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Phenolics and essential mineral profile of organic acid pretreated unripe banana flour

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ABSTRACT

Banana fruit (*Musa* spp) though rich in essential minerals, has also been implicated for the presence of phytochemicals which nonetheless beneficial, can also act as mineral inhibitors when in forms such as phenolic compounds, phytates and tannins. This study assayed the essential macro and trace minerals as well as phenolic compounds present in unripe banana flour (UBF) obtained from the pulp of four different cultivars. Unripe banana flour was processed by oven drying in a forced air oven dryer at 70 °C upon pretreatment with ascorbic, citric and lactic acid. Organic acid pretreatment was done separately on each unripe banana cultivar at concentrations of 10, 15 and 20 g/L. Phenolic compounds were profiled using liquid chromatography mass spectrometry electrospray ion (LC-MS-ESI) while essential minerals were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) and mass spectroscopy (ICP-MS) respectively. Results of LC-MS-ESI assay of phenolics revealed the presence of flavonoids: epicatechin and myricetin 3-O-rhamnosyl-glucoside in varying concentrations in UBF. Essential mineral profile indicated that Zinc had the least occurrence of 3.55 mg/kg ($p < 0.05$), while potassium was the most abundant mineral at 14746.73 mg/kg in UBF of all four banana cultivars. Correlation between phenolic compounds and essential minerals using Pearson's Correlation Coefficient test revealed weak and inverse association between flavonoids and most macro and trace minerals present in UBF samples. Organic acid pretreatment thus exhibited little effect on phenolics and essential minerals of UBF samples, though, inhibitory influence of phenolic compounds was recorded on essential minerals.

1. Introduction

Present day consumer preference on foods is now increasingly determined by factors such as effects on their health, coupled with available and beneficial nutrients inherent in these foods. *Musa* spp., a highly consumed fruit and the fifth most important crop in world export trade (Anyasi, Jideani, & Mchau, 2013; Aurore, Parfait, & Fahrasmame, 2009), is reported to contain nutrients such as dietary fibres, minerals, vitamins, pro-vitamins and phenolic compounds (Arvanitoyannis & Mavromatis, 2009; Aurore et al., 2009; Facundo, Gurak, Mercadante, Lajolo, & Cordenunsi, 2015; Pereira & Maraschin, 2015; Vilela et al., 2014) in varying concentrations. World leading producers of banana include India, China, Uganda, Ecuador, Philippines and Nigeria; with the United States and the European Union implicated as the leading importers of the fruit (Padam, Tin, Chye, & Abdullah, 2014). The works of Anyasi et al. (2013) elucidated several forms into which the fruit can be processed either whole or as composite and they include yoghurt, ice cream, fruit bar, noodles, chips, infant foods, muffins, confectioneries, jam and beer.

Several authors have reported the presence of essential macro (potassium, phosphorus, calcium, sodium and magnesium) and trace minerals (iron, zinc, copper and manganese) in both pulp and peel at different states of ripeness of the fruit (Arvanitoyannis & Mavromatis, 2009; Sulaiman et al., 2011; Wall, 2006). The presence of phenolic compounds such as the flavonoids and its derivatives has also been reported in varying concentrations in banana fruit (Bennett et al., 2010; Del Verde-Mendez, Forster, Rodriguez-Delgado, Rodriguez-Rodriguez, & Diaz-Romero, 2003; Pereira & Maraschin, 2015). Hence, banana is said to contain bioactive compounds with great antioxidant potentials which contributes to physiological defense against oxidative and free-radical-mediated reactions in the biological systems (Singh, Singh, Kaur, & Singh, 2016).

According to Freeland-Graves and Trotter (2003), minerals assist in body catalytic, structural and regulatory functions. Minerals function as electrolytes were they bring about fluid balance, gastric acidity and acid-base balance. Minerals have also been implicated in providing cellular and basal metabolism were they exhibit physiological functions of active transport, regulation of blood pressure, membrane potential of

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cells, muscle contraction and nerve transmission (Freeland-Graves & Trotter, 2003; Sulaiman et al., 2011). Apart from their use as food additives, other physicochemical properties of texture, colour, flavour, pH and nutritive value are properties which the mineral content of the food produce can have a potential effect on (Freeland-Graves & Trotter, 2003; Sulaiman et al., 2011). The bioavailability of these minerals upon processing and consumption is however affected by the presence of anti-nutritional factors (ANFs) and powerful inhibitors such as phenolic compounds present in the fruit (Mascitelli & Goldstein, 2014; Raes, Knockaert, Struijs, & Van Camp, 2014).

Phenolic compounds are known for their antioxidant properties which have beneficial health properties in reduction of coronary heart diseases, inhibition of lipoproteins and antioxidant in humans (Borges et al., 2014; Vijayakumar, Presannakumar, & Vijayalakshmi, 2008). Polyphenols reported to be present in banana includes gallic acid, catechins and epicatechins, anthocyanins and other flavonoid derivatives (Bennett et al., 2010; Del Verde-Mendez et al., 2003; Harnly et al., 2006; Pereira & Maraschin, 2015). The reported presence of these compounds are however in different proportion and their occurrence are in lower concentrations (6.5–18.9 mg GAE/g d.w. at different days of harvest) when compared to other phenolic rich fruits such as cherries, pomegranates, berries, apples and grapes (Bennett et al., 2010; Shahidi & Ambigaipalan, 2015). However, availability and concentration of these health beneficial nutrients is said to vary due to location, climatic factor, agricultural practices, cultural variations and degrees of ripeness of the fruit (Del Verde-Mendez et al., 2003; Englberger et al., 2010; Naczka & Shahidi, 2006). Given the paucity of data on phenolics in banana as well as their inhibitory roles in mineral content, this research seeks to profile the mineral and phenolic concentration of unripe banana flour (UBF) obtained from commercial banana cultivar Williams and non-commercial banana cultivars ‘Luvhele’, ‘Mabonde’ and ‘Muomva-red’ cultivated in Limpopo Province of South Africa. The study also seeks to determine the inhibitory effects of polyphenols on the minerals present in banana.

