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Black bean anthocyanin-rich extracts as food colorants: Physicochemical stability and antidiabetes potential

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ABSTRACT

Black beans contain anthocyanins that could be used as colorants in foods with associated health benefits. The objective was to optimize anthocyanins extraction from black bean coats and evaluate their physicochemical stability and antidiabetes potential. Optimal extraction conditions were 24% ethanol, 1:40 solid-to-liquid ratio and 29 °C ($P < 0.0001$). Three anthocyanins were identified by MS ions, delphinidin-3-*O*-glucoside (465.1 m/*z*), petunidin-3-*O*-glucoside (479.1 m/*z*) and malvidin-3-*O*-glucoside (493.1 m/*z*). A total of 32 mg of anthocyanins were quantified per gram of dry extract. Bean anthocyanins were stable at pH 2.5 and low-temperature 4 °C (89.6%), with an extrapolated half-life of 277 days. Anthocyanin-rich extracts inhibited α-glucosidase (37.8%), α-amylase (35.6%), dipeptidyl peptidase-IV (34.4%), reactive oxygen species (81.6%), and decreased glucose uptake. Black bean coats are a good source of anthocyanins and other phenolics with the potential to be used as natural-source food colorants with exceptional antidiabetes potential.

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1. Introduction

Anthocyanins represent the largest group of phenolic pigments and the most important group of water-soluble pigments in plants, responsible for colors in fruits, vegetables, cereal grains, and flowers (Shipp & Adbel-Aal, 2010). They are formed by two or three chemical units: an aglycon base or flavylium ring (anthocyanidin), sugars, and possibly acylation groups. Cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin are the most frequently occurring anthocyanidins, which may be glycosylated or acylated by different sugars and aromatic or aliphatic acids on their aglycon unit to yield anthocyanins in the plant (Bueno et al., 2012).

Anthocyanins are very unstable and susceptible to degradation. Its color stability is affected by pH, their chemical structure, concentration, storage temperature, light, oxygen, and the presence of enzymes, flavonoids, proteins and metal ions (Castaneda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodrıguez, &

Abbreviations: AE, anthocyanin-rich extract; b*, yellowness/blueness; C*, Chroma; C3GE, cyaniding-3-glucoside equivalent; DW, dry weight; DPP-IV, dipeptidyl peptidase IV; Ea, Arrhenius activation energy; ΔE*, change of color; GAE, gallic acid equivalent; h, Hue; HDC, high energy collision; K, first-order kinetic rate; Ki, inhibition constant; L*, lightness; *m*/*z*, mass/charge; PHL, phloretin; Q_{10}, change in the reaction rate constant for 10C; RSM, response surface methodology; t_R, retention time; $t_{1/2}$, half-life; a*, redness/greenness; t_0, time zero; T2D, type-2 diabetes; ROS, reactive oxygen species.

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Galan-Vidal, 2009; Hernandez-Herrero & Frutos, 2014). Anthocyanins are usually stable at pH 1 to 4 and degrade above pH 7. At pH 1, the predominant structure corresponds to the flavylium cation, conferring red and purple colors, whereas, at values between pH 2 and 4, blue quinoid bases predominate. Some of the ways to optimize anthocyanin stability during storage are to increase anthocyanin concentration, remove oxygen and inactivate enzymes (Castaneda-Ovando et al., 2009).

Anthocyanins are usually located in the seed coat of common beans. In previous studies, we found that Negro-Otomi cultivar (black bean), had the highest anthocyanin concentration (2.5 mg/g coat) (Mojica, Meyer, Berhow, & de Mejía, 2015) among other 14 common bean cultivars. Anthocyanins may provide anti-inflammatory and antidiabetes benefits since they inhibit pro-inflammatory cytokines, decrease their production, and prevent β-cell dysfunction (de Mejia & Johnson, 2013). The potential antidiabetes mechanism of action of anthocyanins and other polyphenols from berries or other food sources can be classified into two groups: insulin-dependent and insulin-independent. The insulin-dependent mechanism involves the improvement of pancreatic β-cell function (reducing oxidative stress, increasing insulin production, reducing β-cell apoptosis and promoting β-cell proliferation), and enhancing tissue sensitivity (changes in peripheral tissue in inflammation and oxidative stress). On the other hand, the insulin-independent mechanism involves the blockage of starch degrading enzymes and the reduction in glucose absorption (inhibition of α-glucosidase, α-amylase, and glucose transporters SGLT1 and GLUT2); and changes in energy metabolism status (AMP-activated protein kinase) (Castro-Acosta, Lenihan-Geels, Corpe, & Hall, 2016; Edirisinghe & Burton-Freeman, 2016). Furthermore, anthocyanins have a wide range of health benefits for the human body such as antioxidant, anticancer, anti-cardiovascular disease, and hepatoprotective activity (Hu, Zheng, Li, & Suo, 2014).

