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Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits

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ABSTRACT

This study aimed to evaluate the chemical composition, identify the bioactive compounds and measure the antioxidant activity present in blackberry, red raspberry, strawberry, sweet cherry and blueberry fruits produced in the subtropical areas of Brazil and to verify that the chemical properties of these fruit are similar when compared to the temperate production zones. Compared with berries and cherries grown in temperate climates, the centesimal composition and physical chemical characteristics found in the Brazilian berries and cherries are in agreement with data from the literature. For the mineral composition, the analyzed fruits presented lower concentrations of P, K, Ca, Mg and Zn and higher levels of Fe. The values found for the bioactive compounds generally fit the ranges reported in the literature with minor differences. The greatest difference was found in relation to ascorbic acid, as all fruits analyzed showed levels well above those found in the literature.

1. Introduction

Berry fruits, are small fleshy fruits, which are commercially cultivated and commonly consumed in fresh and processed forms, include blackberry (*Rubus* spp.), black raspberry (*Rubus occidentalis*), red raspberry (*Rubus idaeus*), blueberry (*Vaccinium corymbosum*) and strawberry (*Fragaria* × *ananassa*) (Seeram, 2008).

Berries are rich in phenolic compounds, such as phenolic acids, tannins, stilbenes, flavonoids and anthocyanins, but berries, in particular, have been the focus of considerable research regarding their anthocyanin-rich properties and according to Seeram (2008), there are many studies claim that that the dietary intake of berry fruits has a positive and profound impact on human health, performance, and disease.

Although it is already well established that berries and cherries are sources of bioactive compounds such as polyphenols and anthocyanins, these studies focused mainly on berries from temperate climates, mainly in the temperate regions of Europe, Asia and North America (Chen, Xin, Zhang, & Yuan, 2013). Knowing that the composition of the fruits varies with a series of factors that includes species, variety, cultivation, region, weather conditions, ripeness, time of harvest and storage conditions (Faniadis, Drogoudi, & Vasilakakis, 2010; Haffner, Rosenfeld, Skrede, & Wang, 2002), is extremely relevant for the characterization and comparison of berries produced in tropical and subtropical climates with traditional berries from a temperate climate.

The raspberry and blackberry cultivation in Brazil has been increasing steadily, especially in the subtropical areas where temperatures are higher in the fall and winter and especially higher in the summer, and previous results show that blackberry plants produce large quantities of fruit in subtropical areas, with some varieties producing higher amounts compared to temperate zones (Campagnolo & Pio, 2012). For raspberries, the productive performance results of the subtropical areas in Brazil are very encouraging because the production of raspberries is constant throughout

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the year with certain cultivars producing large quantities of fruit in the fall and winter (Moura et al., 2012). Thus, the determination of the nutritional composition of the berries and cherries produced in Brazilian subtropical zones is important to know the nutritional and functional properties and to verify that the chemical properties of the fruit are similar when compared to the temperate production zones.

To this end, the aims of the present study were to evaluate the chemical composition, identify the bioactive compounds and measure the antioxidant activity present in blackberry (*Rubus* spp.), red raspberry (*R. idaeus*), strawberry (*Fragaria* × *ananassa*), sweet cherry (*Prunus avium* L.) and blueberry (*V. corymbosum*) fruits produced in the subtropical areas of the states of Minas Gerais and São Paulo, Brazil.

2. Materials and methods

2.1. Fruit samples

The blackberry, red raspberry and strawberry plants were acquired from the south of Minas Gerais, whereas the blueberry and cherry plants were acquired from a producer in São Paulo. The fruits were harvested at their physiological maturity in the morning and transported in Styrofoam boxes to the post-harvest fruit and vegetable laboratory of the Universidade Federal de Lavras. Upon delivery, the fruits were sanitized, and all fruits were stored in a cold room at −18 °C during the analysis time.

2.2. Chemical reagents

The following chemicals were used for the experiments described later: acetone, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), aluminium chloride ($AlCl_3$), β-carotene, (+)-catechin, hydrochloric acid (HCl), 2,4-dinitrophenylhydrazine (2,4-DNPH), chloroform, copper sulphate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethanol, ethyl ether, Folin–Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Kjeldahl reagent, linoleic acid, methanol, nitric acid, perchloric acid, petroleum ether, phenolphthalein solution, phosphate buffer, potassium sulphate, potassium persulphate, sodium carbonate, sodium nitrate ($NaNO_3$), sodium hydroxide (NaOH), sulphuric acid and Tween 40 as well as the thermostable alpha-amylase, protease, and amyloglucosidase enzymes.

