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Analytical Methods

Optimisation of the extraction of phenolic compounds from apples using response surface methodology

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ABSTRACT

The extraction of phenolic compounds from apples was optimised using response surface methodology (RSM). A Box–Behnken design was conducted to analyse the effects of solvent concentration (methanol or acetone), temperature and time on the extraction of total phenolic content, total flavonoids and antioxidant capacity (FRAP and DPPH). Analysis of the individual phenolics was performed by HPLC in optimal extraction conditions. The optimisation suggested that extraction with 84.5% methanol for 15 min, at 28 °C and extraction with 65% acetone for 20 min, at 10 °Cwere the best solutions for this combination of variables. RSM was shown to be an adequate approach for modelling the extraction of phenolic compounds from apples. Most of the experiments with acetone solutions extracted more bioactive compounds, and hence they had more antioxidant capacity, however, chlorogenic acid and phloridzin had higher yields (32.4% and 48.4%, respectively) in extraction with methanol.

1. Introduction

Apples are the second most important fruit in the world (70 million tons) and are produced in temperate climate countries (Tropics of Cancer and Capricorn). They are consumed throughout the year in most countries of the world, not only for their organoleptic qualities, but also due to technological advancements in area of conservation (Braga et al., 2013).

Apples and their products contain significant amounts of phenolic compounds (Khanizadeh et al., 2008), which play an important role in maintaining human health, since they have a preventive effect against various types of diseases such as cancer, cardiovascular diseases, neuropathies and diabetes (Shahidi, 2012). Chlorogenic acid and *p*-coumaroylquinic acid are the main phenolic acids found in apples; epicatechin, catechin, procyanidins (B1 and B2), quercetins glycosides, anthocyanins and phloridzin are the major flavonoids (Khanizadeh et al., 2008; Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005). Tsao et al. (2005) reported that among the main phenols found in apples, cyanidin-3-galactoside and procyanidins have antioxidant activity three times higher and twice as high, respectively, than epicatechin and glycosides of quercitins.

There is growing interest in the study of these bioactive compounds (Kchaou, Abbès, Blecker, Attia, & Besbes, 2013; Spigno, Tramelli, & De Faveri, 2007; Wijekoon, Bhat, & Karim, 2011), and for this purpose, the first step is extracting them from the vacuolar structures and other tissues where they are found (Wink, 1997). The extraction conditions may not be the same for different plant materials since they are influenced by several parameters, such as the chemical nature of the sample, the solvent used, agitation, extraction time, solute/solvent ratio and temperature (Haminiuk, Maciel, Plata-Oviedo, & Peralta, 2012; Luthria, 2008). In addition, the oxidation of phenolic compounds should be avoided, since they are involved in the enzymatic browning reaction and consequently lose their phenol function and antioxidant capacity (Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994). It is advisable to use dry, frozen or lyophilised samples to avoid enzyme action (Escribano-Bailón & Santos-Buelga, 2004).

The optimisation of the extraction of phenolic compounds is essential to reach an accurate analysis. Response surface methodology (RSM) is an effective tool for optimising this process. Moreover, it is a method for developing, improving and optimising processes, and it can evaluate the effect of the variables and their interactions (Farris & Piergiovanni, 2009; Wettasinghe & Shahidi, 1999).

Thus, this study aimed to evaluate the effect of concentrations of the solvents, methanol and acetone, time and temperature on

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the extraction of apple phenolic compounds and their antioxidant capacity using RSM as the optimisation technique.

2. Materials and methods

2.1. Materials

Gala apples (10 kg) used in the experiments were obtained in the city of Ponta Grossa (25° 05′ 42″ S 50° 09′ 43″ O), Paraná, Brazil.

The reagents Folin–Ciocalteau, Trolox (6-hydroxy-2,5,7,8-tetremethychroman-2-carboxylic acid), TPTZ (2,4,6-Tri (2-pyridyl)-s-triazine), DPPH (2,2-diphenyl-2-picrylhydrazyl), chlorogenic acid, *p*-coumaric acid, phloridzin, phloretin, (+)-catechin, (-)-epicatechin, procyanidin B1, procyanidin B2, quercetin, quercetin-3-D-galactoside, quercetin-3-β-D-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-rutinoside, caffeic acid and gallic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol, acetone, acetic acid and acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ, USA) and sodium nitrite and aluminium chloride from Vetec (Rio de Janeiro, RJ, Brazil) and Fluka (St. Louis, MO, USA), respectively. The liquid nitrogen (99%) used was produced with StirLIN-1 (Stirling Cryogenics, Dwarka, New Delhi, India). The aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore, São Paulo, SP, Brazil).

