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Solvent and supercritical carbon dioxide extraction of color from eggplants: Characterization and food applications

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ABSTRACT

Extractions of anthocyanins from peels of eggplant (*Solanum melongena* L.) using GRAS solvents and supercritical carbon dioxide (SC-CO_2) were investigated. Conditions of solvent extraction that provided maximum yield of anthocyanin were water–ethanol (1:1, v/v) in 10% citric acid at 60 °C. The highest yield of the same by SC-CO_2 was a sample size of 10 g of peels at 60 °C, 10 MPa, 1.5 h extracting time and 2 L min^{-1} of CO_2. Delphinidin-3-glucoside and delphinidin-3-rutinoside were tentatively identified as the major anthocyanins in the extracts. The total phenolics, proanthocyanidin content and antioxidant activity of the solvent-extracted color were significantly higher than those in the SC-CO_2 extracted color. Stability studies concluded that the former was more stable at high temperature regimes. Trace metal content in the SC-CO_2 extracted color was significantly lower. Usage of the SC-CO_2 extracted color in non-thermal food applications is recommended owing to its higher stability and low metal contamination.

1. Introduction

Anthocyanins generally occur in plants as glycosides and as acylglycosides of anthocyanidins, also known as aglycones (Mazza, Cacace, & Kay, 2004). Although these pigments are water soluble, non-toxic moieties conferring health benefits such as anti-carcinogenicity, anti-viral and anti-allergenic properties; they have disadvantages in their tinctorial power and stability in food systems compared to azo dyes (MacDougall, 2002; Mukhopadhyay, 2000).

Eggplant (*Solanum melongena* L.), commonly known as brinjal, has dark purple peels with high anthocyanin content (FAO, 2008). Major acylated anthocyanins present in eggplant peels are delphinidin-3-(p-coumaroylrutinoside)-5-glucoside (nasunin) and 3-caffeoylrutinoside-5-glucoside, while the major non-acylated anthocyanins are delphinidin-3-glucoside, delphinidin-3-rutinoside and petunidin (Shahidi, Chandrasekara, & Zhong, 2011).

Various solvent systems have been reported for extraction of anthocyanins from flowers, fruits and vegetables of which 0.01–1% HCl in methanol is known to yield the most stable red anthocyanin extract (Takeoka & Dao, 2002). Delphinidin-3-rutinoside has been extracted from eggplants using methanol–acetic acid–water (Wu & Prior, 2005) and aqueous solutions of tartaric acid and malic acid (Todaro et al., 2009). Most of these methods extract anthocyanins in organic solvents of environmental and health concerns. Although, tartaric and malic acid solutions are not of health concern, the yields of anthocyanin reported with these extracting solvents are not appreciable to merit commercial feasibility in food applications. Therefore, our investigation envisages designing extraction procedures using suitable food grade reagents for improved recovery of anthocyanins from eggplant peels for food applications.

The current work focuses on optimization of composition of a solvent system using GRAS status reagents such as water, ethanol and citric acid. This work also envisages reporting for the first time the use of green technology of SC-CO_2 for extracting the eggplant peel color for better yield and improved quality for best suitability in food applications. Characterization of the extracted colors was carried out by densitometry and HPLC–MS–MS analyses. Colors extracted by either method were comparatively evaluated for their phytochemical properties such as total phenolics, flavonoids and proanthocyanidin content and for their antioxidant potency. Stability studies of the color extracts were conducted and their specific half-life values were determined. Finally food applications were also designed using the color extracts, post assay of trace metal constituents therein.

2. Materials and methods

The eggplant variety with the local name of *Muktakeshi*, procured from Jadavpur market of Kolkata, India, was selected for

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our study owing to its ready availability, bright color and easy peelable skin.

2.1. Preparation of samples for color extraction

Bright purple color eggplants, free from wilts, discolored spots and white fungal patches were selected for our work. Post-peeling, the peels (moisture content 87%) were cut to uniform dimensions (0.5 × 0.5 mm for solvent extractions and 10 × 10 mm for SC-CO_2 extraction). The shredded peels were then subjected to lyophilization in a bench top freeze dryer (M/s Eyela, Tokyo, Japan) and stored at −18 to −20 °C prior to color extraction.

2.2. Optimization of extraction parameters using solvent and SC-CO_2

To obtain aqueous extracts of food grade colors – amount of citric acid, water:ethanol (v/v) (all of GRAS status), solvent:peels (v/w), rotary shaker rpm (100, 150 and 180) and extraction temperature [ambient (23 °C), 40 °C, 60 °C and 80 °C] were varied to obtain the best yield of color from the peels.

The extracts were then centrifuged at 13,200 g for 5 min at 4 °C and the total anthocyanin content of each of the extract was determined by pH differential method. These were initially concentrated using a rotavac system (M/s Buchi, Flawil, Switzerland) at 40–45 °C and 0.005 MPa Hg and finally by purging a gentle stream of nitrogen. The extracts were then filtered through 0.2 μm filters and stored at 4 °C until analyzed.

