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Determination of lipid composition of the two principal cherimoya cultivars grown in Andalusian Region

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ABSTRACT

Cherimoya (*Annona cherimola*) is a tropical fruit, native to inter-Andean valleys from Peru and Ecuador. The main cherimoya growing in the Mediterranean basin is the coast of Granada–Malaga (Spain), the so-called Costa Tropical.

Recently, the number of studies related to economic exploitation of seeds and other by-products proceeding from new oleaginous vegetable sources has increased. Therefore, the aim of this work was to characterize the lipid fraction of the edible portion of the cherimoya and its by-products.

Different fatty acids were identified in pulp, seed, and skin. The major fatty acids were palmitic, oleic, linoleic, and α-linolenic. On the other hand, α-tocopherol was identified in seeds and peel. Moreover, three different phospholipids were identified. Finally, β-sitosterol was the principle sterol in all samples and, to our knowledge, γ-sitosterol was identified only in pulp and peel of cherimoya for the first time.

1. Introduction

Cherimoya (*Annona cherimola* Mill.) is one of the tropical fruit that has become an important crop due to its excellent sensorial properties and because it is a source of bioactive compounds.

The main cherimoya growing in the Mediterranean basin is the coast of Granada–Malaga (Spain), the so-called "Costa Tropical". Spain is considered one of the major producers worldwide. Two different cultivars are resident in "Costa Tropical", 'Fino de Jete', which is the most wide spread in the world, and 'Campa' (Alique & Oliveira, 1994; Barreca et al., 2011).

The high potential of tropical fruit pulps and their by-products in human nutrition has led food research to investigate the isolation of specific phytochemicals for application in nutraceutical

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supplements, dietary additives, and new foods and pharmaceutical products (Ribeiro da Silva et al., 2014).

Special attention has been focused by food researchers on the lipid fraction in fruits and vegetables due to the presence of antioxidant compounds that play a natural preventive role in cardiovascular disease and several degenerative illness (Ayala-Zavala et al., 2011; Fadavi, Barzegar, & Azizi, 2006; Melgarejo & Artes, 2000). In fact, lipophilic characterization at fruits and by-products has opened a promising field of research in the cosmetic and food industries for their potential uses as nutritional supplements in functional foods (Villaverde et al., 2013). Plant seed oil is one of the most interesting essential oils due to its properties; nevertheless there are relatively reports few studies in the literature about the lipid composition of fruit seeds such as prickly pear, apple, and some *Annona* species (Bada, Leon-Camacho, Copovi, & Alonso, 2014; Monteiro & Alves, 2011; Ramadan & Morsel, 2003). It has been found that fruit peel lipids can be a source of essential fatty acids, sterols, and lipid soluble antioxidants (Fiorentino et al., 2009; Ramadan et al., 2003). Previous studies about lipid composition of cherimoya fruit have been developed. Gutierrez et al. reported a general study about lipids fraction of the fruit mesocarp studying only the fatty acids content (Gutiérrez, Sola, & Vargas, 2005). Cordeiro and co-workers studied the fatty acids, sterols, and δ-tocopherol content also in pulp samples (Cordeiro, Sousa, Freitas, & Gouveia, 2013). Recently, Albuquerque et al. reported the vitamin E content in some cherimoya cultivars and its by-products (Albuquerque et al., in press).

There are few research studies about the determination of lipid compounds in *Annona cherimola* and its by-products. For this reason, the aim of this work was to determine the lipid composition (FA, sterols, tocopherols, and phospholipids) of cherimoya pulp and by-products, from two different cultivars from the Granada coast (Spain).

2. Materials and methods

2.1. Samples

Two different cherimoya cultivars, 'Fino de Jete' and 'Campa', were collected in February 2012. These cultivars were selected because they are the most commercialized in Andalucia (Spain). The.

cultivars were grown under the same agronomic and environmental conditions in the same experimental field located in Motril (Spain) (36°44′43″N 3°31′14″O). Trees of each cultivars (10 trees) is planted at 7 × 7 m spacing.