Consumers may have a preference towards natural pigments versus synthetic colorants due to their perception of being a healthier and safer option. Besides, anthocyanins exert a wide range of colors and hydro-solubility, making them an important alternative as a food pigment. Also, these anthocyanins could promote important health benefits when consumed. Therefore, the objective of this study was to optimize the extraction conditions of anthocyanins from black bean coats, evaluate their shelf-life and thermal stability at different pHs and temperatures and evaluate their antidiabetes potential.

2. Materials and methods

2.1. Materials

Black bean (*Phaseolus vulgaris* L.) "Negro Otomi" cultivar was obtained from INIFAP research center in Mexico. The 7-up cherry beverage (Dr. Pepper Snapple Group) contained artificial flavors and red 40 among other ingredients. Chemicals used for extraction were all ACS grade and purchased from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA). All solvents for chromatographic techniques were of HPLC-grade. For sample preparation, five kg of black beans were soaked in drinking potable water (1:2 beans/water ratio), at room temperature for 16 h; the hulls (coats) were manually removed from cotyledons and dried at 50 °C in a conventional oven, ground in a commercial blender, sieved in mesh 40 (Advantech, USA standard testing sieve), mean particle size 0.420 mm and stored in a double plastic bag at 4 °C until analysis (not more than one month). The yield obtained was 100 g of coats per kg of processed beans. The cotyledons were used for the production of bioactive peptides (Mojica, Gonzalez de Mejia, Granados-Silvestre, & Menjivar, 2017). Enzymes human dipeptidyl peptidase IV (EC 3.4.14.5), α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), α-amylase (EC 3.2.1.1), acarbose, phloretin, and sitagliptin were purchased from Sigma-Aldrich (St. Louis, MO). DPPIV-GLO® protease assay kit was purchased from Promega (Madison, WI). 2-NBDG (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose) was purchased from ThermoFisher (Carlsbad, CA). Human colon epithelial cells Caco-2 [Caco2] (ATCC®HTB-37], Eagle's Minimum Essential Medium (EMEM), and 0.25% (w/v) trypsin-0.53 mM EDTA were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Penicillin-streptomycin was purchased from Corning Inc. (Corning, NY, USA). Fetal bovine serum (FBS) was purchased from Hyclone (Thermo Scientific Hyclone, Logan, UT, USA).

2.2. Physicochemical stability

2.2.1. Optimization of extraction of anthocyanins from bean coat by response surface methodology (RSM)

Anthocyanins and total polyphenols were extracted from bean coats using either only water or two different concentrations of ethanol (0, 12.5 and 25%) in acidified water with 2% formic acid (pH = 2.0). Extractions were performed by stirring coat beans and the respective solution at 600 rpm for two h at different temperatures (4, 22 and 40 °C), and different solid-to-liquid ratios (1:30, 1:40 and 1:50). After extraction, the mixtures were filtered using Whatman No. 1 filter paper. All extracts were immediately analyzed for total monomeric anthocyanins, color, and total polyphenols.

Response surface methodology was used to optimize the extraction of anthocyanin and polyphenols by using functional relationships between the dependent variable and the independent variables as previously reported (Khuri & Mukhopadhyay, 2010). Factorial 3^3 experimental design was used with three independent randomized replications. Ethanol concentration (x1), solid-to-liquid- ratio (x2) and extraction temperature (x3) were chosen for independent variables. The range and center point values with actual and coded values of variables used for the optimization of anthocyanins and total polyphenols extraction from black bean coat were coded levels −1, 0, +1, for ethanol concentration (x1, %), 0, 12.5 and 25; for solid-to-liquid ratio (x2, mL/g), 1:30, 1:40 and 1:50; and for extraction temperature (x3, °C), 4, 22 and 40. Anthocyanin concentration and total polyphenols were selected as the responses for the combination of the independent variables as presented in Table 1. The variables were coded according to the following equation:

$$x = \frac{x_i - x_o}{\Delta x}$$

Where x is the coded value; x_i, the corresponding actual value; x_0, the actual value at the center of the domain; and Δx, the increment of x_i corresponding to a variation of 1 unit of x. The polynomial second-degree equation is described below:

$$y = b_0 + \sum_{i=1}^{3} b_i x_i + \sum_{i=1}^{3} b_{ii} x_i^2 + + \sum_{i=1}^{2} \sum_{j=i+1}^{3} b_{ij} x_i x_j$$

2.2.2. Anthocyanins extraction and measurement of total anthocyanin concentration

Anthocyanin-rich extracts (AE) were obtained from black bean coats using the parameters found from the optimization analysis previously mentioned. Three consecutive extractions were performed to evaluate the percent recovery of anthocyanins and polyphenols after extraction with 24% ethanol in acidified water (2% formic acid), 1:40 solid-to-liquid ratio and 29 °C during two h. Ethanol was removed using a rotary vacuum evaporator at

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