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Analytical Methods

Odour-active compounds in papaya fruit cv. Red Maradol

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ABSTRACT

Application of solid-phase microextraction and simultaneous distillation–extraction combined with GC–FID, GC–MS, aroma extract dilution analysis, and odour activity value were used to analyse volatile compounds from papaya fruit cv. Red Maradol and to estimate the most odour-active compounds. The analyses led to the identification of 137 compounds; 118 of them were positively identified. Twenty-five odorants were considered as odour-active compounds and contribute to the typical papaya aroma, from which ethyl butanoate, benzyl isothiocyanate, 1-hexen-3-one, (*E*)-β-ionone, and methyl benzoate were the most odour-active compounds.

1. Introduction

Papaya (*Carica papaya* L.) is a native fruit of tropical America, but it is currently disseminated throughout the tropics. It is a very popular fruit with consumers for its high content of sugar, vitamin C and carotenoids, as well as for its pleasant aromatic odour (Bari et al., 2006). Many cultivars are grown in various parts of the world and are known to vary markedly in their flavour characteristics, including cv. Red Maradol, which has shown great success in cultivation and has become a prominent papaya for ripe consumption.

Analytical research on the aroma compounds of this fruit carried out for 40 years was summarised and published by TNO (1996) and reviewed by different authors (Nursten, 1970; Ortega & Pino, 1997; Shibamoto & Tang, 1990; Winterhalter, 1991). More than 300 volatile constituents have been identified in fresh fruit; however, only some of them have been recognised as papaya flavour contributors. Pino, Almora, and Marbot (2003) used GC–olfactometry (GC–O) to evaluate volatiles isolated from cv. Red Maradol papaya and found that esters from short-chain acids contributed to the fruit aroma. Jirovetz, Buchbauer, and Shahabi (2003) analysed by solid-phase microextraction combined with GC–O the volatile profile from unripe and ripe fruits grown in Cameroon. They concluded that papaya fruit aroma is the result of a combination of high concentration of linalool and short-chain alcohols and esters (exotic fruity, floral odour is additionally known from linalool and its derivatives), plus some C6 compounds (e.g., (*E*)-3-hexen-1-ol) in medium or low amounts (green-notes of unripe fruit), while a pungent-sour odour impression, found in the unripe fruit, is due to benzyl isothiocyanate (mustard-oil-aroma) in high amounts. The aroma of diluted benzyl isothiocyanate was described as fruity and papaya-like (Fischer, 1996). Recently, Ulrich and Wijaya (2010) analysed the volatiles from four Indonesian and one Brazilian papaya cultivars by GC–MS and GC–O and found that character impact odorants were hexanal (herbaceous), (*Z*)-2-penten-1-ol (chemical), nonanal (herbaceous), (*Z*)-linalool oxide (floral), linalool (flowery), dimethyl sulfoxide (sweetish), butanoic acid (stinky), verbenone (flowery), phenylmethyl butanoate (sweetish, floral), δ-octalactone (flowery), and benzyl isothiocyanate (smokey).

It is important to identify the trace compounds contributing significantly to a food aroma. For this purpose, it is necessary to achieve proper isolation (using adequate solvent and solventless methods) and identification of odour-contributing constituents in combination with sensory evaluation of the fruit and its individual components. It has been shown for a considerable number of foods that all the volatiles present are not able to interact with human olfactory receptors. Instead, only a smaller number of the so-called key odorants is obviously detected by the human odorant receptors and, consequently, participate in the creation of the respective aroma impression in the brain (Schieberle, 1995). An approach to separate odour-active volatiles from the bulk of odourless food volatiles is GC–O on serial dilutions of aroma distillates, by, for example, aroma extract dilution analysis (AEDA) (Schieberle, 1995). Dilution-based odour threshold techniques, such as AEDA, are useful methods for the screening of important odorants in foods, but these methods neither permit a study on the influence of the food matrix on odorant binding nor on the interactions of odorants when matching the overall odour impression of the food.

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These limitations are resolved when the concentrations of the individual odorants are correlated with the respective odour thresholds using the odour activity value (OAV) concept (Schieberle, 1995).

Because a knowledge of the key odorants in the final product is a prerequisite for studies on the influence of processing steps, the aim of the present study was to determine aroma profile and odour-active compounds of papaya cv. Red Maradol, which is considered the most aromatic Cuban papaya, by application of the aroma extract dilution analysis and odour activity values.

2. Materials and methods

2.1. Fruits

Papaya fruits cv. Red Maradol were harvested at three-quarters-ripe stage (yellow with trace of green) in the experimental station of the Tropical Fruit Institute in Alquizar (Cuba) from the 2012 crop season and immediately transported to the laboratory. Fruits were incubated to ripen at ambient temperature (26–33 °C) and 70–85% RH until full ripening (fully yellow) at the fifth day. Fruits were cut longitudinally, and the peel, placenta and seeds were removed. The pulp was homogenised using a blender. Three batches of three fruits each were prepared and immediately used for subsequent analyses.