The fruits were collected at commercial maturation. A total of 30 fruits from 6 individual trees for each cultivar were taken. The pulp, seeds, and peel of fresh samples were manually separated and freeze dried by a lyophilizer (Christ Alpha 1–2 LD Freeze dryer, Shropshire, UK). Afterwards, the dried samples were milled and kept at −18 °C until use.

2.2. Chemicals

All solvents and reagents were purchased from Merck (Darmstadt, Germany). The following standards were supplied by Sigma–Aldrich (Saint Louis, MO, USA): L-α-phosphatidylethanolamine (PE), L-α-phosphatidylcholine (PC), L-α-phosphatidylinositol (PI), α-, δ-tocopherol and dihydrocholesterol. The GLC-463 mix for FA was from Nu-Check (Elysian, MN, USA).

2.3. Determination of oil content

To determine the total oil content, cherimoya pulp, seeds, and peel were extracted with diethyl ether in a Soxtech instrument, and the remaining solvent was removed by vacuum evaporation. Each extraction was carried out three times for each sample.

2.4. Lipid extraction for analysis

The pulp, seeds, and peel oils were extracted using the procedure described by Boselli, Velazco, Caboni, and Lercker (2001) using a chloroform/methanol solution. The lipid extract was stored at −18 °C until it was analysed.

2.5. Total fatty acid composition analysis

The FA composition of cherimoya samples was determined from extracted oil as FAMEs by capillary gas chromatography analysis as reported by Verardo, Gomez-Caravaca, Gori, Losi, and Caboni (2013) with minor modifications as follows: the sample volume injection was 0.2 μL with a split ratio set at 1:400.

2.6. Tocopherol analysis

Approximately 50 mg of oil from the pulp, seed or peel, was dissolved in 500 μL of *n*-hexane. α-, γ-, and δ-tocopherol were determined by HPLC (Agilent 1200 series, Agilent Technologies, Palo Alto, CA, USA) equipped with a fluorescence detector (Agilent Technologies, Palo Alto, CA, USA) under the conditions as reported by Gomez-Caravaca, Verardo, and Caboni (2010). A calibration curve was constructed with α-tocopherol standard solution ($r = 0.9999$) and it was used for quantification.

2.7. Phytosterol and triterpenic alcohol analysis

To determine the phytosterols and triterpenic alcohols, 100 μL of dihydrocholesterol ($c = 2.0$ mg/mL) was added to 200 mg of each oil sample and saponification was conducted at room temperature (Iafelice, Verardo, Marconi, & Caboni, 2009). The unsaponifiable matter was sylilated according to Sweeley, Bentley, Makita, and Wells (1963) and it was analysed using a GC/MS (GCMS-QP2010 Plus, Shimadzu, Tokyo, Japan) with the chromatographic conditions reported by Cardenia, Rodriguez-Estrada, Baldacci, Savioli, and Lercker (2012).

Compound identification was achieved by comparing chromatographics peaks and peak mass spectra with peaks in a standard mixture and with GC–MS data reported by Pelillo, Iafelice, Marconi, and Caboni (2003). Quantification of identified phytosterol and triterpenic alcohol compounds was performed in relation to dihydrocholesterol used as an internal standard.

2.8. Phospholipid determination

To determine the phospholipids in the pulp and by-products of cherimoya fruit, approximately 100 mg of oil were weighed and dissolved in 1 mL of 88/12 (*v*/*v*) chloroform/methanol and used for HPLC analysis.

Quantitation of the phospholipid classes was performed using HPLC–ELSD. The chromatographic method used by Verardo et al. (2013) to separate the polar lipids was carried out.

2.9. Determination of phospholipid fatty acids

HPLC–MS analyses were carried out on the same extract analyzed by HPLC–ELSD. A liquid chromatography apparatus HP