Food Research International 100 (2017) 423–434

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Use of HPLC- and GC-QTOF to determine hydrophilic and lipophilic phenols in mango fruit (*Mangifera indica* L.) and its by-products

Ana López-Cobo [a,b], Vito Verardo [c,d], Elixabet Diaz-de-Cerio [a,b], Antonio Segura-Carretero [a,b], Alberto Fernández-Gutiérrez [a,b], Ana M Gómez-Caravaca [a,b,*]

[a] Department of Analytical Chemistry, University of Granada, c/Fuentenueva s/n, 18071 Granada, Spain
[b] Research and Development of Functional Food Centre (CIDAF), PTS Granada, Avda. del Conocimiento s/n, Edificio Bioregión, 18007 Granada, Spain
[c] Department of Chemistry and Physics (Analytical Chemistry Area), Agrifood Campus of International Excellence, ceiA3, University of Almería, Carretera de Sacramento s/n, E-04120 Almería, Spain
[d] Research Centre for Agricultural and Food Biotechnology (BITAL), Agrifood Campus of International Excellence, ceiA3, University of Almería, Carretera de Sacramento s/n, E-04120 Almería, Spain

ARTICLE INFO

Article history:
Received 27 October 2016
Received in revised form 15 February 2017
Accepted 17 February 2017
Available online 17 February 2017

Keywords:
Mango
Mangifera indica L.
HPLC-DAD-ESI-QTOF-MS
GC-QTOF-MS
Phenolic compounds
Alk(en)ylresorcinols
By-products

ABSTRACT

Mango industry processing generates high quantities of mango by-products such as peels and seeds (35%–60% of the fruit). Indeed, it is known that mango and its by-products contain different families of bioactive compounds that possess several health benefits. Thus, the aim of this study has been the determination of different families of phenolic derivatives (free and bound phenolic compounds and alk(en)ylresorcinols (ARs)) in mango edible part and its by-products (peel, seed and seed husk) from three different cultivars. This is the first study that evaluates the phenolic compounds and ARs in the four fractions of mango of three different cultivars. Special attention has been paid to the determination of anthocyanins and ARs, because these families of compounds had not been studied in depth in mango. In fact, petunidin rutinoside-(*p*-coumaric acid) gallate was found in mango pulp, peel, seed and seed husk of the three cultivars and, it had never been described in mango before. It is also important to highlight that this is the first time that the identification and quantification of ARs have been performed in mango seed and seed husk; besides, four and five out of eleven alk(en)ylresorcinols detected in peel and pulp, respectively, were identified for the first time in these mango fractions. Furthermore, antioxidant activity was measured by ABTS and FRAP assays. Seed free and bound phenolic extracts showed the highest antioxidant capacity.

1. Introduction

Mango (*Mangifera indica* L.), which belongs to the family Anacardiaceae, is grown naturally or mainly cultivated in tropical and subtropical regions. It is one of the most popular edible fruit and its production, at present, ranks seventh in world fruit production, just after watermelons, bananas, apples, grapes, oranges and coconuts (Food and Agriculture Organization of the United Nations. Statistics division, 2016).

Mango is generally consumed as a dessert fruit, but there has been an increase in consumption of mango products such as juice, nectar, powder, canned mango slices in syrup, and chutneys. Processing of mango fruits generates a significant amount of by-products such as peels and seeds (Ayala-Zavala et al., 2011), which represent from 35% to 60% of the fruit, approximately. There are many previous research works that have studied the bioactive compounds content such as phenolic compounds, carotenoids, tocopherols, or sterols (Ajila, Naidu, Bhat, & Prasada Rao, 2007; Ajila & Prasada Rao, 2013; Maisuthisakul & Gordon, 2009; Masibo & He, 2008). The daily intake in the diet of these bioactive compounds has been shown to have possible health benefits due to their antiviral, antibacterial, analgesic, anti-inflammatory, and immunomodulatory activities (Makare, Bodhankar, & Rangari, 2001). Indeed, they have demonstrated in vitro antiamoebic activity (Tona, Kambu, Ngimbi, Cimanga, & Vlietinck, 1998), interesting R-amylase and R-glucosidase inhibitory activities (Prashanth, Amit, Samiulla, Asha, & Padmaja, 2001) and cardiotonic and diuretic properties (Scartezzini & Speroni, 2000).

