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Analytical Methods

Lipophilic phytochemicals from banana fruits of several *Musa* species

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ABSTRACT

The chemical composition of the lipophilic extract of ripe pulp of banana fruit from several banana cultivars belonging to the *Musa acuminata* and *Musa balbisiana* species (namely 'Chinese Cavendish', 'Giant Cavendish', 'Dwarf Red', 'Grand Nain', 'Eilon', 'Gruesa', 'Silver', 'Ricasa', 'Williams' and 'Zelig') was studied by gas chromatography–mass spectrometry for the first time. The banana cultivars showed similar amounts of lipophilic extractives (ca. 0.4% of dry material weight) as well as qualitative chemical compositions. The major groups of compounds identified in these fractions were fatty acids and sterols making up 68.6–84.3% and 11.1–28.0%, respectively, of the total amount of lipophilic components. Smaller amounts of long chain aliphatic alcohols and α-tocopherol were also identified. These results are a relevant contribution for the valorisation of these banana cultivars as sources of valuable phytochemicals (ω-3 and ω-6 fatty acids, and sterols) with well-established beneficial nutritional and health effects.

1. Introduction

Musa is a plant genus from the family Musaceae and order Zingiberales (Arvanitoyannis & Mavromatis, 2009) that produces the fourth most important food crop in the world after rice, wheat and maize, i.e. bananas and plantains (Sharrock & Frison, 1998). Although the genus *Musa* is composed of four taxonomic sections, namely *Australimusa*, *Callimusa*, *Musa* (formerly known as *Eumusa*) and *Rhodochlam*, most cultivated varieties (cultivars) originate from intra- and inter-specific hybridisations between two diploid wild species of the section *Musa*: *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) (Lim, 2012). The most important banana cultivars are triploid AAA and plantains are mostly AAB, ABB or BBB (Ball, Vrydaghs, Van Den Hauwe, Manwaring, & De Langhe, 2006). Due to the difficulty of breeding infertile plants, only a few cultivars have been introduced in the last 50 years. However, the advent of clonal propagation, combined with selection programs, led to the singling out of "elite" clones in terms of yield and fruit quality, adapted to the agro-ecological conditions. 'Dwarf Cavendish', 'Giant Cavendish' and 'Gros Michael' are among the most important banana cultivars for fresh consumption (Po & Po, 2012).

Banana and plantain plants are cultivated in more than 130 countries throughout the tropical and subtropical regions, over a harvested area of approximately 10 million hectares (FAOSTAT, 2011). The annual world production accounts for about 145 million tonnes (ca. 106 million tonnes for banana and 39 million tonnes for plantain), with India as the major producer (29 million tonnes), Equador the main exporter and the European Union and United States of America the major importers of bananas (FAOSTAT, 2011). Banana plants were successfully introduced to Madeira Island (Portugal) in the middle of the 16th century and are one of the most important crops for the island's economy, representing 20% of the agricultural production and one third of the exports from the Island. Among the cultivated species, 'Dwarf Cavendish' (*M. acuminata* Colla var. *cavendish*) is by far the most important in terms of occupied area and productivity, comprising ca. 60% of the total banana production in Madeira. However, changes in the European Union banana policy prompted farmers to select and grow varieties that fulfil the needs of consumers (Council Regulation, 2006). As a response to these requirements, in addition to 'Dwarf Cavendish', new banana cultivars adapted to the edaphic

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conditions of the region have been introduced to the island (Nuno, Ribeiro, & Silva, 1998).

The banana fruit, with an average annual per capita consumption that varies from 4.5 kg in USA to 16 kg in Sweden and up to 151 kg in São Tomé e Principe (Po & Po, 2012), has a high nutritional value, contributing to an elevated intake of carbohydrates, fibre, vitamins, and minerals, together with a very low intake of fats (Arvanitoyannis & Mavromatis, 2009; Po & Po, 2012). In addition, banana fruits are also a rich source of phytochemicals (Po & Po, 2012), including unsaturated fatty acids and sterols (Knapp & Nicholas, 1969; Oliveira, Freire, Silvestre, & Cordeiro, 2008), with a recognised role in human diets and health (Moreau, Whitaker, & Hicks, 2002; Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000). Our interest in ripe banana pulp results from the several studies concerning the valorisation of banana agro-industry residues, including different morphological parts of the plants (Oliveira et al., 2005), fruit peels and unripe pulp (Oliveira et al., 2008; Villaverde et al., 2013), from which several lipophilic phytochemicals with valuable nutraceutical properties, such as phytosterols, steryl glucosides, tocopherols and unsaturated fatty acids, among others, were identified.

