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Analytical Methods

Polyphenol extraction optimisation from Ceylon gooseberry (*Dovyalis hebecarpa*) pulp

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ABSTRACT

Originally from Asia, *Dovyalis hebecarpa* is a dark purple/red exotic berry now also produced in Brazil. However, no reports were found in the literature about phenolic extraction or characterisation of this berry. In this study we evaluate the extraction optimisation of anthocyanins and total phenolics in *D. hebecarpa* berries aiming at the development of a simple and mild analytical technique. Multivariate analysis was used to optimise the extraction variables (ethanol:water:acetone solvent proportions, times, and acid concentrations) at different levels. Acetone/water (20/80 v/v) gave the highest anthocyanin extraction yield, but pure water and different proportions of acetone/water or acetone/ethanol/water (with >50% of water) were also effective. Neither acid concentration nor time had a significant effect on extraction efficiency allowing to fix the recommended parameters at the lowest values tested (0.35% formic acid v/v, and 17.6 min). Under optimised conditions, extraction efficiencies were increased by 31.5% and 11% for anthocyanin and total phenolics, respectively as compared to traditional methods that use more solvent and time. Thus, the optimised methodology increased yields being less hazardous and time consuming than traditional methods. Finally, freeze-dried *D. hebecarpa* showed high content of target phytochemicals (319 mg/100 g and 1421 mg/100 g of total anthocyanin and total phenolic content, respectively).

1. Introduction

Ceylon gooseberry or *Ketembilla* (*Dovyalis hebecarpa*, *Salicaceae* family), originally from Sri Lanka and introduced into USA (Florida) in 1920, is a small spherical fruit (0.5–1 inch) characterised by a deep purple-red colour, sour juice, and small seeds enclosed in the pulp. Fruits can be consumed as jams, juice, or fresh after skin removal. Despite an attractive appearance, skins are unpleasant in the mouth due to a velvety texture and could be considered co-product (Morton & Dowling, 1987). Recently, it is being cultivated as an exotic fruit in the southwest regions of Brazil. High production is obtained from March to May, but it is cultivated until August with satisfactory harvesting yields.

A hybrid of *D. hebecarpa* with *Dovyalis abyssinica* was reported as a source of vitamin C (120.3 mg/100 g of fresh fruit) and with good physical quality for market, showing an average of 75% pulp (Cavalcante & Martins, 2005). Phenolic composition of this hybrid revealed higher contents of anthocyanins in fruit peels and carotenoids in the pulp (De Rosso & Mercadante, 2007). Nevertheless, no reports were found about extraction and phytochemicals content in *D. hebecarpa* species.

Phenolic compounds are secondary metabolites in fruits and vegetables acting as a defense barrier against microorganism, insects, and UV radiation, or as attractants to promote pollination and seed dispersal. Some phytochemicals, as anthocyanins and phenolic acids, are very important for food acceptance acting as

Abbreviations: SLD, simplex lattice design; RSM, response surface methodology; TMA, total monomeric anthocyanin; TPC, total phenolic content; GAE, gallic acid equivalent; CGE, cyanidin-3-glucoside equivalent.

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natural pigments or adding specific flavours, such as astringency and acidity (Shahidi & Naczk, 2003; Lattanzio, Kroon, Quideau, & Treutter, 2009).

Potential health benefits of phenolic compounds in the human diet have been extensively discussed in the literature and are still being investigated mainly linked to antioxidant activity and degenerative disease prevention (Zafra-Stone et al., 2007). The biological activity of berry phytochemicals is believed to be a result of multiple mechanisms that initially were believed to be mainly linked to antioxidant effects. However, nowadays the questionable polyphenols bioavailability has given rise to the discussion of different mechanisms by which it could act in the onset and in the development of degenerative diseases. Some of them are their anti-inflammatory activity, their enzyme inhibition capacity, or their potential modulation of the gut microbiota (Chiva-Blanch & Visioli, 2012).

Anthocyanins are glycosylated forms of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium, acylated or not, which are found as red to purple natural pigments in fruit and vegetables. Usually, extraction procedures are conducted using acidified solvents due to the higher anthocyanin stability at low pH values (Giusti & Jing, 2008). However, degradation of native chemical structure due to acid hydrolysis or oxidation can occur if the acid concentration is high or if the extraction time is long (Naczk & Shahidi, 2006; Revilla, Ryan, & Martín-Ortega, 1998). Thus, extraction conditions should be studied to ensure high extraction yields and minimal degradation in native chemical structure of these pigments. Finally, results obtained under optimised extraction conditions will better represent the real composition of the vegetable matrix being used for characterisation studies.