Phenolic compounds are secondary metabolites that are synthesized during normal plant development in response to stress conditions (Beckman, 2000; Cheynier, 2012). They play a protective role and because of that, they are mainly distributed in the outer layers of the fruit. The main phenolic and other polar compounds identified in mango fruits have been flavonol glycosides (quercetin and kaempferol derivatives and rhamnetin hexoside), xanthone glycosides (mangiferin derivatives), and gallotannin and benzophenone derivatives (maclurin and iriflophenone glycosides). Anthocyanins and alk(en)ylresorcinols

* Corresponding author at: Department of Analytical Chemistry, University of Granada, c/Fuentenueva s/n, 18071 Granada, Spain.
E-mail address: anagomez@ugr.es (A.M. Gómez-Caravaca).

http://dx.doi.org/10.1016/j.foodres.2017.02.008
0963-9969/

(ARs) are two other important families of phenolic compounds contained in mango that have not been studied in depth yet.

Anthocyanins are widely distributed in most vascular plants where they serve as inducible sunshields (Grotewold, 2006). Cyanidin-3-O-galactoside and 7-O-methylcyanidin 3-O-β-D-galactopyranoside are the main anthocyanin compounds found in the red color of mango fruit peel (Berardini, Fezer, Conrad, Beifuss, Carle, et al., 2005; Berardini, Schieber, Klaiber, Beifuss, Carle, et al., 2005b).

Alk(en)ylresorcinols represent a class of amphiphilic phenolic lipids which are predominantly produced by higher plants such as cereals as well as in mango peels and pulp, peas (*Pisum sativum* L.), and ginkgo (*Ginkgo biloba* L.) (Kienzle, Carle, Sruamsiri, Tosta, & Neidhart, 2014; Knödler, Kaiser, Carle, & Schieber, 2008; Kozubek & Tyman, 1999; Ross, 2012a; Zarnowski & Kozubek, 1999). Several potential health effects related to the intake of ARs, such as anti-microbial, anti-inflammatory, anti-mutagenic, and anti-carcinogenic activities, have been demonstrated in animal and model systems (Knödler, Conrad, Wenzig, Bauer, Lacorn, et al., 2008; Ross, 2012b; Stasiuk & Kozubek, 2010; Zarnowski & Kozubek, 1999). Due to their amphiphilic nature, ARs interact with membranes and DNA and, thus, they have been suggested as potential inhibitors of bacterial, fungal, and protozoal growth (Geerkens, Matejka, Carle, & Schweiggert, 2015). Mango peel and pulp alk(en)ylresorcinols have previously been studied by Geerkens, Matejka, Carle, & Schweiggert (2015) and Kienzle et al. (2014)); however, to the best of our knowledge, the present study is the first evidence for their presence in mango seed and husk.

Some studies have shown that the qualitative and quantitative phenolic composition of the extracts studied depends mainly on the variety, the maturity stage and the part of the mango analyzed (peel, pulp or seed) (Barreto et al., 2008; Kozubek & Tyman, 1999; Maisuthisakul & Gordon, 2009; Ribeiro, Barbosa, Queiroz, Knödler, & Schieber, 2008; Schieber, Berardini, & Carle, 2003).

Taking into account the few studies on the determination and quantification of anthocyanins and alk(en)ylresorcinols in mango, and the important role of phenolic compounds in health, the aim of this study was to determine the free and bound phenolic compounds profile, paying special attention to anthocyanins profile, and alk(en)ylresorcinols profile of pulp, peel, seed and seed husk of three cultivars of mango fruit ("Keitt", "Osteen" and "Sensación") by HPLC-DAD-ESI-QTOF-MS and GC-QTOF-MS. Furthermore, the antioxidant activity of the phenolic extracts of the four parts of the three mango cultivars was measured by ABTS and FRAP methods.

2. Materials and methods

2.1. Samples

Three mango cultivars ("Keitt", "Osteen" and "Sensación") cultivated under the same agronomical and environmental conditions were provided by Miguel García Sánchez e Hijos, S.A. (Motril, Spain) in July 2015. About 10 kg of fruit from each cultivar at optimal consumption ripeness established by the company (13–16°Brix) were manually separated in pulp, peel, seed and seed husk, and then, freeze-dried in a lyophilizer (Advantage Plus EL-85 freeze dryer, SP Scientific, Ipswich, Suffolk, UK). The samples were milled (IKA M20-IKAWERKE GmbH & Co. KG, Staufen,Germany) and kept at −18 °C until use.

