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Nutritional characteristics and bioactive compound content of guava purees and their effect on biochemical markers of hyperglycemic and hypercholesterolemic rats

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ABSTRACT

The nutritional characteristics and bioactive compound content in purees elaborated with guava-strawberry, guava-blackberry, guava-soursop or guava-passion fruit were evaluated as well as their effect on biochemical markers of hyperglycemic and hypercholesterolemic rats. Over a 4-week period, the effects of each puree were examined. All purees presented a high content of indigestible fraction (70.6–82.3 g/100 g), vitamin C (500–534.6 mg/100 g), soluble polyphenols (32.8–33 mg/g) and antioxidant capacity. Several phenolic acids and flavonoids were identified. The addition of purees in the diet increased the body weight of hyperglycemic rats (~7%), but decreased the body weight of hypercholesterolemic rats (~15%). All the purees decreased the levels of plasma glucose, urea and creatinine in hyperglycemic rats, as well as the total cholesterol and triacylglycerol levels in hypercholesterolemic rats. The hepatic damage was reduced for all purees. These guava-purees represent a therapeutic alternative for individuals with diet-related diseases problems such as hyperglycemia and hypercholesterolemia.

1. Introduction

Noncommunicable diseases (NCDs) are defined as chronic diseases that are not transmitted from person to person. Alarmingly, these diseases are increasing worldwide, affecting all age groups (WHO, 2015). NCDs include obesity, hyperglycemia, hypercholesterolemia, hyperlipidemia, arteriosclerosis, diabetes mellitus (DM) type II, hypertension and other cardiovascular diseases (WHO, 2015). These metabolic disorders are known as a metabolic syndrome, which increases the morbidity and mortality that along with an increasingly aging society, creates a serious medical and socioeconomic problem (Elleuch et al., 2011). It is known that these pathologies decrease with the consumption of fresh fruits and vegetables due to the significant amounts of vitamins, minerals, dietary fibre, indigestible fraction and bioactive compounds that they have; which turn can control and prevent non-degenerative diseases. Moreover the diets that are rich in these vegetables may cause lower rates of mortality caused by NCDs (Elleuch et al., 2011).

Therefore many fruits and their components (stem, leaves, seeds and by-products) have been investigated to study their effects on health issues that include anti-hyperglycemic, hepatic steatosis, anti-inflammatory, anti-cancer, cardioprotective, anti-obesity, among others. The fruits that have been most studied are: guava (Huang, Yin, & Chiu, 2011; Liu, Wang, Hsieh, Lu, & Chiang, 2015), berries (Afrin et al., 2016; Aqil et al., 2016; Mazzoni et al., 2016), passion fruit (Kandandapani, Balaraman, & Ahamed, 2015) and soursop fruit (Coria-Téllez, Montalvo-Gónzalez, Yahia, & Obledo-Vázquez, 2016). These fruits are widely accepted by the consumers and are an important source of vitamin C, vitamin E, pigments (anthocyanins or carotenoids), dietary fibre (DF) and polyphenols (Huang et al., 2011; Meireles et al., 2015; USDA, 2011).

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While the majority of the available scientific evidence is based on the effects of the intake of fresh fruits or extracts of its components, the consumers increase the demand of elaborated and processed products that are easily accessible, ready to eat and that provide health benefits. The combination of fruits enhance the benefits of a food, providing an increase in the content of macro and micronutrients, DF and a big number of phytochemicals, which individually or combined may have important biological activities that promote health benefits (Swada, Keeley, Ghane, & Engeseth, 2016). The synergistic potential of papaya and strawberry nectar blends has been studied and was demonstrated that the ascorbic acid, carotenoid concentrations and antioxidant capacity were best retained in these blends (Swada et al., 2016). These same authors reported better characteristics of the product than either fruit processed individually. Chavez-Tapia et al. (2016) found that the concentration of guava purees mixed with a *Hibiscus sabdariffa* (Roselle) extract under vacuum pressure had higher sensory quality and conserved their nutrients and bioactive compounds in comparison with a control puree. However the conservation of bioactive compounds in processed guava puree in mixture with exotic fruits has not been characterized neither their effects on biochemical markers have been evaluated. In this work, we evaluated the nutritional characteristics and bioactive compounds of guava puree addionated with strawberry, blackberry, passion fruit or soursop fruit as well as their effects on biochemical markers of hyperglycemic and hypercholesterolemic wistar rats submitted to a diet with these purees.

