Food Chemistry 127 (2011) 689–693

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Volatile compounds of leaves and fruits of *Mangifera indica* var. *coquinho* (Anacardiaceae) obtained using solid phase microextraction and hydrodistillation

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ARTICLE INFO

Article history:
Received 4 February 2010
Received in revised form 29 October 2010
Accepted 29 December 2010
Available online 8 January 2011

Keywords:
Mangifera indica var. *coquinho*
Essential oil composition
Solid phase microextraction
Hydrodistillation

ABSTRACT

Volatile compounds present on fruits and leaves of *Mangifera indica* var. *coquinho* were investigated by headspace solid phase microextraction (HS-SPME) and hydrodistillation (HD). Conventional techniques, such as hydrodistillation, may impart chemical changes to the original oil composition being also time-consuming. On the other hand, HS-SPME provides solvent-less extractions, shorter extraction times and may supply complementary information about the composition of the compounds. The HS-SPME technique was previously evaluated by the comparative study among the fibres: commercial PDMS, NiTi-ZrO_2 and NiTi-ZrO_2-PDMS. The fibre NiTi-ZrO_2-PDMS showed better sensitivity and precision and was used on the extraction of components. The influence of several parameters like the time and temperature of extraction and desorption time were examined to obtain better efficiency. Fruits and leaves were analysed in mature and immature stages. The profiles corresponding to the volatile compounds detected by both techniques are discussed.

1. Introduction

Original from the east, *Mangifera indica* is an exotic tree that was very well adapted in Brazil. It is considered one of the best tropical fruit, popularly known as mango. Ethnobotanical studies indicate that *M. indica* is widely used in Brazil to treat back ache and bronchitis (Albuquerque et al., 2007). The use of medicinal plants is a direct consequence of ancient habits involving the search of natural healing against sickness and pathologies. These plants have active substances in their composition with high therapeutic potential. The essential oils, like some of those substances, are extremely powerful. The solid phase microextraction (SPME) is an efficient technique used on the extraction of volatile oils. Introduced by Arthur and Pawliszyn (1990), it is a solvent-free sample preparation technique for the extraction of volatile and semi volatile compounds (Bicchi, Cordero, Liberto, Sgorbini, & Rubiolo, 2007; Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2003). The nature of the fibre coating strongly influences the HS-SPME sampling, as well as physical factors such as headspace equilibration temperature, extraction time and analyte diffusion rate from the vapour phase to the fibre surface (Bianchi, Nuzzi, Leva, & Rizzolo, 2007). The hydrodistillation (HD) was used as a complementary technique for the elucidation of possible misinterpretations as a result of processes that may promote artifact formation (Schossler, Schneider, Wunsch, Soares, & Zini, 2009). The volatile oil composition of fruits and leaves from *M. indica* var. *coquinho* has been reported previously, but analysed only through one method of extraction, the HD and at one stage of maturation (Helena et al., 2000; Torres, Talens, Carot, Chiralt, & Escriche, 2007). Simionatto, Peres, Hess, Silva, and Chagas (2010) found for the first time an interesting anticancer activity in the leaf oil of *M. indica* var. *coquinho*, suggesting the presence of very active sesquiterpenes. In this context, the aim of the present work is to compare the volatile oil composition of fruits and leaves collected during different periods of vegetation. The results show the differences and similarities obtained by two extraction techniques, the hydrodistillation and HS-SPME. Budziak, Martendal, and Carasek (2007a, 2008) reported the preparation and application of two new fibres used in HS-SPME technique, the NiTi-ZrO_2 and NiTi-ZrO_2-PDMS which have as main characteristics thermal stability and excellent sensitivity. They were successfully applied in the extraction of some analytes (Budziak, Martendal, & Carasek, 2007b) and here are evaluated on the extraction of essential oils. The parameters of HS-SPME technique were optimised to improve analysis efficiency.

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doi:10.1016/j.foodchem.2010.12.123

2. Experimental

2.1. Plant material

The leaves and fruits of *M. indica* var. *coquinho* were collected in September 2008 (immature period) and February 2009 (mature period), from the biological reserve of Federal University of Mato Grosso do Sul, in the town of Campo Grande, Mato Grosso do Sul state, Brazil. Voucher specimens (20206) were collected at the Herbarium of Federal University of Mato Grosso do Sul. Geographic coordinates of the biological reserve of UFMS are 30° 10′ 47″ S and 51° 23′ 33″ W. Leaves and fruits were sampled of the same bush in the two seasons. After collected, the samples were immediately fractioned in two parts and submitted to hydrodistillation and HS-SPME.

2.2. Hydrodistillation

Fresh leaves (200 g) and fruits (150 g) were subjected to hydrodistillation in a modified Clevenger apparatus for 4 h, followed by exhaustive extraction of the distillate with hexane. Anhydrous sodium sulphate, previously heated to 400 °C, was employed to eliminate essential oil humidity. After removal of the solvent, the average yield of the crude oils was 0.12%.

2.3. HS-SPME

2.3.1. Fibre selection

The experiments were performed using a SPME holder and fibre assemblies for manual sampling (Supelco, Bellefonte, PA, USA). Three different fibres were evaluated for the extraction of the essential oil as follows: commercial fibre of polydimethylsiloxane (PDMS, 100 μm) and the two new fibres, NiTi-ZrO_2 (1.35 μm) and NiTi-ZrO_2-PDMS (35 μm) prepared in the Laboratory of the Federal University of Santa Catarina (Budziak, Martendal, & Carasek, 2007a; Budziak, Martendal, & Carasek, 2008). Before measurements the fibres were conditioned according to Supelco's recommendations.

