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Accumulation of primary and secondary metabolites in edible jackfruit seed tissues and scavenging of reactive nitrogen species

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ABSTRACT

Studies involving jackfruit tree (*Artocarpus heterophyllus* Lam.) focus on its fruit. Nevertheless a considerable part of jackfruit weight is represented by its seeds. Despite being consumed in several countries, knowledge about the chemical composition of these seeds is scarce. In this work, the accumulation of primary and secondary metabolites in jackfruit seed kernel and seed coating membrane was studied. Sixty-seven compounds were identified, sixty of them being reported for the first time in jackfruit seed. Both tissues had a similar qualitative profile, but significant quantitative differences were found. The capacity of aqueous extracts from jackfruit seed kernel and seed coating membranes to scavenge nitric oxide radical was also evaluated for the first time, the extract prepared from the seed coating membrane being the most potent. This work increases the potential revenue from a food that is still largely wasted.

1. Introduction

Artocarpus heterophyllus Lam. (synonyms: *Artocarpus brasiliensis* Gomez, *Artocarpus heterophylla* Lam., *Artocarpus maxima* Blanco, *Artocarpus philippensis* Lam., *Polyphema jaca* Lour., *Soccus arboreus major* Rumph., *Artocarpus integra* (Thunb.) Merr., *Artocarpus integrifolia* L. f., *Artocarpus integrifolius* auct., *Artocarpus integer* auct. and *Artocarpus jaca* Lam.) (Baliga, Shivashankara, Haniadka, Dsouza, & Bhat, 2011; Jagtap & Bapat, 2010), popularly known as jackfruit tree or "jaqueira", is an evergreen tree belonging to the Moraceae family, widely found throughout many tropical and subtropical regions (Ajayi, 2011; Olaifa, Ajayi, & Raji, 2013; Rajiv, Prakash, Rajesh, & Anurag, 2009). Beyond its traditional medicinal uses, every part of the plant is useful as source of fuel and timber, and leaves and fruit wastes are employed as feed for goats, cattle and pigs (Ajayi, 2011; Jagtap & Bapat, 2010; Rajah, Reddy, Sushma, Rani, & Reddy, 2010).

The most striking characteristic of this tree is its capacity to provide a notable yield of fruits, commonly known as "jaca", considered to be the largest tree-borne fruit in the world (Ajayi, 2011; Jagadeesh et al., 2007). The fruits are consumed mainly in Bangladesh and India, as well as in many parts of Southeast Asia (Rahman, Nahar, Mian, & Moshiuzzaman, 1999; Swami, Thakor, Haldankar, & Kalse, 2012). Although jackfruit can be eaten raw or processed in wine and several delicacies, a considerable amount of the harvest is discarded, including their seeds and the thin whitish membranes in which they are enclosed (Olaifa et al., 2013; Sharma, Bhutia, & Aradhya, 2013). Up to 500 seeds can be found in a single fruit, corresponding to approximately 15% of its total weight (Rajiv et al., 2009). Although the consumption of jackfruit seeds is still scarce, they are part of some local dishes in Asian countries (Biswas & Rahmatullah, 2011; Olaifa et al., 2013; Rahman et al., 1999; Soong & Barlow, 2004; Swami et al., 2012). As fresh seeds cannot be kept for long periods of time, flour from dried seeds is an alternative for use in some food products (Rajiv et al., 2009).

Although anticancer, antibacterial, antioxidant and other remarkable health benefits have been attributed to jackfruits seeds (Baliga et al., 2011; De Miranda-Santos, Mengel, Bunn-Moreno, & Campos-Neto, 1991; Jagtap & Bapat, 2010; Soong & Barlow, 2004), their chemical composition is still little explored. Only the composition of jackfruit seeds concerning vitamins, proteins, carbohydrates (mainly starch), carbohydrate-binding proteins, namely lectins (jacalin being the most representative), fat and dietary fibre (Jagtap & Bapat, 2010; Olaifa et al., 2013; Soong & Barlow,

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2004; Swami et al., 2012) have been described previously. Thus, a more comprehensive characterisation of the seeds composition is required to assess potential exploitation of this food.

The relationship between regular consumption of fruits and vegetables and the reduced risk of chronic diseases is well established, as is the relationship with bioactive compounds from these foods (Liu, 2013). An extensive number of studies has suggested that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are associated with the development of several chronic diseases (Kehrer & Klotz, 2015; Liu, 2013). Free radicals can be generated in a wide variety of chemical and biological systems in the human body, including endogenous processes and normal cell metabolism, or result from exogenous sources (Kehrer & Klotz, 2015), performing useful roles, for example, in cell signalling and defence against pathogens. However, when there is an imbalance between production of free radicals and their neutralization, their accumulation leads to oxidative stress, which is associated with chronic diseases, as well as with ageing (Liu, 2013).

Considering that the chemical profile of jackfruit seed is still underexplored, the purpose of this work was to deepen knowledge about its composition. For this, the distinct accumulation of primary and secondary metabolites in jackfruit seed kernel and its coating membrane was studied. Organic acids, amino acids, phenolic compounds, carotenoids and sterols were determined using high pressure liquid chromatography coupled to a diode-array detector (HPLC-DAD) and, for fatty acids characterisation, gas chromatography/ion trap-mass spectrometry (GC/IT-MS) was employed. Furthermore, since the chemical profile can greatly influence the biological effects, and in order to add value to these materials (Milella et al., 2011), the activity against nitric oxide (•NO), a radical with an essential role in inflammation, was also assessed.