2. Materials and methods

2.1. Reagents

All chemicals were of HPLC or analytical grade, indigestible fraction (IF) assay kit, pepsin, α-amylase, pancreatin, dinitrophenylhydrazine, Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ), 2 ,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic) acid diammonium salt (ABTS), 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) and the standards were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

2.2. Puree samples

Four purees (Requested patent: MX/a/2015/016803) were elaborated and donated by Purés y Derivados de Nayarit (PDN), Company, Mexico. Briefly, the process is described as in the requested patent: concentrated guava pulp under vacuum pressure was supplemented with 1–25 g of strawberry pulp (guava-strawberry puree, GSP), 1–20 g of blackberry pulp (guava-blackberry pure, GBP), 1–15 g of soursop pulp (guava-soursop puree, GGP) or 1–10 g of passion fruit pulp (guava-passion fruit puree, GPP) for each 100 g of puree. The mixtures were sweetened with 2 g of stevia (97% of purity of rebaudioside A, Metco, Mexico DF, Mexico). The puree samples were vacuum packed in multi-laminated bags of high-density polyethylene (0.940–0.970 $g \cdot cm^{-3}$, Fast Sincere International Industrial, Hong Kong, China) pasteurized and then stored a 4 °C for three weeks. Three bags of 450 g of each puree were freeze-dried (LABCONCO freeze dryer, Model 77522020, Kansas, USA) for their posterior analysis.

2.3. Nutritional composition of purees

Moisture (Method 934.06), protein (Method 920.152), fat (method 948.22) and ash (Method 940.26) were measured according to the AOAC methods (2005). The soluble carbohydrates were determined according to the method of Dubois, Gilles, Hamilton, Rerbers, and Smith (1956). All data were reported in grams per 100 g of dry matter (DM) of puree, except the moisture.

The soluble indigestible fraction (SIF), insoluble indigestible fraction (IIF) and total dietary indigestible fraction (TIF) contents were measured according to Saura-Calixto, García-Alonso, Goni, and Bravo (2000). Briefly, each sample (300 mg) was digested with 0.2 mL of pepsin (300 mg/mL solution in HCl-KCl, buffer, 0.2 M, pH 1.5, 1 h, 40 °C). Then, the mixture was cooled and 1 mL of pancreatin solution (5 mg/mL) in phosphate buffer was added, (0.1 M, pH 7.5, 6 h 37 °C). Then, 1 mL of α-amylase (120 mg/mL solution in a tris-maleate buffer, 0.1 M, pH 6.9, 16 h, 37 °C) was added. The sample was centrifuged (15 min, 3000*g*) and washed with distilled water to subsequently it transfer into a dialysis bag (D9652-3 0.48 m, 12,000–14,000 Da, Sigma Aldrich). The residues were dried and quantified gravimetrically, being IIF. Supernatants were dialyzed in water for 48 h at 25 °C. Once the dialysis was carried out, the SIF was hydrolysed with concentrated H_2SO_4 (90 min, 100 °C) and total carbohydrates were quantified (Englyst & Cummings, 1988). TIF, was considered as the sum of the SIF + IIF. Results are calculated in gram per 100*g* of DM.

2.3.1. Ascorbic acid (AA) content

Each sample (0.1 g) was mixed with 20 mL of H_3PO_4. The mixture was centrifuged (3000*g*, 20 min at 4 °C). The supernatant was filtered (0.22 μm) and 20 μL was injected to a HPLC (Agilent Technologies 1260 infinity, Waldbron, Germany). The stationary phase was a C18 column (4.6 × 100 mm ZORBAX Eclipse plus, Santa Clara, USA). A monobasic sodium phosphate solution (NaH_2PO_4) at pH 2.7 and a flow of 0.5 mL/min as mobile phase was used. AA was detected with a UV–VIS diode array detector at 250 nm. The results were expressed as milligram of AA per 10s) (Osuna-García, Wall & Wadell, 1998).

2.4. Analysis of total carotenoids, total soluble polyphenols, hydrolysable polyphenols, total anthocyanins, profile and phenolic content and antioxidant capacity of purees

2.4.1. Total carotenoids

The total carotenoid (TC) content was performed with 2 g of sample, 10 mL of an ether-acetone mixture (80 and 20 mL, respectively for 100 mL) and 0.5 g $MgCO_3$. The mixture was stirred for 1 min and centrifuged at 11,000*g* and 4 °C for 30 min. The supernatant was recovery and homogenized with 15 mL of a NaCl solution (200 g/L). The absorbance of the ethereal extract was measured at 448 nm. The quantification was performed using a calibration curve of β-carotene standard. The results were expressed as micrograms β-carotene per gram of DM (De Ancos, Gonzalez, & Cano, 2000).

2.4.2. Total soluble polyphenol (TSP) content

An organic aqueous extraction was performed with 500 mg of lyophilized sample in 20 mL of an acidified methanol solution (0.8 M HCl, 50:50, v/v, 60 min). The extract was centrifuged (Hermle Z32HK, Wehingen, Germany) at 8000*g* for 10 min at 4 °C. The supernatant was recovered and the residue was re-extracted with 20 mL of an acetone:water solution (70:30, v/v, 60 min) with centrifugation at the same conditions. The supernatants were combined and carried out at 25 mL with an acidified methanol solution and an acetone:water solution (50:50, v/v). The TSP content was determined in the extracts using the methodology proposed by Montreau (1972). Aliquots of the TPS extraction (250 μL) were mixed with 1000 μL of a Na_2CO_3 (sodium carbonate, 75 g/L) solution. After 3 min, Folin–Ciocalteu's reagent (1250 μL) was added and heated in water-bath for 15 min. A microplate reader (Bio-Tek® Synergy HT, Winooski, VT, USA) in a multi-