2.2. Chemicals and reagents

HPLC-grade acetic acid, HPLC-MS-grade acetonitrile, hexane, diethyl ether, ethyl acetate and dichloromethane were purchased from Fisher Scientific (Leicestershire, UK), and methanol, ethanol, sodium acetate, hydrochloric acid were purchased from Panreac (Barcelona, Spain). Solvents were filtered using a Solvent Filtration Apparatus 58061 (Supelco, Bellefonte, PA, USA). Double-deionized water with conductivity lower than 18.2 MΩ was obtained with a Milli-Q system from Millipore (Bedford, MA, USA). The following standards and reagents were supplied by Sigma-Aldrich (St. Louis, MO, USA): gallic acid, coumaric acid, ferulic acid, vanillic acid, catechin, quercetin-glucoside, quinic acid, citric acid, mangiferin, ellagic acid, cyanidin 3-O-β-D-galactopyranoside, methylbehenate, nonadecylresorcinol, acetic acid, hydroxide sodium, ABTS, potassium persulfate, trolox, TPTZ, ferric chloride and ferrous sulfate. Pyridine was purchased from VWR (Chemicals Prolabo, Fontenay-sous-Bois, France). Trimethylchlorosilane and anhydrous sodium sulfate were supplied by Merck KGaA (Darmstadt, Germany), and hexamethyldisilazane was supplied by Alfa Aesar GmbH & Co KG (Karlsruhe, Germany).

2.3. Extraction of the free polar fraction of mango

The extraction of the free polar fraction was carried out according to Gómez-Caravaca, López-Cobo, Verardo, Segura-Carretero, and Fernández-Gutiérrez (2016). Briefly, 0.5 g of sample powder were dissolved in 10 mL of a solution of methanol/water (80:20, *v*/v). The mixture was placed in an ultrasonic bath for 15 min and then it was centrifuged for 15 min at 1000*g*; the supernatant was removed, and the extraction was repeated twice more.

The supernatants were collected, evaporated, and reconstituted in 3 mL of methanol/water (80:20, *v*/v). The final extracts were filtered with regenerated cellulose filters 0.2 μm (Millipore, Bedford, MA, USA) and stored at −18 °C until the analyses. Three extraction replicates were done for each sample.

2.4. Extraction of the bound polar fraction of mango

Bound compounds are linked to the cellular walls and they are not taken into account only with a conventional extraction. Thus, a second extraction was performed following the method previously proposed by Verardo, Gómez-Caravaca, Marconi, and Caboni (2011). Once the extraction with aqueous methanol was performed to discharge free polar compounds, mango samples were digested with 100 mL of 2 M NaOH. The mixture was then brought to pH 2–3 and extracted with 500 mL of hexane. The final solution was extracted three times with 100 mL of diethyl ether/ethyl acetate (1:1, *v*/v). The organic fractions were pooled and evaporated to dryness. The phenolic compounds were reconstituted in 2 mL of methanol/water (1:1 *v*/v). The final extracts were filtered and stored at −18 °C until the analyses. Three extraction replicates were done for each sample.

2.5. Extraction of alk(en)ylresorcinols in mango

0.5 g of sample powder were extracted using 10 mL of dichloromethane in an ultrasonic bath during 10 min. Afterwards, it was centrifuged for 10 min at 1000*g*; the supernatant was removed, and the extraction was repeated twice more. The supernatants were collected, evaporated, reconstituted in 1 mL of dichloromethane and stored at −18 °C until the analyses. Three extraction replicates were done for each sample.

2.6. HPLC-DAD-ESI-QTOF-MS analysis of the polar fraction

The free and bound polar extracts obtained from pulp, peel, seed and seed husk of the three mango cultivars were analyzed by HPLC-DAD-ESI-QTOF-MS. The chromatographic determination was performed by an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) consisting of a vacuum degasser, a binary pump, an autosampler, a column heater, and a diode array detector (DAD). This instrument was equipped with an Agilent Poroshell 120 EC-C18 column (4.6 × 100 mm, 2.7 μm) from Agilent Technologies. Separation was performed using different gradient elution programmes depending on the phenolic classes. Previously optimised and validated methodologies were used to perform the analyses.