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Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry of monoterpenoids as a powerful tool for grape origin traceability

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Abstract

The establishment of the monoterpenoid profile of *Vitis vinifera* L. cv. 'Fernão-Pires' white grape was achieved by headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC × GC–ToF-MS). The plot of the first dimension *versus* the second dimension retention times using the *m/z* 93, 121, and 136 was used. The grapes were found to contain 56 monoterpenoids identified by GC × GC–ToF-MS. From these, 20 were reported for the first time in grapes. According to their chemical structure, the compounds were organized in different groups: monoterpene hydrocarbons and monoterpene oxygen-containing compounds, this later divided in oxides, alcohols (monoterpenols and monoterpendiols), aldehydes, esters, and ketones. A database composed by the retention indices of monoterpenoids calculated in the bi-dimensional column set was created, representing a developmental step in monoterpenoid analysis using a GC × GC system. Remarkable results were also obtained in terms of compound classification based on the organized structure of the peaks of structurally related compounds in the GC × GC contour plot. This information represents a valuable approach for future studies, as the ordered-structure principle can considerably help the establishment of the composition of samples. This study proposes a methodology and provides data that can be applied to determine the monoterpenoid profile of grapes, and its extension to the analysis of musts, and wines. As monoterpenoids are secondary metabolites whose synthesis is encoded by variety-related genes, the terpenoid profile may be used as a way to trace its varietal origin.

Keywords: *Vitis vinifera* L.; Fernão-Pires; White grapes; Monoterpenoids; HS-SPME; GC × GC–ToF-MS

1. Introduction

Several studies carried out on grapes characterization recognized a relationship between the wine varietal character and the grape and musts volatile and semi-volatile compounds, namely monoterpenoids [1–6]. Thus, these compounds play an important role in the differentiation of wine varieties [3]. Different types of monoterpene compounds have been reported to be present in grapes, which include monoterpene hydrocarbons and monoterpene oxygen-containing compounds, particularly monoterpenols, monoterpendiols, and monoterpene possessing cyclic structures [6]. The monoterpenols appear as the dominating group, especially in white varieties, represented by linalool, hotrienol, α-terpineol, geraniol, and nerol [3,6]. These compounds, which contribute to the varietal characteristics, have specific aroma descriptors: linalool has characteristic citrus-like, sweet and flowery notes, hotrienol, α-terpineol, and geraniol exhibit flowery and sweet aromas [1,3,7], and nerol has a rose scent [8]. The monoterpendiols are the polyhydroxylated forms of the monoterpenes, being 3,7-dimethyl-1,5-octadien-3,7-diol (terpendiol I) and 3,7-dimethyl-1,7-octadien-3,6-diol (terpendiol II) the most widespread in grapes. These compounds make no direct contribution to the aroma, although some of them are reactive and can breakdown to give pleasant volatiles. For example, terpendiol I is odourless but represent a major potential source of hotrienol by dehydration at wine pH [6,9]. As con-

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cerns the monoterpene cyclic structures, the pyran and furan forms of the linalool oxides were the main compounds detected in *Vitis vinifera* L., which may contribute to the floral and citrus aromas [6,10]. The different monoterpenoids may appear in the free and odourant form, and/or in a glycosidically-linked and odourless form. They are located essentially in the skin of the grape [6].

The monoterpenoids of grapes are generally present in trace amounts ($\mu g\,kg^{-1}$) and their analyses require a previous step of isolation and/or concentration. In the last years, fast, simple and solvent-free methodologies have been used, such as solid-phase microextraction (SPME) [11,12] and stir bar sorptive extraction [13]. This step is normally followed by a capillary one-dimensional gas chromatography coupled with quadrupole mass spectrometry detection (GC–qMS). In spite of the great separation power of the conventional one-dimensional modern chromatographic techniques, the complex nature of the samples, including different kinds of chemical classes beyond monoterpenoids, such as aromatic and aliphatic alcohols, sesquiterpenoids, and C_{13} norisoprenoids [11–13], requires extended GC runs. Furthermore, deep analyses of the chromatograms frequently indicate that some peaks are the result of two or more co-eluting compounds. As a consequence of chromatographic co-elution, reliable MS identification is not possible.

