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Main differences between volatiles of sparkling and base wines accessed through comprehensive two dimensional gas chromatography with time-of-flight mass spectrometric detection and chemometric tools

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ABSTRACT

The main changes in the volatile profile of base wines and their corresponding sparkling wines produced by traditional method were evaluated and investigated for the first time using headspace solid-phase microextraction combined with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry detection (GC × GC/TOFMS) and chemometric tools. Fisher ratios helped to find the 119 analytes that were responsible for the main differences between base and sparkling wines and principal component analysis explained 93.1% of the total variance related to the selected 78 compounds. It was also possible to observe five subclusters in base wines and four subclusters in sparkling wines samples through hierarchical cluster analysis, which seemed to have an organised distribution according to the regions where the wines came from. Twenty of the most important volatile compounds co-eluted with other components and separation of some of them was possible due to GC × GC/TOFMS performance.

1. Introduction

Sparkling wines produced by the traditional method, similar to Champenoise method used for the production of Champagne, are prepared by double fermentation followed by ageing of the wine with the yeast in the bottle. The first fermentation transforms grape must into base wine; however the essence of traditional method is the second fermentation, which takes place in the bottle and increases the alcohol content and the internal bottle pressure up to 5–7 atmospheres. The base wine resulting from the first phase undergoes a second alcoholic fermentation inside a sealed bottle by adding a suspension of yeasts and sugar (*liqueur de tirage*) (Ribéreau-Gayon, Dubourdieu, Donche, & Lonvaud, 2007). This step is followed by ageing on yeast lees under anaerobic conditions for several months. Autolysis of the yeast occurs during this prolonged contact. This is a slow process, associated with cell death and involves hydrolytic enzymes that act to release cytoplasmic and cell wall compounds including peptides, fatty acids, nucleotides and amino acids. During ageing, the organoleptic and foam properties of wine are modified, reflecting changes in the wine composition (Alexandre & Guilloux-Benatier, 2006).

The aroma development in base wines and in the respective produced sparkling wine has been researched for rosé sparkling wines produced with the Garnacha Tinta grape variety in Spain (Hidalgo et al., 2004) and also for base and sparkling wine produced by a blending of *Vitis vinifera* varieties including Macabeo, Parallada and Xarel-lo in Spain (Pozo-Bayon, Pueyo, Martin-Álvarez, Martínez-Rodríguez, & Polo, 2003; Torrens, Riu-Aumatell, Vichi, López-Tamames, & Buxaderas, 2010). In these research works one-dimensional gas chromatography (1D-GC/MS) was used for the analysis of wine volatiles. A closer look at these 1D-GC results shows that there are many unresolved peaks, due to the high complexity of the samples. Two or more co-eluting compounds may prevent the achievement of a correct identification of these volatile compounds and this is especially cumbersome when traces of aroma active compounds are hidden by other co-eluting compounds. This means that important information on volatile composition may be missing and consequently misidentification and quantification of target compounds may occur. Furthermore, the complex nature of these samples, including compounds of different kinds of chemical classes requires long GC run times to obtain the

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maximum separation power (Bianchi, Careri, Mangia, & Musci, 2007; Rocha, Coelho, Zrostlikova, Delgadillo, & Coimbra, 2007). Comprehensive two-dimensional gas chromatography (GC × GC) is an excellent choice to reveal the composition of complex samples, as it allows the analysis of the whole sample in the same analysis time required for a normal 1D-GC run, providing the selectivity of two different stationary phases, along with superior sensitivity and structured 2D plots (Beens & Brinkman, 2005).

GC × GC has been used to determine volatile compounds in some types of grapes and wines, such as Muscat wines (Bordiga et al., 2013), Cabernet Sauvignon wines (Robinson, Boss, Heymann, Solomon, & Trengove, 2011), Pinotage wines (Weldegergis et al., 2011), Madeira wines (Perestrelo, Barros, Câmara, & Rocha, 2011) and Fernão-Pires grapes (Rocha et al., 2007). A former work of this research group on Merlot volatiles has shown the advantages of GC × GC/TOFMS through a detailed characterisation of Merlot volatiles and also with a preliminary approach of the use of multivariate analysis for discrimination of 24 wine samples according to their original grape cultivars (Welke, Manfroi, Zanus, Lazarotto, & Zini, 2012). Further work discriminated 54 Brazilian wines made from five different grape varieties (Welke, Manfroi, Zanus, Lazarotto, & Zini, 2013).

The production of sparkling wines by a traditional method is a long process encompassing many different steps, and most of them require a long time and expensive manual labour. Consequently, sparkling wine is a high added value product and winemakers are constantly seeking for product improvement, as customers are becoming more demanding regarding its quality. Changes of volatile compounds profile after second fermentation and ageing have not yet been investigated using GC × GC and may provide a better understanding of what is really happening during these important stages of sparkling wine production, unveiling the important volatiles that may be elected as markers for achieving a high aroma quality during vinification (Alexandre & Guilloux-Benatier, 2006).