2.3. Chemical analyses

Three repetitions were performed for all chemical analyses. The values of the titratable acid, total soluble solids, total sugar, pH, moisture, ash, protein, lipid, carbohydrate and total dietary fibre contents were determined (AOAC—Association of Official Analytical Chemists, 1998).

2.4. Minerals

The mineral levels were assessed in crushed and homogenized samples prepared by organic wet digestion in accordance with the methodology described by Salinas and Garcia (1985). For organic digestion, the samples were treated with a mixture of concentrated nitric and perchloric acids at a high temperature. The macro- and microelements were solubilized, subjected to different treatments and diluted for further quantitative evaluation. The quantification of elements was performed by spectrophotometry using a standard curve for each mineral. To determine the concentration of calcium, iron and manganese, we used an atomic absorption spectrophotometer and acetylene. A flame photometer was used to determine potassium (768 nm), and a visible-light spectrophotometer was used to determine phosphorus (420 nm).

2.5. Preparation of antioxidant and phenolic extracts

The extracts were obtained according to the method described by Larrauri, Ruperez, and Saura-Calixto (1997). Briefly, samples were weighed (in grams) in centrifuge tubes and extracted sequentially with 40 mL of methanol/water (50:50, v/v) at room temperature for 1 h. The tubes were centrifuged at 25,400*g* for 15 min, and the supernatant was recovered. Then, 40 mL of acetone/water (70:30, v/v) was added to the residue at room temperature. The samples were extracted for 60 min and centrifuged. To determine the antioxidant activity as well as total flavonoid, total monomeric anthocyanin and phenolic contents, the methanol and acetone extracts were combined and brought to a final volume of 100 mL with distilled water.

2.5.1. Antioxidant activity

The antioxidant activity was determined using the ABTS, DPPH and β-carotene methods. For the ABTS assay, the procedure followed the method of Re et al. (1999) with minor modifications. The ABTS radical cation (ABTS•+) was generated by the reaction of 5 mL of aqueous ABTS solution (7 mM) with 88 μL of 140 mM (2.45 mM final concentration) potassium persulphate. The mixture was kept in the dark for 16 h before use and then diluted with ethanol to obtain an absorbance of 0.7 ± 0.05 units at 734 nm using a spectrophotometer. The fruit extracts (30 μL) or a reference substance (Trolox) were allowed to react with 3 mL of the resulting blue–green ABTS radical solution in the dark. The decrease of absorbance at 734 nm was measured after 6 min. Ethanolic solutions of known Trolox concentrations were used for calibration. The results are expressed as micromoles of Trolox equivalents (TEs) per gram of fresh weight (μmol of TEs/g of f.w.).

The DPPH free radical-scavenging capacity was estimated using the method of Brand-williams, Cuvelier, and Berset (1995). Briefly, the solution of DPPH (600 μM) was diluted with ethanol to obtain an absorbance of 0.7 ± 0.02 units at 517 nm. The fruit extracts (0.1 mL) were allowed to react with 3.9 mL of the DPPH radical solution for 30 min in the dark, and the decrease in absorbance from the resulting solution was monitored. The absorbance of the reaction mixture was measured at 517 nm. The results were expressed as EC_{50} (g f.w./g of DPPH).

The antioxidant activity was also determined by the β-carotene method, following the procedure described by Marco (1968) with minor modifications. Briefly, an aliquot (50 μL) of the β-carotene chloroform solution (20 mg/mL) was added to a flask containing 40 μL of linoleic acid, 1.0 mL of chloroform, and 530 μL of Tween 40 and then mixed. The chloroform was evaporated using an oxygenator. After the evaporation, oxygenated distilled water (approximately 100 mL) was added to obtain an absorbance of 0.65 ± 0.5 units at 470 nm. An aliquot (0.4 mL) of Trolox solution (200 mg/L) or diluted fruit extract (200 mg/L) was added to 5 mL of the β-carotene solution and incubated in a water bath at 40 °C. The measurements were performed after 2 min and 120 min at an absorbance of 470 nm using a spectrophotometer. The antioxidant activity was calculated as the percent inhibition relative to the control.

2.5.2. Total phenolic content

The total phenolic content was determined according to the adapted Folin–Ciocalteu method (Waterhouse, 2002). The extracts (0.5 mL) were mixed with 2.5 mL of Folin–Ciocalteu reagent (10%) and 2 mL of sodium carbonate solution (4%). The mixture was stirred and kept at room temperature for 2 h in the dark. The absorbance was measured at 750 nm against a blank. Aqueous