One-dimensional chromatographic processes are widely applied in the analysis of food products. Although such methods often provide rewarding analytical results, the complexity of many naturally occurring matrices exceeds the capacity of any single separation system. As a consequence, in the past years considerable research has been dedicated to the combination of independent techniques with the aim of strengthening resolving power [14]. Comprehensive two-dimensional gas chromatography ($GC \times GC$) employs two orthogonal mechanisms to separate the constituents of the sample within a single analysis. The technique is based on the application of two GC columns coated with different stationary phases, such as one apolar and one polar, connected in series through a special interface (modulator). The interface cuts small (several seconds) portions of the first dimension eluate by cryofocusing, and re-injects it onto the second column. Each first dimension peak is modulated several times, which allows the preservation of the first dimension separation. The second column is very short and narrow and consequently each modulated portion is "*flash*" separated before the next modulation starts. Using this instrumental approach, compounds co-eluting from the first column undergo additional separation on the second one [15]. Therefore, the separation potential is greatly enhanced when compared to the one-dimensional GC. Besides chromatographic separation, sensitivity and limits of detection are also improved due to the focusing of the peak in the modulator and the separation of analytes from chemical background [16]. $GC \times GC$ also offers new opportunities to develop relationships between molecular structure and retentions in the two-dimensional separation space defined by the $GC \times GC$ retention in the combined dimensions [17].

Since the second column produces peaks as narrow as 0.1 s, a detection technique must be fast enough to describe the peaks properly. This represents a problem for classical scanning mass spectrometers, which are capable of scanning rates up to 50 spectra s^{-1}. On the other hand, the high-speed time-of-flight mass spectrometry (ToF-MS), with the maximum acquisition rates of 500 spectra s^{-1}, provides sufficient data density to address the requirements of $GC \times GC$ separations [15]. Besides that, ToF-MS brings other advantages such as full mass spectra acquisition at trace level sensitivity and mass spectral continuity, which allows for deconvolution of spectra of co-eluted peaks. The $GC \times GC$ has recently been used for food analysis [14,16,18,19] and, more recently, for wines [20], although it is not yet applied on grapes characterization.

The aim of this study is to develop a methodology based on the headspace SPME (HS-SPME) coupled with comprehensive $GC \times GC$–ToF-MS in order to obtain a deep qualitative characterisation (profile) of the monoterpenoids of grape. Although this methodology allows to study the whole volatile and semi-volatile composition of the grapes, considering the complexity of the data obtained, this manuscript was focused only on the monoterpenoid's fraction. Thus, to reduce the complexity and the time of analysis, specific *m/z* and a $GC \times GC$ space characteristic of monoterpenoids were established. The one-dimensional GC–qMS detection mode was also applied as a comparative approach.

2. Experimental

2.1. Samples

Healthy mature-state *Vitis vinifera* L. cv 'Fernão-Pires' (FP) grapes from the 2002 harvest were collected in Bairrada Appellation, from Talhão da Avenida vineyard, in Portugal. Samples were transported immediately to the laboratory and were stored in a freezer at −80 °C until analysis.

2.2. HS-SPME methodology

The SPME coating fibre and the experimental parameters were established according to a methodology previously developed in our laboratory for the grape analysis [11]. The SPME holder for manual sampling and the fibre used were purchased from Supelco (Aldrich, Bellefonte, PA, USA). The SPME device included a fused silica fibre coating partially cross-linked with 65 μm Carbowax-divinylbenzene (CW-DVB). The SPME fibre was conditioned at 250 °C for 30 min in the GC injector, according to the manufacturer's recommendations. For headspace sampling, 50 g of grapes was crushed manually in a plastic bag and introduced into a 120 ml glass vial, which corresponds to a ratio of the volume of the liquid phase to the headspace volume ($1/\beta$) of 0.5. The vial was capped with a PTFE septum and an aluminium cap (Chromacol, Welwyn Garden City, UK). After the addition of 8 g of NaCl and stirring (25 × 5 mm bar) at 1000 rpm, it was placed in a thermostatted bath adjusted to 40.0 ± 0.1 °C for 60 min to promote the transference of the compounds from the sample to the headspace. After this step, the SPME fibre was manually inserted into the sample vial headspace for 60 min.