The experiment for the selection of the fibre was performed with 0.1 mg of fresh chopped leaves inside 4 mL amber vials capped with PTFE-coated septa, at temperature controlled of 30 °C and headspace extraction of 5 min. Desorption of the analytes was carried out at 240 °C during 60 s. Fig. 1 shows the results for the extraction efficiency evaluated by the peak areas in chromatograms obtained with the three studied fibres. The NiTi-ZrO_2-PDMS (35 μm) fibre has a higher adsorptive capacity compared to the other fibres evaluated. It proved sensitivity and precision, with coefficient of variation (CV%) smaller than 10%, and was chosen for the study of the chemical composition of the essential oil of leaves and fruits of *M. indica* var. *coquinho*.

2.3.2. Optimisation of HS-SPME method

Using the NiTi-ZrO_2-PDMS fibre the following parameters that could affect the extraction efficiency were optimised: mass of sample, extraction time and temperature and desorption time. Fruit's experiments were performed with masses of 10 and 100 mg of sample and leaves' experiments were performed with 1, 10, 50 and 100 mg of sample. The effect of the temperature on the extraction process was evaluated by testing successive conditions at room temperature (30 °C), 40, 50 and 60 °C. The influence of the exposure time of the fibre was studied in experiments performed at 15, 30, 45, and 60 min. The time required for desorption of the substances from the fibre coating was determined testing the times of 30, 60, 90 and 120 s at an injector temperature of 250 °C. For each experiment, at least three replicates of extraction were performed.

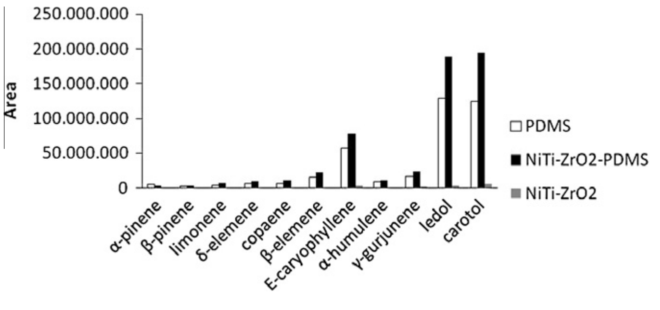

Fig. 1. Comparison of the fibers on the extraction of some substances.

2.4. Chromatographic analysis

The HS-SPME/GC-MS analyses were performed with Varian GC–MS system comprising a CP-3900 gas chromatograph (Walnut Creek, CA, USA) with 1177 injector and ion-trap mass spectrometer (Saturn 2100-T/MS/MS). Chromatographic separation was performed on a Factor Four VF-5 ms fused-silica capillary column (30 m × 0.25 mm, d_f 0.25 μm), from Varian (Walnut Creek, CA, USA). A SPME liner (72 mm × 0.75 mm i.d) purchased from Varian was used. The initial temperature of oven was of 50 °C (2 min) and increased to 250 °C at 3 °Cmin^{-1}, and the injector was kept at 250 °C. Helium (99.999% purity) was used as carrier gas at a constant flow of 1.0 mL min^{-1}. The temperatures of the manifold, GC–MS interface and the ion trap were 50, 250 and 200 °C, respectively. The MS scan parameters included electron impact ionisation voltage of 70 eV, mass range of 40–450 m/z and scan interval of 0.5 s. Saturn GC/MS 5.52 workstation software was used for instrument control and data treatment. The HS-SPME/GC-MS analyses were made in the splitless mode, 60 s closed valve, 15 min more with the split valve open (ratio 1:50) to clean the fibre, followed by a split ratio of 1:20 to the analysis end. The retention times of n-alkanes to calculate retention index were obtained of headspace extraction performed during 30 min, at 60 °C, of a solution with C_9–C_{22} in n-hexane.

Analyses with injection of 1 μL of hexanic solutions of essential oils were made in the split mode (1:20) in the same chromatographic conditions used in the HS-SPME/GC-MS analysis.

Linear temperature programmed retention indexes (RI) were calculated using the retention data of linear alkanes (C_9–C_{22}), along with retention data of the substances of the essential oils. The identification of the volatile components was based on comparison of their mass spectra with those of NIST 2.0 and Saturn Libraries and those described by Adams (1995), as well as by comparison of their retention indexes with literature data.

2.5. Statistical analysis

Statistical analysis was performed using the MINITAB 14 for Windows statistical software to characterise and describe the homogeneity among oils samples of two stages of maturation. Cluster's statistical analysis with Average Linkage and Euclidean Distance was applied in the normalised percentage value of the substances. The similarity index was calculated as similarity$_{ab}$ = $(1 - d_{ab}/d_{max}) \times 100$, where d_{ab} is the Euclidean distance of samples a and b, and d_{max} is the largest Euclidean distance in the data set.

3. Results and discussion

The analytical parameters adopted for the analysis of fruits and leaves were 45 min (extraction time), 40 °C (extraction temperature), 60 s (desorption time) and 50 mg of leaves and