As far as our literature survey could ascertain, no studies about the chemical composition of lipophilic extracts of ripe banana pulp of 'Chinese Cavendish', 'Giant Cavendish', 'Dwarf Red', 'Grand Nain', 'Eilon', 'Gruesa', 'Silver', 'Ricasa', 'Williams' and 'Zelig' cultivars have been published. In this vein, the present study aimed to determine the phytochemicals from several cultivars of *M. acuminata* (AAA) and *M. acuminata* × *M. balbisiana* (ABB) cultivated in Madeira Island, by assessing the lipophilic fraction chemical composition of ripe banana pulp through gas chromatography–mass spectrometry (GC–MS).

2. Material and methods

2.1. Chemicals

Dichloromethane (99% purity), pyridine (99% purity), trimethylchlorosilane (99% purity), *N,O*-bis(trimethylsilyl)trifluoroacetamide (99% purity), stigmasterol (95% purity), octadecanoic acid (99% purity), nonadecan-1-ol (99% purity), and tetracosane (99% purity) were supplied by Sigma–Aldrich (Madrid, Spain).

2.2. Samples preparation

Ten varieties of ripe bananas, namely 'Chinese Cavendish', 'Giant Cavendish', 'Dwarf Red', 'Grand Nain', 'Eilon', 'Gruesa', 'Silver', 'Ricasa', 'Williams' and 'Zelig', were kindly provided by the Bananicultura Center of the Regional Government, Lugar de Baixo, in the Madeira Island (Portugal, 32° 40′ 47″ N, 17° 5′ 13″ W). For each variety a minimum of 50 ripe fruits were collected. Peels were separated from the fruit, and the pulps were cut, immediately frozen with liquid nitrogen, freeze dried and milled to pass through a 40–60 mesh sieve. The freeze dried samples were kept at −18 °C until extraction.

2.3. Extraction

Three powdered samples (20 g) of each cultivar were Soxhlet extracted with dichloromethane (600 mL) for 6 h. The solvent was evaporated to dryness, the lipophilic extracts were weighed and the results were expressed in percent of dry material. Dichloromethane was selected as a fairly specific solvent for lipophilic compounds isolation for analytical purposes.

2.4. GC–MS analysis

Before GC–MS analysis, two aliquots of each dried extract (20 mg each) and an accurate amount of internal standard (tetracosane, 0.25–0.50 mg) were dissolved in 250 μL of pyridine. The compounds containing hydroxyl and carboxyl groups were converted into trimethylsilyl (TMS) ethers and esters, respectively, by adding 250 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide and 50 μL of trimethylchlorosilane, standing the mixture at 70 °C for 30 min (Freire, Silvestre, Neto, & Cavaleiro, 2002). The derivatised extracts were analysed by GC–MS following previously described methods (Freire et al., 2002; Oliveira et al., 2008) on a Trace Gas Chromatograph 2000 Series, equipped with a Thermo Scientific DSQII single-quadrupole mass spectrometer and a DB-1 J&W capillary column (30 m × 0.32 mm inner diameter, 0.25 μm film thickness). The chromatographic conditions were as follows: initial temperature, 80 °C for 5 min; temperature gradient, 4 °C min^{-1}; final temperature, 260 °C; temperature gradient, 2 °C min^{-1}; final

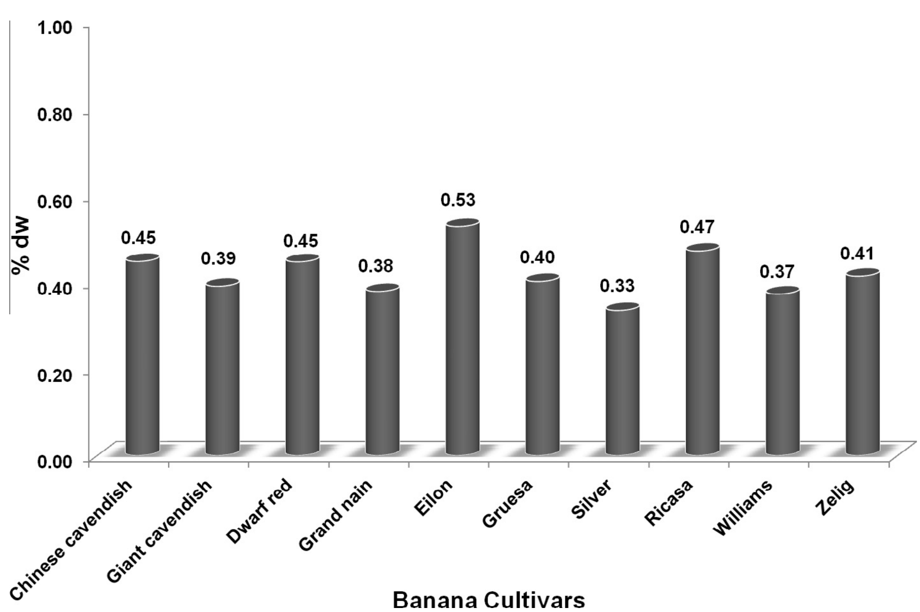

Fig. 1. Lipophilic extractive yields in dry material % for each ripe pulp from the studied cultivars.