracting medium (solvents and their mixture), different extracting media such as water, acidified water, mixture of ethanol

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and water (at different ratios) and mixture of ethanol and acidified water (at different ratios) are used.

Membrane processes have potential to be alternative for pigment concentration. Several research groups are working on the concentration of anthocyanin by employing membrane processes. For instance, Rodriguez-saona et al. [15] studied the concentration of anthocyanin from red radish extract by employing centitherm evaporation or direct osmosis process separately and also in combination with each other. Gilewicz-Lukasik et al. [16] employed ultrafiltration and nanofiltration for the concentration of anthocyanin from aronia (black chokeberry) fruit. Integrated approach for the concentration of anthocyanin from red radish was reported by Patil and Raghavarao [17]. In the present study, an attempt has been made for the first time to dealcoholization of the alcoholic anthocyanin extract by a membrane process called membrane pertraction (MP) and further concentration (anthocyanin) by osmotic membrane distillation (OMD). The main objective of the present study is to develop an efficient alternate method for the extraction and concentration of anthocyanin.

2. Materials and methods

2.1. Chemicals and membranes

Alcohol (98%) was procured from Hayman Limited, Essex, England; HCl was obtained from Merk Limited, Mumbai, India, calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) was procured from Ranbaxy Limited, Punjab, India. Doubled distilled water was used for all the extractions. Hydrophobic polypropylene (PP) membrane of pore size 0.05 μm and 0.2 μm manufactured by Accurel, Enka, Germany was used for both the MP and OMD membrane processes.

2.2. Preparation of anthocyanin (red radish) extract

Fresh red radishes (*Raphanus sativus* L.) were purchased from local market and washed with distilled water at room temperature ($25 \pm 2\,^{\circ}C$). The outer layer (skin containing color) was removed manually with the help of a peeler. The pigment from peels was extracted with different extracting media using a food processor (Singer, India FP-450). The extracted pigment was filtrated to remove the fibrous particles and then it was centrifuged at 10,000 rpm for about 10 min to remove the tiny suspended particles [17]. The anthocyanin content in all the extract was calculated using the following equation [15,17,18].

$$\text{Anthocyanin pigment (mg/L)} = \frac{A \times \text{Mw} \times \text{DF} \times 10^3}{\varepsilon \times L} \quad (1)$$

where $A = A_{510}$ (pH 1.0)–A_{510} (pH 4.5), Mw is the molecular weight of anthocyanin (433.2 g/mol), DF is the dilution factor, ε is the extinction coefficient (31,600 L/cm mol) and L is the path length (1 cm).

2.3. pH and concentration measurements

pH meter (Control Dynamics, India, APX 175) was used for the measurement of pH of anthocyanins extracts. A UV–vis spectrophotometer (Double beam Spectrophotometer, Shimadzu, Japan, Model UV-160A) was employed for spectral analysis. Experiments were carried out in triplicates and the standard deviations (3%) obtained were indicated in terms of error bars.

2.4. Ethanol estimation

The percentage of ethanol present in the feed was estimated using specific gravity bottle, using water as a standard, at $25 \pm 2\,^{\circ}C$. The measured density values were compared with standard values according to AOAC method [19].

2.5. Color analysis

The color characteristics (Hunter CIE L^*, a^*, b^*) of anthocyanin obtained in different extraction processes were measured using a Hunter colorimeter (LabScan XE, Hunterlab, Virginia). The sample were placed in a 1 cm path length optical glass cell and CIE L^*, a^*, b^* values were noted in duplicates in the total transmission mode, using illuminant C and 2° observer angle.

3. Experimental

3.1. Extraction of anthocyanin

Fifty gram of red radish peels were mixed with the 100 ml of the extracting medium and the mixture was ground using a food processor. The solid–liquid ratio of 1:2 and the grinding time of 3 min were maintained same for all the extractions. However, the extracting medium was different in each extraction. The extracting media used for the extractions include water, acidified water (1% HCl), mixture of water and ethanol at different ratios (0–80% of ethanol in water) and mixture of ethanol and acidified water at different ratios (0–80% of ethanol in acidified water (1% HCl)). The anthocyanin extract obtained in each extraction was filtered through the muslin cloth to remove fibrous particles. In order to know the efficiency of the extracting medium, the number of extractions is restricted to only one (after the first extraction the obtained filter cake was discarded). The filtrates obtained for different extracting medium were centrifuged separately at 10 000 rpm for about 10 min to remove the suspended particles. The anthocyanin content in each of these extract was measured using pH differential method and the results were compared.

3.2. Membrane processes

The MP/OMD experiments were performed using a flat membrane module (fabricated at CFTRI, Mysore) having a membrane area of 0.01 m^2. The module consists of polyester mesh, viton gasket and hydrophobic membrane (polypropylene) placed in between two stainless steel frames [17,20]. Feed solution (anthocyanin extract) and stripping solution were circulated on either side of the membrane in co-current mode using peristaltic pumps. The transmembrane flux was calculated by measuring change in weight once in every hour. The pure water and calcium chloride dihydrate solution were used as the stripping solutions in membrane pertraction and osmotic membrane distillation, respectively.

3.3. Mechanism of membrane pertraction and osmotic membrane distillation

MP, which is used to dealcoholize the red radish alcohol extract is an athermal membrane process [21] and employs a hydrophobic membrane to separate two solutions. In this process, the alcoholic anthocyanin extract is circulated on one surface of the membrane, while another liquid in which alcohol is highly soluble (usually termed stripping solution) is circulated on the other side of the membrane. The liquid most frequently proposed for use as the stripping solution is pure water. The driving force for the MP is the difference in partial pressure of an alcohol across the membrane. Alcohol evaporates from the surface of the solution having higher partial pressure (high concentration of alcohol), diffuses in the form of vapor through the membrane and condenses on the surface of the other solution (pure water), which results in dealcoholization

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