Aiming to develop efficient and fast new extraction techniques, sonication and subcritical fluids have been tested with promising results (Adil, Cetin, Yener, & Bayındırlı, 2007). However, these fluids increase the extraction costs and require equipment that is not typically present in most laboratory facilities. Moreover, in some cases, traditional methods still give better yields. Previous literature data revealed that solvent extraction showed higher results than an optimised subcritical fluid method (CO_2 plus ethanol) in apple and peach pomace for total phenolics and antioxidant activity (Adil et al., 2007). Nevertheless, the presence of even relatively safer solvents (such as alcohol) in the composition of natural colorants may limit its application in food. Thus, even that solvent extraction generates reliable results and satisfactory yields it should be optimised aiming the reduction or depletion of organic dissolvent agents. In addition, most extraction procedures were not optimised or validated and few details are reported about the selection of extraction variables. Considering it, anthocyanin and phenolic extraction could be one of the sources of high variation between reported values in a same plant material.

Furthermore, anthocyanins can be present in different compartments in the plant tissue, including vacuoles or in vacuolar inclusions. Thus, depending on the specific tissue characteristics, the optimum conditions for extraction to maximise yields may vary (Giusti & Jing, 2008).

Simplex lattice and central composite design are two multivariate experimental designs that allow investigating the effect of a mixture or a set of factors under one or more responses. The advantage of these techniques is that it allows the researcher to obtain knowledge of the whole system behaviour inside of the ranges, to evaluate simultaneously multiple variables, and to predict results using statically valid models (Ferreira et al., 2007). There is a wide range of applications of this statistical tool in the literature from bioprocesses in enzyme production (Ries & Alves Macedo, 2011) to extraction conditions for phenolics (Cacace & Mazza, 2002; Monrad, Srinivas, Howard, & King, 2012).

Due to the increasing need for efficient, simple, and mild extraction procedures for anthocyanins and other phenolics to allow its further evaluation and characterisation, this work was focused on optimising extraction methods for these types of compounds from *D. hebecarpa* pulp. Within this study, multivariate experimental designs were used to develop procedures investigating extraction variables to improve knowledge in this field.

2. Material and methods

2.1. Chemicals and equipment

The phenol reagent Folin–Ciocalteu and gallic acid were obtained from Sigma–Aldrich. A Büchi rotary evaporator and an UV-1600 spectrophotometer from Proanálise (São Paulo, Brazil) were used for concentration and quantification purposes. A food processor (Philips Mini Food Processor, model HR7625) Terroni Freeze-dryer, model LS-3000E (São Carlos, Brazil) and a Qhimis analytical grinder, model Q298A (São Paulo, Brazil) were used for sample preparation.

2.2. Sample preparation

Ripened samples (12.5 °Brix) were obtained from Bragança Paulista city (São Paulo, Brazil) in April of 2010 (see Supplementary material for fruit images). After harvesting, fruits were manually peeled while frozen (−20 °C) to minimise enzymatic degradation and decrease juice loss. Frozen pulps were crushed using a food processor and placed into trays to re-freeze. Materials were then freeze-dried until the pressure was reduced to stable values lower than 22μHg. Since particle size is extremely relevant to extraction effectiveness, freeze-dried samples were grinded until a fine and visually homogenous powder was obtained.

2.3. Initial extraction procedure

A water/acetone (25:75 v/v) mixture, acidified with 2% of formic acid was the initial solvent tested (Mertz, Cheynier, Günata, & Brat, 2007). Freeze-dried powder samples (0.5 g) were mixed with the extraction solvent (15 ml) in a ratio of 1:30 w/v for 15 min, filtered and re-extracted under the same conditions. Both filtrates were combined, taken to a volume of 100 ml in a volumetric flask, and analysed for total phenolic and monomeric anthocyanins using the methods described below.

2.4. Extraction optimisation

2.4.1. Selection of the solid-to-liquid ratio

Solid–liquid ratios of 1:30; 1:60, 1:90, 1:120, 1:200 were studied in order to investigate the amount of solvent need to maximise the yield of anthocyanin and phenolic compounds extraction. Freeze-dried pulp powder (0.5 g) was extracted under agitation for 1 h with 75% aqueous acetone solution containing 2% formic acid in the different solid–liquid ratios chosen. Extraction media was filtered under vacuum, solids were discarded, and the filtrate was taken to an exact volume (100 mL) with the extraction solvent. The number of extractions was restricted to only one to avoid loses during the filtration process that could jumble the results. The solid–liquid ratio with highest extraction yield of anthocyanin and total phenolic compounds content was fixed for the next experimental step in the optimisation process. Three independent replications were performed and analysed in triplicate. ANOVA tests and means comparisons using Tukey test ($p \leqslant 0.05$) were performed in Statistica 7.0 (StatSoft, Inc.).