For SC-CO_2 extraction, a SPE-ED SFE 2 model of M/s Applied Separations, Allentown, USA, was used. 10 g peels were pre-treated with 10% citric acid solution for 1 min and then loaded to the extraction vessel. Optimization of yield of color from eggplant peels was conducted using a mixed-level factorial design and parameters such as extraction pressure (10, 12, 15 MPa), temperature (60, 80 °C), time (90 min = 60 min static time + 30 min dynamic time) and 2 L min^{-1} flow rate of CO_2. All extracts were collected in amber-colored collection vials in an ice bath, sealed in an inert atmosphere of nitrogen and stored at 4 °C in dark until further usage.

2.3. Characterization of the color extracted from the eggplant peels

2.3.1. Densitometric estimation of anthocyanin in the color extracts

Identification of the different anthocyanins present in the color extracts was conducted by densitometric method with Camag HPTLC unit (Camag Linomat V and TLC scanner III of M/s Camag, Muttenz, Switzerland). The color extracts were spotted on silica gel 60 (F_{254}) coated Al plates. The plates were developed at 23 °C with butanol:acetic acid:water (4:1:5, v/v/v). Delphinidin-3-rutinoside and delphinidin-3-glucoside recorded R_f values of 0.19 (Tatsuzawa et al., 2000) and 0.26 (Takeoka & Dao, 2002) respectively at 530 nm.

2.3.2. HPLC–electrospray ionization (ESI)–mass spectrometry (MS/MS) analyses of the color extracts

Anthocyanins in the extracts were separated on an analytical C18 Phenomenex Gemini column (50 × 4.6 mm i.d., 5 μm) in a HPLC–MS/MS system (M/s Shimadzu, Kyoto, Japan) equipped with an autosampler L-2200 and an ATI 2000 LC–MS–MS (M/s Applied Biosystem, Canada, USA) in accordance to Wu and Prior (2005).

2.4. Evaluation of phytochemical properties of the color extracted from eggplant peels

Total anthocyanin content (TA) of the color extracts was estimated using pH differential method (Giusti & Wrolstad, 2000) and expressed as g delphinidin-3-glucoside/kg of dry peel weight; total phenolic compound as g gallic acid/kg of dry peel weight using Folin–Ciocalteu reagent (Spanos & Wrolstad, 1990), total flavonoid content as g quercetin/kg of dry peel weight using $AlCl_3$ colorimetric method (Aiyegoro & Okoh, 2010), total proanthocyanidin (Aiyegoro & Okoh, 2010) as g catechin/kg of dry peel weight and antioxidant activity of 60 g kg^{-1} dry peel weight of color extracts by measuring the radical scavenging activity of DPPH (Aiyegoro & Okoh, 2010) and by FRAP assay using 0.176 mg/mL ascorbic acid as standard (Benzie & Strain, 1999).

2.5. Trace metal analyses of the extracted colors

Prior to food applications of extracted colors, it was necessary to analyze the same for hazardous metals. It is likely that soil and fertilizers could possibly contribute to trace metals in eggplants, it is therefore necessary to detect whether the levels of the same in the extracted colors are below the permissible limits of the PFA (2004) and EU Scientific Committee on Food (SCF, 2002). Trace metal analyses in the extracts were carried out for Pb, Cd, Hg, Cu and As (Ogimoto, Uematsu, Suzuki, Kabashima, & Nakazato, 2009) by flame atomic absorption spectrophotometer.

2.6. Stability studies of the color extracts

Stability of the color extracts was determined by calculating the specific half-life value ($T_{1/2}$) at low and high temperature regimes. For the low temperature study, the samples were stored in dark at 4 °C. TA of the colors was measured at an interval of 20 days for 60 days and $T_{1/2}$ was determined. Accelerated stability studies of the extracted colors were performed at 95 °C. TA of each sample was measured and $T_{1/2}$ was determined for 1–7 h (Sadilova, Stintzing, & Carle, 2006).

2.7. Food applications using eggplant peel color extract

From the stability studies, it was found that the color extracts of eggplant peel, obtained by either procedure were reasonably stable over a long range of temperature. Therefore both non-thermal and thermal food applications were designed using these extracted colors on jelly crystals and custard respectively. According to PFA, application of synthetic red colors such as Ponceau 4R, Carmoisine and Erythrosine are banned in these two food commodities. Therefore these food applications were selected for our study and no colored food sample was considered as positive control. Of the three sets of jelly crystals and custards prepared, one set served as blank (control). The other two sets were individually colored with solvent-extracted color and with that obtained by SC-CO_2.

2.7.1. Non-thermal food application of eggplant peel color extract on jelly crystals

To 5 g of edible grade gelatin (M/s Finagel, Maharashtra, India), 30 g milled sugar, 0.5 g citric acid and 0.25 g Na-citrate were added. The whole dry mix was transferred to 75 mL boiling water and heating was continued until a clear solution was obtained. The mixture was then cooled to ambient (23 °C) conditions and 15 mg/kg jelly weight of anthocyanin was then added to the same and color measurements of the 10 mm deep jelly crystals was measured by Lovibond Tinctometer Model F (M/s The Tintometer Ltd., Salisbury, Wilts, UK). The jelly crystals were organoleptically tested by a semi-trained panel of five members.

2.7.2. Thermal food application of eggplant peel color extract in custard

Thermal food application of the eggplant peel color was carried out in custard prepared from a commercial sample of custard powder (M/s Pillsbury, Minnesota, USA). To 10 g of commercial