



## Comparative study of free and glycoconjugated volatile compounds of three banana cultivars from French West Indies: Cavendish, Frayssinette and Plantain

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### ABSTRACT

Free and glycoconjugated volatile composition of three bananas from cultivars of *Musa* sp. grown in the French West Indies (FWI): Cavendish, Frayssinette and Plantain were investigated. They were extracted from the pulp of bananas thanks to an accelerated solvent extraction method for the free volatile compounds. Glycosides were isolated from aqueous extracts then aglycons were obtained after enzymatic hydrolysis of glycosidic extracts. Free volatile compounds and aglycons were analysed by GC–MS. The main volatile compounds found in Cavendish banana were (*E*)-2-hexenal and acetoin, in Plantain: (*E*)-2-hexenal and hexanal, and in Frayssinette: 2,3-butanediol and two diastereomer solerols. The most abundant of aglycons were 3-methyl-butanol, 3-methyl-butanoic acid, solerol (two diastereoisomers) and acetovanillone. This compound, rarely identified in fruits, is detected for the first time in glycoconjugated volatile compounds of fruits. The abundance of these two isomers in the extracts of Frayssinette seemed to be characteristic of this variety of banana.

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### 1. Introduction

In French West Indies (FWI), circa 9100 ha are dedicated to the culture of bananas. In 2009, the production amounted to a total of 251,000 tons. Two main products are to be distinguished.

Dessert bananas of the Cavendish sub-group, the typical sweet dessert banana measuring 15–17 cm long, is the main cultivar both economically and in production volume and almost all of this production is exported to the European Union.

Bananas of the Plantain sub-group, cooking bananas, long and oval measuring 20–25 cm long, are essentially consumed locally. In FWI, efforts have been made to develop other cultivars that would add market value, for instance the variety Frayssinette, Sucrier sub-group.

The Frayssinette banana which is considered as a dessert banana (Aurore, Parfait, & Fahrasmane, 2009; Fahrasmane, Ganou, & Aurore, 2007), has only recently been exported. This banana is small-sized, 8–10 cm long, very pleasant in mouth, very sweet and distinguished through a fruity and soft aroma.

In general, the aromatic properties of banana have contributed to its attractiveness as a fresh fruit and make it a valued ingredient in culinary preparations, pastries and cream products. Many studies have been performed on the aromatic components of fresh des-

sert banana (*Musa acuminata*, *Musa sapientum*, *Musa cavendish*) (Baldry, 1982; Boudhrioua, Giampaoli, & Bonazzi, 2003; Cosio & René, 1996; Engel, Heidlas, & Tressl, 1990; Macku & Jennings, 1987; Maltini & Giangiaco, 1976; Mattei, 1973; Quast, 1976). The volatile profile of dessert banana has been established using different extraction and analytical methods (Brat et al., 2004; Pérez, Cert, Rios, & Olias, 1997; Schiota, 1993), on different varieties from Ivory Coast (Cosio et al., 1996), from Madeira (Nogueira, Fernandes, & Nascimento, 2003; Quast, 1976), from Cuba (Pino, Ortega, Marbot, & Aguero, 2003) and from Honduras (Jordan, Tandon, Shaw, & Goodner, 2001). As a result of these studies, 246 volatile components have been identified: 57 alcohols, 10 aldehydes, 10 ketones, 39 acids, 112 esters, eight bases but only 12 of them contribute significantly to banana aroma (five acetates, four butyrates and three alcohols).

Many studies have determined the sensorial contribution of each component to the aroma of Cavendish banana. The ester fraction contributes to fruity note (Brat et al., 2004; Pérez et al., 1997; Salmon, Martin, Renaud, & Fourel, 1996). 3-Methylbutyl acetate, isoamyl butanoate and isoamyl isovalerate were reported to be key components of the banana's fruity odour (Cosio et al., 1996; Jordan et al., 2001; Quast, 1976; Schiota, 1993). Salmon et al. (1996) showed that isoamyl acetate, isoamyl butyrate, and pentan-2-one are the esters which are characteristic of geographic origin of the Cavendish variety. The 3-methylbutyl butanoate is the predominant ester of banana Cavendish (Nogueira et al., 2003).

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Carbonyl and alcoholic compounds contribute to the green-woody and herbal note (Engel et al., 1990; Schiota, 1993). The aroma was associated with compounds such as 3-methyl, 1-methylhexyl, 2-methylpropyl (or isobutyl), 3-methylbutyl (or isoamyl), and hexyl esters of acetic, butanoic and isovaleric acids (Cosio et al., 1996; Schwab, Davidovich-Rikanati, & Lewinsohn, 2008). Also, recent works showed that endogenous esters could be formed during the extraction of the volatiles (Birtić, Ginies, Causse, & Renard, 2009).

A significant proportion of potential contributors to flavour has been reported to occur as non volatile compounds, most often as glycosides in which a glycosyl unit is attached through a  $\beta$ -glycosidic linkage to an aglycon (Stahl-Biskup, Intert, Holthuijzen, Stengele, & Schulz, 1993). Glycoconjugated volatiles have been reported for Valeria and Pequena enna variety bananas from Madeira (Pérez et al., 1997).

So far, no studies have been carried out on the volatile compounds of the other bananas cultivated in FWI. Therefore, the purpose of this work was to identify the major free and glycoconjugated volatile compounds in Plantain and Frayssinette, two banana cultivars grown in the FWI and compare their aromatic potential with the one of the Cavendish variety.