2. Materials and methods

2.1. Plant material

Non-commercial banana cultivars: ‘Luvhele’ (*Musa ABB*), ‘Mabonde’ (*Musa AAA*) and ‘Muomva-red’ (*Musa balbisiana*) indigenous and obtained from communities and household farms in Limpopo Province of South Africa, as well as commercial cultivar Williams obtained at unripe green stage 2 of maturity (Aurore et al., 2009) from the Agricultural Research Council (ARC) Station in Levubu, South Africa were used for this research. The commercial and non-commercial banana cultivars were characterized at the ARC Levubu Station, and compared with the *Musa* Germplasm Information System database and other literatures (Aurore et al., 2009; Daniells, Jenny, Karamura, & Tomekpe, 2001). Two banana bunches each, of the commercial and non-commercial cultivars were obtained from the ARC and household farms. From the bunch of each cultivar, 15 fingers severed from the banana hands randomly selected from each bunch were used for the research. Peeled individual fingers of the banana hands, were then cut to 4 mm size and pretreated with organic acids: ascorbic, citric and lactic acid at concentrations of 10, 15 and 20 g/L for 10 min. The mixture containing organic acid pretreatment and sliced pulp were allowed to drain for 2 min after which sliced banana fruit pulp were oven dried in a forced air oven dryer at a temperature of 70 °C for 12 h.

2.2. Sample preparation

Oven dried pretreated pulp of all four banana cultivars were used in obtaining homogenized unripe banana flour through milling (Retsch ZM 200miller, Haan, Germany) of the dried pulp at 16,000 rpm for

30 s. About 30–50 g of UBF were obtained from milled unripe banana pulp of each cultivar which were then used for profiling the total polyphenol, antioxidant capacity and individual phenolic compounds. All analysis for the commercial and non-commercial UBF samples were conducted in triplicates.

2.3. Mineral analysis

In order to solubilise the acid-extractable elemental content of the sample, digestion was performed on a MARS microwave digester (CEM Corporation, North Carolina, USA), using ultra-pure HNO₃ at elevated temperature and pressure. After a cooling period, the extract was made up to 50 mL volume with deionised water, then analysed by ICP-AES and ICP-MS for the selected analytes. Analysis of essential elements-sodium, potassium, calcium, magnesium, phosphorus and Sulphur-were measured by a Thermo iCAP 6200 ICP-AES (Thermo Fisher Scientific, Waltham, USA). The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards to quantify selected elements. A NIST-traceable quality control standard of a separate supplier than the main calibration standards were analysed to verify the accuracy of the calibration before sample analysis. Where samples had undergone a digestion step, the results were corrected for the dilution factor resulting from the digestion procedure.

Trace elements of iron and zinc were analysed using Agilent 7900 quadrupole ICP-MS. Samples were introduced via a 0.4 mL/min micromist nebulizer into a peltier-cooled spray chamber at a temperature of 2 °C, with a carrier gas flow of 1.05 L/min. The elements were analysed under He-collision mode to remove polyatomic interferences. The instrument was calibrated using NIST traceable standards to quantify selected elements. A NIST-traceable quality control standard of a separate supplier than the main calibration standards were analysed to verify the accuracy of the calibration before sample analysis. Where samples had undergone a digestion step, the results were corrected for the dilution factor resulting from the digestion procedure.

2.4. Determination of antioxidant capacity of unripe banana flour

In determining the ability of phenolic compound extracts from UBF samples to scavenge the unstable free radical 1,1-diphenyl-2-picrylhydrazyl, the method proposed by Anyasi, Jideani, and Mchau (2015) for unripe banana cultivars was adopted. The extraction solvent used was methanol while gallic acid was used as the standard and results of analysis measured in mg GA/g (d.w.). Dilution of different concentrations of 10, 20, 30, 40 and 50 mg/mL of the sample was used to determine the IC₅₀ of the sample with final values of IC₅₀ obtained by plotting the percentage disappearance of DPPH as a function of the sample concentration. Absorbance was read at 517 nm using a UV spectrophotometer microplate reader (Zenyth 200rt Biochrom, UK). According to Brito, Ramirez, Areche, Sepúlveda, and Simirgiotis (2014), DPPH radicals absorb at 517 nm but undergoes a decrease in absorption upon reduction by an antioxidant compound.

2.5. Assay of total polyphenol content of non-commercial unripe banana flour

Assay of total polyphenol content (TPC) was conducted for flour of banana cultivars used in this study. Total phenolics were determined using the Folin-Ciocalteu colorimetric methods with slight modifications as stated in the work of Anyasi et al. (2015). This classical method is based on the reduction of MoO₄²⁻ to MoO₃²⁻ which is detected by colour change from yellow to blue and measured at 760 nm using a UV spectrophotometer microplate reader (Zenyth 200rt Biochrom, UK). Acetone was used as the extraction solvent while gallic acid was used as the standard phenolic compound. Final results of total phenolics were expressed as gallic acid equivalent (mg GAE/100 g d.w.).

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