2.2. Methods

2.2.1. Extraction of phenolic compounds

The apples were fragmented in a microprocessor (Metvisa, Brusque, SC, Brazil), immediately frozen with liquid nitrogen (1:2, w/v) to avoid the oxidation of the phenolic compounds (Guyot, Marnet, Sanoner, & Drilleau, 2001), and lyophilised (LD 1500, Terroni, São Paulo, SP, Brazil). The freeze-dried material (without seeds) was homogenised by crushing in a mortar. 1 g of the crushed apple was extracted with 60 mL of methanol or acetone in different concentrations, followed by incubation at different temperatures and times (Table 1). Then, the mixture was centrifuged (8160*g*, 20 min at 4 °C) (HIMAC CR-GII, Hitachi, Ibaraki, Japan), concentrated by evaporation under vacuum (40 °C) in a rotary evaporator (Tecnal TE-211, Piracicaba, SP, Brazil), and freeze-dried. The samples were reconstituted with 2 mL of 2.5% acetic acid and methanol (3:1, v/v) and filtered through a 0.22 μm (Nylon) syringe filter (Waters, Milford, MA, USA) prior to analysis.

2.2.2. Total phenolic content (TPC)

The total phenolic content (TPC) was determined by colorimetric analysis using Folin–Ciocalteau reagent, as described by Singleton and Rossi (1965). In a test tube, 8.4 mL of distilled water, 100 μL of sample, and 500 μL of Folin–Ciocalteau reagent were added. After 3 min, 1.0 mL of 20% sodium carbonate was added into each tube, which was agitated in a vortex (Vision Scientific CO. LTD., Korea). After 1 h, the absorbance (720 nm) was measured by spectrophotometer (model Mini UV 1240, Shimadzu, Kyoto, Japan). The measurement was compared to a calibration curve of chlorogenic acid [total phenolic concentration = 1473.3 × absorbance; $R^2 = 0.998$; $p < 0.001$] and the results were expressed as milligrams of chlorogenic acid equivalents (CAE) per kilogram of apple [mg CAE/100 g].

2.2.3. Total flavonoid content (TFC)

The total flavonoid content (TFC) of the phenolic extracts was determined using a method described by Zhishen, Mengcheng, and Jianming (1999) with modifications. 250 μL of the samples was mixed with 2.72 mL of ethanol (30%, v/v) and 120 μL of sodium nitrite solution (0.5 mol/L). After 5 min, 120 μL of aluminum chloride (0.3 mol/L) was added. The mixture was stirred and was allowed to react for 5 min. Then, 800 μL of sodium hydroxide (1 mol/L) was added and the absorbance was measured at 510 nm using a spectrophotometer (model Mini UV 1240, Shimadzu, Kyoto, Japan). The measurement was compared to a calibration curve of catechin (CT) [flavonoid concentration = 755.37 × absorbance; $R^2 = 0.996$; $p < 0.001$] and the results were expressed as milligrams of catechin equivalents (CTE) per kilogram of apple [mg CTE/100 g].

2.2.4. Measurement of in vitro antioxidant capacity

Free-radical scavenging activity of the extracts was determined in triplicate by the DPPH assay according to the Brand-Williams method, Brand-Williams, Cuvelier, and Berset (1995) with minor adaptations. This method determines the hydrogen donating capacity of molecules and does not produce oxidative chain reactions or react with free radical intermediates. Diluted samples (100 μL) were mixed with 3.9 mL of 60 μmol/L methanolic DPPH.

Table 1
Box–Behnken design applied for apple phenolic compounds extraction.

Run	Factors		
	Time (min)	Temperature (°C)	Solvent concentration (%)
1	−1	−1	0
2	+1	−1	0
3	−1	+1	0
4	+1	+1	0
5	−1	0	−1
6	+1	0	−1
7	−1	0	+1
8	+1	0	1
9	0	−1	−1
10	0	+1	−1
11	0	−1	+1
12	0	+1	+1
13	0	0	0
14	0	0	0
15	0	0	0
True values[a]			Methanol / Acetone
−1	10	10	70 / 50
0	15	25	85 / 65
+1	20	40	99.9 / 80

[a] Values adopted for each factor in the phenolic extraction experiment.