## 2. Materials and methods

### 2.1. Fruit materials

Bananas of Cavendish, Frayssinette and Plantain cultivars were obtained from plants grown in Guadeloupe (FWI). Cultivar “Cavendish” was harvested at 90 days after flowering (the commercial harvesting stage). Cultivars Frayssinette and Plantain were harvested between 90 and 100 days after flowering (agricultural customs of this region). After postharvest, the fruits (15–30 fingers) were kept for 24 h in chambers ventilated with humidified air, then exposed to 10,000 ppm of acetylene for 24 h at 20 °C in the dark. They were stored for 11 days in a relative humidity of about 85% at a temperature of 21–25 °C. Samples (3 kg) were taken from these mature fruits, being yellow with brown spots (maturation degree seven according to the commercial peel colour scale of Chiquita®, Brands, Inc.). Maturity of the fruits was confirmed by measuring fruit colour and firmness. Firmness was measured using a TA-XT2 texturometer (Lloyd Instruments) (1–4 N/s). Colour was measured with a Minolta CR-200 model tristimulus colorimeter (value  $a^*$  between 0 and 3).

From each variety, three samples (3 × 362 g) were constituted, as replicate materials, by pooling five (for Cavendish and Plantain) or 10 (for Frayssinette) bananas each, i.e. a total of 15–30 fruits for analysis of volatiles and for extraction of glycosides. The fruits were peeled and their pulp was sliced, frozen in liquid nitrogen and stored at –80 °C for subsequent analysis.

### 2.2. Extraction of free volatile compounds

Frozen pulps (362 g) were partially thawed and blended at room temperature for approximately 1 min to obtain a homogeneous paste. Approximately 12 g of each paste were mixed with Hydromatrix (Varian, Les Ulis France), a drying agent consisting of pelletised diatomaceous earth material, used in a ratio of 1:1 (w/w), and 32  $\mu$ g of 4-nonanol as internal standard. Volatile compounds were extracted with a Dionex ASE 200 (Dionex, Voisins Le Bretonneux, France), using a combination of elevated pressure and temperature with dichloromethane as extraction solvent. The extraction conditions were the following: temperature: 40 °C – pressure: 100 bars – heat-up time: 5 min; static time: 5 min; flush

volume: 60% of cell volume (15 ml cell size); purge time: 60 s; static cycle: 1; total extraction time: 12 min per sample.

The volatile extracts obtained were dried over anhydrous sodium sulphate, concentrated to 2 ml on a Kuderna-Danish evaporator and then to 1 ml under a gentle stream of purified nitrogen. Three extractions were done for each sample. Volatile extracts analyses were performed in triplicate.

### 2.3. Extraction of glycoconjugated volatile compounds

#### 2.3.1. Isolation of glycosidic extracts

The rest of the frozen pulp paste (350 g) was slurred with 700 g deionised water. The mixture was then centrifuged (13,000g, 30 min, 4 °C). The supernatant was vacuum filtered using Celite 11 g (Interchim SA, Montluçon France) and Whatman No. 1 filter paper. The juice obtained was passed through a 20 × 50 mm column packed with Amberlite XAD2 (Supelco, Bellefonte, USA) as reported by Pérez et al. (1997). After column washing with 200 ml of distilled water then 75 ml of pentane/dichloromethane (2:1 v/v), the glycosides were extracted by eluting with 50 ml of methanol. The methanolic fraction was concentrated under reduced pressure to dryness and re-dissolved in 5 ml of 0.2 M citrate-phosphate buffer (pH 5.0). Remaining volatile compounds were removed by pentane-dichloromethane extraction (2:1 v/v; 4 × 2 ml). Glycosidic extracts were then cleaned of residual extraction solvent by a gentle stream of purified nitrogen. They were supplemented with 20  $\mu$ g of an internal standard (4-nonanol).

#### 2.3.2. Enzymatic hydrolysis

To release aglycons, the glycosidic extracts were incubated at 38 °C, over 48 h, with an enzymatic preparation of pectinase AR2000 (40 mg/ml citrate-phosphate buffer), as reported by Günata, Dugelay, Sapis, Baumes, and Bayonove (1993) and Sarry, Gil, and Günata (2004). AR 2000 is an enzymatic preparation, for pectolytic activities with secondary glycosidase activities, derived from *Aspergillus niger* and produced by DSM Food Specialties (Séclin, France) in agreement with the standards ISO 9002. It transforms the glycosidic non aromatic precursor compounds into free volatile (aglycons). The aglycons were extracted three times with 2 ml dichloromethane. The organic layer was dried over anhydrous sodium sulphate, concentrated to a final volume of 1 ml under a gentle stream of purified nitrogen. The extracts were stored at –20 °C until analysis. All analyses were performed in triplicate.

### 2.4. Analysis and identification of free and glycoconjugated volatile compounds

Samples of volatile extracts or aglycons (2  $\mu$ l) were injected in the port of a GC–MS (CP2010; Shimadzu, Kyoto) with a CPWAX 52 CB capillary column (10 m, 0.1 mm i.d., 0.2  $\mu$ m film-thickness) for free volatile and (30 m, 0.25 mm i.d., 0.5  $\mu$ m film-thickness) for aglycons. The injection port (240 °C) was operated in splitless mode for the first 30 s, and then the carrier gas (He) velocity was constant at 35 cm s<sup>-1</sup>. The initial oven temperature of 35 °C was held for 2 min and then ramped 5 °C per min to 230 °C. This final temperature was held for 10 min.

The mass spectrometer was operated in the electron impact mode at 70 eV with continuous scans (every 0.25 s) from mass to charge ratio ( $m/z$ ) 29–250. Data were collected with GC–MS Solution Software.

The levels of compounds were expressed as  $\mu$ g 4-nonanol equivalent/kg of pulp banana fresh weight by integrating the total ion current. The concentrations were considered as relative estimates of recoveries after extraction and calibration factors related to the standards were not determined. Note that all data are presented on a fresh weight basis.

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