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Authentication of juices from antioxidant and chemical perspectives: A feasibility quality control study using chemometrics

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ABSTRACT

In this work, Brazilian juices (n = 38) from distinct botanical species were analyzed for the physicochemical properties, major phenolic classes, and antioxidant activity using high-throughput assays. Principal component analysis [PCA] was applied to study the data structure, while classification methods based on partial least squares-discriminant analysis [PLS-DA] and dual data-driven PCA/soft independent modeling of class analogy [DD-SIMCA] were used to predict the class membership of juices. In addition, multiple linear regression [MLR] models were proposed to explain the antioxidant activity of juices. PLS-DA was successfully used to authenticate the class membership of juices, enabling the identification of the main variables responsible for the discrimination. Similarly, DD-SIMCA was shown to be useful for the authentication of juices. Additionally, the main phenolic classes responsible for each of the antioxidant activity were revealed by MLR. Overall, the characterization of juices was reached by the application of relatively simple analytical methods supplemented with modern chemometric tools.

1. Introduction

In 2013, Brazil produced a total of 43.6 million tons of fruits, in which about 23.8 million tons were used to produce juices, nectars, and pulps. According to the European Fruit Juice Association (AIJN, 2014), the consumption of the fruit juices and nectars in Europe reached 10 billion liters, while fruit juices and nectars accounted for 38.9 billion liters in 2014, generating more than €94 billion. From this amount, Latin America and North America held 9% and 25% of the production, respectively, while EU countries accounted for 26%. The orange and other citric juices (*i.e.,* tangerine/mandarins, lemon/limes) represent about 40% of the worldwide share, while the juice blends (*i.e.,* berries, passion fruit, and peach) and the apple juices gather 33% of the share. Brazil plays an important role in manufacturing of *Citrus*, tropical and apple juices. Development of juice blends is a common practice, but nowadays we observe a new trend in retail stores, the so-called *super juices,* which are juices made from berries and combinations with some vegetables, such as red beet, pomegranate, and cranberry. These products are regarded as healthy because of a high purported nutritive value and high content of bioactive compounds (Medina, 2011).

When the quality of juices is analyzed, many parameters can be determined, such as carotenoids, phenolic compounds, volatile organic compounds, organic acids and sugars, antioxidant activity, physical properties (rheological parameters, color), among others (Jandrić & Cannavan, 2016; Meléndez-Martínez, Vicario, & Heredia, 2007; Spinelli et al., 2016). In a general view, multiple analytical markers can be analyzed and for a large amount of juices, producing a complex data set. Aiming to increase the understanding of the interconnection between intrinsic characteristics of fruits and their technological products, as well as the effects of ripeness, farming system, year of cultivation, and other agronomical factors, multivariate statistical methods are successfully applied in food science and technology (Marseglia et al., 2016; Nyarko, Puzey, & Donnelly, 2014). In addition, these statistical tools are also used to characterize juices the multiple botanical and geographical origin of the juices. Thus, statistical analysis is, *de fato*, an essential tool for quality control purposes (Tassoni, Tango, & Ferri, 2013; Zhao et al., 2016).

Although high-performance liquid chromatography (HPLC coupled or not with mass spectrometry) of individual compounds (*i.e.*, phenolics, pigments, and organic acids) is very reliable and

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well established, it is known that not all food companies perform the quality control based on the use of HPLC (Jandrić & Cannavan, 2016). Therefore, application of alternative methods for the routine analysis of physicochemical parameters, major phenolic classes, and antioxidant activity measured by high-throughput assays can be a low-cost and rapid alternative. Additionally, as Brazilian juices are exported to many countries worldwide, it is important to monitor their authenticity and quality by measuring their chemical composition, physicochemical properties, and antioxidant activity. Therefore, the objectives of this study are to characterize Brazilian juices coming from different species and to classify these juices based on physicochemical and chemical data. The characterization of samples should be reached by the application of relatively simple analytical methods supplemented with modern multivariate data processing.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu's phenol reagent, tripyridyl-2,4,6-*s*-triazine (TPTZ), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid, sodium molybdate dihydrate, vanillin, (+)-catechin, chlorogenic acid, 3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid disodium salt (ferrozine), quercetin, ferric chloride hexahydrate, and ascorbic acid were obtained from Sigma (St. Louis, MO, USA), whereas $K_3[Fe(CN)_6]$, was obtained from Merck (Germany). All the other reagents used in the experiments were of analytical grade and ultrapure water (Millipore, São Paulo, Brazil) was used.