2.2. Chemicals and reagents

Standards of chemicals were purchased from Sigma–Aldrich (St. Louis, MO) and Fluka (Buchs, Switzerland), and some were supplied by Dallant (Barcelona, Spain). An *n*-alkane solution (C_8–C_{32}) was purchased from Sigma–Aldrich. To this solution, a mixture of C_5–C_7 *n*-alkanes was added. Anhydrous sodium sulfate, sodium chloride and diethyl ether were purchased from Merck (Darmstadt, Germany); the solvent was redistilled and checked for purity.

2.3. Standard chemical analysis

Soluble solids, total acidity (as anhydrous citric acid) and pH value were performed in the fruit pulp according to standard methods (AOAC, 1997).

2.4. Headspace solid-phase microextraction (HS-SPME) analysis

Volatile compounds from the fresh fruit homogenate headspace were extracted using four SPME fibre coatings: 100 μm PDMS, 65 μm PDMS/DVB, 50/30 μm DVB/CAR/PDMS, and 85 μm CAR/PDMS (Supelco, Bellefonte, PA). All the fibres were conditioned before use and cleaned between analyses by inserting them into the GC injector, where they were kept at the recommended temperature, and to prevent contamination, were used immediately after conditioning. SPME extraction was performed at 40 °C on 3 g of stirred homogenate and 1 g NaCl contained in a 15-mL vial sealed with a PTFE-lined screw cap, at constant magnetic stirring (600 min^{-1}). A pre-extraction time of 10 min and an extraction time of 30 min were applied. Analyses were made in triplicate. The sampling conditions were chosen after preliminary GC–FID analyses and were similar to those reported in other fruit studies (Marković, Vahčić, Ganić, & Banović, 2007; Pino & Febles, 2013; Quijano & Pino, 2009; Thaiphanit & Anprung, 2010; Wang, Zhi, Chen, Bao, & Yang, 2007).

2.5. Isolation of volatile compounds by simultaneous distillation–extraction (SDE)

Volatile compounds were isolated according to a previously reported procedure (Pino, 2012; Pino, Mesa, Muñoz, Martí, & Marbot, 2005). Two hundred grams of fresh papaya homogenate were mixed with 600 mL of distilled water; 1.5 mg of methyl nonanoate was added as internal standard, and the volatiles were isolated by means of SDE using 25 mL of diethyl ether (previously redistilled and checked as to purity) for 1 h. The aroma extract was dried over anhydrous sodium sulfate and concentrated to 0.6 mL in a Kuderna-Danish evaporator with a Vigreux column (12 × 1 cm) and then to 0.2 mL with a gentle nitrogen stream. Extractions were made from each of the three batches. The recovery and repeatability of the extraction procedure was tested for some compounds (1-butanol, 3-methyl-1-butanol, methyl butanoate, ethyl butanoate, methyl hexanoate, limonene, and benzyl isothiocyanate). Triplicate analyses were performed. The average recovery was 70%, and the relative standard deviations were <10%.

2.6. GC–FID and GC–MS analysis

A Konik 4000A gas chromatograph (Konik, Barcelona), equipped with a 30 m × 0.25 mm × 0.25 μm HP-5 ms (Agilent, Santa Clara, CA) or DB-Wax column (30 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA) fused-silica capillary columns and with a flame ionization detector (FID) was used. Oven temperature was held at 50 °C for 2 min and then raised to 280 °C at 4 °C min^{-1} and held for 10 min. Carrier gas (hydrogen) flow rate was 1 mL min^{-1}. Injector and detector temperatures were 250 °C. For the SDE extracts 1 μL was injected in 1:10 split mode and for SPME extracts splitless mode (2 min) was applied. The retention times of a series of *n*-alkanes (C_8–C_{32}) was used to calculate the retention indices for all identified compounds and for reference standards. Concentrations were expressed as mg methyl nonanoate equivalents kg^{-1} of fresh weight, response factors being taken as 1.0 for all compounds with reference to the internal standard and a recovery factor of 70% was considered. All analyses were replicated three times.

GC–MS analyses were performed on a Hewlett–Packard 6890N series II (Agilent) gas chromatograph with a similar fused capillary column as for the GC–FID. The temperature program and carrier gas flow rate were the same, as for the GC–FID. Electron impact mass spectrometry was performed, with electron energy of 70 eV and ion source and interface temperature of 250 °C. The acquisition was performed in scanning mode (mass range *m*/*z* 35–400). Compounds were preliminarily identified by use of NIST 05, Wiley 6, NBS 75 k, Adams (2001), and in-house Flavorlib libraries, and then the identities of most were confirmed by comparison of their linear retention indices with those of reference standards or with published data (Adams, 2001).

2.7. SPME with GC–O

A Hewlett–Packard series 6890N GC (Agilent Technologies) connected with a fused silica capillary tube (25 cm × 0.25 mm i.d.) to an ODO II sniffing port (SGE International Pty. Ltd., Ringwood, Australia) was used. The flow rate of the carrier gas (H_2) was 25 mL min^{-1}, and the oven temperature was kept at 250 °C. The SPME extracts were introduced into the GC port (splitless mode for 2 min, injector temperature at 250 °C). Because no chromatographic separation was carried out by the short capillary, volatile compounds arrived simultaneously at the sniffing port. Here, for each SPME extract, a trained panel of three assessors perceived and evaluated the resulting global odour. Fibres were kept in the GC inlet until the end of the sensorial stimulus. Sensory analysis sessions were performed only after a suitable training: panellists