2. Materials and methods

2.1. Standards and reagents

Standards were purchased from various suppliers: fatty acid methyl esters (FAME) of dodecanoic (C12:0), tridecanoic (C13:0), tetradecanoic (C14:0), *cis*-10-pentadecenoic (C15:1*n*-5c), pentadecanoic (C15:0), *cis*-9-hexadecenoic (C16:1*n*-7c), hexadecanoic (C16:0), *cis*-10-heptadecenoic (C17:1*n*-7c), heptadecanoic (C17:0), *cis*-9,12-octadecadienoic (C18:2*n*-6c), *cis*-9-octadecenoic (C18:1*n*-9c), *trans*-9-octadecenoic (C18:1*n*-9t), octadecanoic (C18:0), *cis*-9,12,15-octadecatrienoic (C18:3*n*-3c), eicosanoic (C20:0), heneicosanoic (C21:0), docosanoic (C22:0), tricosanoic (C23:0) and tetracosanoic (C24:0) acid were obtained from Supelco (Bellefonte, PA, USA); oxalic, citric, malic, quinic, shikimic, fumaric, succinic, aspartic, and glutamic acids, asparagine, glutamine, serine, threonine, glycine, alanine, valine, proline, arginine, isoleucine, leucine, tryptophan, phenylalanine, cysteine, ornithine, lysine, histidine, tyrosine and lutein were from Sigma-Aldrich (St. Louis, MO, USA); sinapic, chlorogenic, *p*-coumaric, ferulic, aconitic and pyruvic acids were purchased from Extrasynthèse (Genay, France); acetic and sulphuric acids were from Fisher Scientific (Loughborough, UK).

HPLC grade methanol, acetonitrile, trichloromethane, *n*-hexane, ethanol, phosphoric acid, and N-(1-naphthyl)ethylene-diamine dihydrochloride were obtained from Merck (Darmstadt, Germany); formic acid was from BDH Prolab (Dublin, Ireland); methanol, acetone, anhydrous sodium sulphate and hydrochloric acid were purchased from Panreac Quimica SA (Barcelona, Spain); potassium hydroxide was from Pronalab (Lisboa, Portugal); *tert*-butyl methyl ether (MTBE), isooctane, boron trifluoride (BF_3) 10% methanolic solution, dabsyl chloride, sodium hydrogen carbonate, sodium dihydrogenophosphate, dimethylformamide, trimethylamine and sulphanilamide were from Sigma (St. Louis, MO, USA); sodium nitroprusside dihydrate was from Riedel-de Haën (St. Louis, MO).

Water was deionised in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Sampling

Seeds including their coating membranes were taken from different *A. heterophyllus* fruits collected in February 2014, in Ossoala, East Timor and identified by Cristóvão Belo (National University of East Timor). The seeds were placed in sterile plastic bags and immediately transported to the laboratory in insulated sealed ice-boxes and dehydrated at 40 °C for 5 days. After exsiccation, the seed kernels and coating membranes were separated and each material was powdered separately in a commercial mill (Moulinex®). The resulting powdered material (mean particle size lower than 910 μm) was stored in the dark. Voucher specimens were deposited at Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade do Porto (Portugal): seed kernel was labelled as Ahet_SK-020214 and seed coating membrane as Ahet_SCM-020214).

2.3. Extraction

For a broader insight on the chemical composition of jackfruit seed, different extracts were prepared.

2.3.1. Aqueous extracts

Aqueous extract of jackfruit seed kernel was prepared by boiling 5.0 ± 0.0099 g of powdered material for 30 min, in 400 mL of water. For seed coating membrane, 2.5 ± 0.0002 g of material were boiled in 300 mL of water for 30 min. The resulting extracts were filtered using a Büchner funnel (porosity 3) and then freeze-dried in a Virtis SP Scientific Sentry 2.0 apparatus (Gardiner, NY 12525, USA). The lyophilized extracts were kept in a desiccator in the dark until analysis. The yields (g extract/g sample, dry matter) obtained were 0.76 for seed kernel and 0.14 for seed coating membrane.

2.3.2. Methanolic extracts

The methanolic extract of jackfruit seed kernel was prepared by mixing 5.0 ± 0.0088 g of powdered material with 100 mL of methanol under the following conditions: 30 min of sonication and 1 h of stirring maceration (300 rpm), at room temperature, followed by 30 min of sonication. For seed coating membrane, 2.5 ± 0.068 g of powdered material was extracted with 50 mL of methanol following the same conditions. The obtained extracts were then filtered under vacuum (by Büchner funnel, porosity 3) and evaporated at reduced pressure (Rotavapor® R-215, BÜCHI Labortechnik, Flawil, Switzerland) until dryness, at 30 °C. The dried extracts were kept at -20 °C and protected from light until analysis. The yields (g extract/g sample, dry matter) obtained were 0.04 for seed kernel and 0.09 for seed coating membrane.

2.3.3. Fatty acids extraction

Each dried material (0.25 ± 0.0010 g) was extracted with 5 mL of chloroform:methanol (2:1), under magnetic stirring at 500 rpm, for 10 min, at 40 °C. The extraction procedure was repeated five times and the resulting extracts were pooled, filtered through a 0.45 μm size pore membrane (Millipore) and concentrated to dryness under reduced pressure (40 °C) on a Rotavapor device. The dried extracts were kept at −20 °C and protected from light until analysis. The yields (g extract/g sample, dry matter) obtained were 0.16 for seed kernel and 0.15 for seed coating membrane.