The purpose of this study is, for the first time, to investigate the main changes that occur in the volatile profile of sparkling wines during their production, using GC × GC/TOFMS data and chemometric tools. The volatile compounds which are the main contributors to differences in base and sparkling wine are presented and discussed under the light of process changes and their potential odour contribution.

2. Material and methods

2.1. Samples, analytical reagents, and supplies

The samples (12 Chardonnay base wines and their respective sparkling wines) were provided by Empresa Brasileira de Pesquisa Agropecuária Uva e Vinho (EMBRAPA) in sealed 750-mL bottles and were chosen as the best wine samples in the "National Evaluation of Wines of 2010" event promoted by the Brazilian Association of Enology. The samples were produced in wineries from cities of Rio Grande do Sul, the most southern state of Brazil: Farroupilha, Caxias, Bento Gonçalves, Garibaldi, Pinheiro Machado and Pinto Bandeira. Sparkling wines have undergone nine months of ageing in contact with lees. Two wine bottles were randomly collected from different production batches in each winery and a sample of each one of them was chosen for extraction and analysis of volatiles that represented that specific wine.

Model wine was prepared with (+)-tartaric acid (6 g L^{-1}) supplied by Synth (São Paulo, Brazil) and 10% of ethanol in MilliQ deionised water. Twenty-two standard compounds were purchased from Aldrich (Steinheim, Germany) and individual stock solutions of each component were prepared in double-distilled ethanol purchased from Nuclear (São Paulo, Brazil). These compounds were employed for positive identification of volatile compounds. The final concentrations of each one of the 22 standard compounds in the model wine solution are listed between parentheses, as follows: ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl decanoate, diethyl succinate and propanol (1000 μg/L of each standard compound), ethyl propanoate, ethyl 2-methylpropanoate, ethyl lactate, ethyl octanoate, ethyl 3-hydroxybutanoate, hexanol, isoamyl acetate, α-terpineol and eugenol (100 μg/L of each standard compound); ethyl 2-methylbutanoate and 2-phenylethyl acetate (50 μg/L of each standard compound); 2-phenylethanol, hexanoic acid, octanoic acid, decanoic acid and dodecanoic acid (5000 μg/L of each standard compound). The pH was adjusted to 3.5 with sodium hydroxide (Nuclear, São Paulo, Brazil). Ultra-pure water was prepared using a MilliQ water purification system (Millipore, Bedford, MA).

The SPME fibre (50/30 divinylbenzenecarboxen-polydimethylsiloxane (DVB/CAR/PDMS) StableFlex) was purchased from Supelco (Bellefonte, PA). The fibre was conditioned according to the manufacturer"s recommendation prior to its first use. Sodium chloride (NaCl) of analytical grade was purchased from Nuclear and was oven dried at 110 °C overnight before use. Twenty-millilitre headspace vials with magnetic screw caps sealed with silicone septa were purchased from Supelco.

2.2. Instrumentation

Extraction of volatile compounds from the headspace of the vials containing samples was performed with a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with an agitator and SPME syringe. The GC × GC system consisted of an Agilent 6890 N (Agilent Technologies, Santa Clara, CA) equipped with a Pegasus time-of-flight mass spectrometric detector (Leco Corporation, St. Joseph, MI). A Carbowax column (100% polyethylene glycol; 30 m × 0.25 mm × 0.25 μm, J&W Scientific Inc., Folsom, CA) was used as the first-dimension (^{1}D) column, and a DB-17 ms ((50%-phenyl)-methylpolysiloxane; 1.70 m × 0.18 mm × 0.18 μm; J&W Scientific Inc.) was used as the second-dimension (^{2}D) column. The same GC system (Agilent 6890 N) was equipped with a secondary column oven and non-moving quadjet dual stage thermal modulator. During modulation, cold pulses were generated using dry nitrogen gas cooled by liquid nitrogen, whereas heated dry air was used for hot pulses. The modulation period was set for 7 s with a 1.4 s hot pulse time. The injector, transfer line and ion source temperature were at 250 °C. The oven temperature programme conditions were as follows: initial temperature of 35 °C for 5 min, programmed at 3 °C min^{-1} to 250 °C (5 min). The secondary oven was kept at 10 °C above the primary oven throughout the chromatographic run. The modulator was offset by +25 °C in relation to the primary oven. Ultra-high-purity helium was used as carrier gas at a constant flow of 1 mL/min. The MS parameters included electron ionisation at 70 eV with ion source temperature at 250 °C, detector voltage of −1750 V, mass range of *m/z* 45–450, and acquisition rate of 100 spectra/s.

2.3. Conditions for the extraction of volatiles

Extraction of volatile compounds was performed with HS-SPME, and experimental conditions are described in a former work of this research group (Welke, Zanus, Lazarotto, Schmitt, & Zini, 2012). The SPME extraction conditions were 1 mL of sample in 20-mL glass headspace vials, 30% of NaCl (m/v), without sample agitation, extraction time of 45 min and extraction temperature of 55 °C. All samples were kept at 55 °C for 10 min prior to extraction. The headspace was sampled using a 2-cm DVB/CAR/PDMS 50/30 μm fibre. The volatile and semi-volatile compounds were desorbed in the GC inlet at 250 °C for 5 min. In order to avoid