2.2. Juice samples

In this study, a total of n = 38 samples of juices from different botanical species were either acquired from local shops (Ponta Grossa, PR, Brazil) or squeezed in the laboratory. There are n = 14 orange juices (*Citrus sinensis*), in which n = 8 were commercial 100% juice, n = 1 organic orange juice, and n = 1 orange cv. Bahia, n = 1 orange cv. Pera Rio, n = 2 orange cv. Lime, n = 1 orange cv. Rosada, n = 3 lemon juices (*Citrus limon*) from three different varieties (cv. Cravo, cv. Taiti, and cv. Galego), n = 3 tangerine juices (*Citrus reticulata*), in which two tangerine samples were organic (certified by IBD, Brazil). The commercial *Citrus* samples referred to pasteurized juices (no additives added) marketed in different plastic bottles, while the other *Citrus* juices were squeezed in the laboratory (no thermal process was applied) and centrifuged at 740 *g* for 5 min. The sample set also consisted of n = 1 yellow passion fruit juice (*Passiflora edulis;* pasteurized), n = 6 apple juices (*Pyrus malus;* 2 and 4 freshly-squeezed and centrifuged pasteurized), in which one was organic (certified by IBD; freshly-squeezed and centrifuged), n = 4 pomegranate nectars (*Punica granatum;* sterilized) in which 3 contained 35% pulp and one contained 11%, n = 2 cranberry nectars containing 21.5% fruit pulp (*Vaccinium macrocarpon;* sterilized), n = 2 blackberry juices (*Morus nigra;* 1 pasteurized and 1 freshly-squeezed and centrifuged), n = 2 blueberry (*Vaccinium* sp; pasteurized), and n = 1 red beet juice (*Beta vulgaris;* freshly prepared by cooking 1 kg of roots at 121 °C/15 min and then the content was processed using a juicer machine and centrifuged) was also used for comparison purposes.

2.3. Physicochemical analysis

The pH at 25 °C was measured according to the AOAC (2005) using a pH meter with a previously calibrated electrode. The titratable acidity was determined by potentiometric titration and expressed as g/100 mL (AOAC, 2005) and the total soluble solids content (TSS) was estimated using a refractometer (model Atago N-1α, Japan) according to the AOAC (2005) and the results were expressed as °Brix.

2.4. Chemical composition (phenolic classes)

Total phenolic content of juices was determined using the Prussian Blue assay as described by Margraf, Karnopp, Rosso, and Granato (2015) and results were expressed as mg gallic acid equivalent/L. Total *ortho*-diphenols content was estimated by the colorimetric method that uses sodium molybdate diluted in water and ethyl alcohol (1:1 v/v) (Durán, Padilla, Martín, Fiestas Ros de Ursinos, & Mendoza, 1991) and results were expressed as mg chlorogenic acid equivalent/L. The total flavonoid content was determined using the colorimetric method described by Herald, Gadgil, and Tilley (2012) and results were expressed as mg (+)-catechin equivalent/L. Condensed tannins content was estimated using the method that employs the reaction of vanillin and condensed tannins in acidic medium (Horszwald & Andlauer, 2011) and results were expressed as mg (+)-catechin equivalent/L. The total flavonols content was estimated using the $AlCl_3$ method outlined by Yermakov, Arasimov, and Yarosh (1987) and results were expressed as mg quercetin equivalent/L. The content of monomeric anthocyanins (apple, blackberry, cranberry, pomegranate, and blueberry juices) was determined by UV–Vis spectrophotometry (λ = 520 nm and λ = 700 nm) using the differential pH method as described by Lee, Durst, and Wrolstadt (2005) and expressed as mg/L. Betalains were quantified in red beet juice in aqueous medium using the colorimetric method described by Stintzing, Schieber, and Carle (2003). Absorbance values were recorded at λ = 485 nm (betaxantins) and λ = 536 nm (betacyanins) and total betalains content was expressed as the sum of betaxantins and betacyanins (mg/L). Results of anthocyanins and betalains were expressed as total pigments (mg/L). Details on these spectrophotometric methods are fully explained by Granato, Santos, Maciel, and Nunes (2016).

2.5. In vitro antioxidant activity

The free radical scavenging activity toward DPPH was quantified using the conditions described by Granato, Karnopp, and van Ruth (2015). The assay was conducted at pH 6.0 using 50 mmol/L sodium phosphate and ethyl alcohol at a 1:1 (v/v) proportion as solvents of the DPPH radical (0.10 mmol/L) (Zheng, Lin, Su, Zhao, & Zhao, 2015). Results were expressed as mg ascorbic acid equivalent/L. The scavenging of $ABTS^{\bullet+}$ was determined as described by Re et al. (1999) and the values were expressed as mg ascorbic acid equivalent/L.

The Fe^{2+} chelating ability of juices was assessed using a spectrophotometric assay that employs ferrozine and $FeSO_4$ using a protocol proposed by Santos, Brizola, and Granato (2017). Ultrapure water was used as control and the percentage of Fe^{2+}-ferrozine complex formation was calculated as: Fe^{2+} chelating rate (%) = $[(Abs_{sample} - Abs_{solution\ without\ ferrozine})/Abs_{control}] \times 100$ and an analytical curve using different EDTA-Na_2 concentrations was plotted. Results were expressed as mg EDTA equivalents/L.

The Folin–Ciocalteu's reducing capacity of juices was assessed using the modified Folin–Ciocalteu assay and results were expressed as mg gallic acid equivalent/L (Singleton, Orthofer, & Lamuela-Raventos, 1999). Ferric reducing antioxidant power (FRAP) of juices was determined according to the method described by Benzie and Strain (1996) and results were expressed as mg ascorbic acid equivalent/L. Total reducing capacity (TRC) was used to measure the potential of both water-soluble and lipophilic