Food Research International 109 (2018) 99–111

Contents lists available at ScienceDirect

Food Research International

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

Impact of storage time and temperature on volatomic signature of Tinta Negra wines by LLME/GC-$_{IT}$MS

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ARTICLE INFO

Keywords:
Volatomic signature
Storage time
Storage temperature
LLME/GC-$_{IT}$MS

ABSTRACT

The current study reports the effect of storage temperature, storage time and glucose content on the volatomic signature of Tinta Negra wines using liquid-liquid microextraction (LLME) combined with gas chromatography–ion trap mass spectrometry (GC–$_{IT}$MS). A total of 65 volatile organic compounds (VOCs) were identified in Tinta Negra, of which only 14 appear during storage. Based on the results, the freshness and fruitiness odours that are related to the presence of some varietal and fermentative components (e.g. terpenic compounds, esters) are lost during wine storage, while other descriptors such as caramel, dried fruit, spice, toast and wood arise due to Maillard reactions (e.g. furanic compounds), among other chemical reactions (e.g. lactones). The results obtained in this study may be applied as a useful tool in the winemaking field in order to introduce changes in the baking (*estufagem*) process and/or predict the effects of storage time when applying high temperatures. In addition, the VOCs identified in this study may help winemakers and wine chemists better understand the aroma composition and profile of Tinta Negra wines.

1. Introduction

Aroma is one of the most remarkable parameters of wine quality and it is responsible for consumer acceptance or rejection. The complexity of wine aroma results from the presence of several volatile organic compounds (VOCs) that originate from biochemical and technological processes/processing (e.g., grape destemming, crushing and pressing), as well as from several factors such as grape variety, climate conditions, winemaking technology, aging and storage (Alañón et al., 2013; Moreno et al., 2017; Sánchez-Palomo, Trujillo, Ruiz, & Viñas, 2017).

Madeira wine is a fortified wine produced on the Island of Madeira. It is characterized by supplementation with distilled grape spirit at different fermentation stages to obtain an alcoholic level between 18 and 21% (*v*/v) and different glucose levels ranging from dry (up to 25 g/L, fermented to low sugar levels) to sweet (130 g/L, partial fermentation) wines. The wine is submitted to a baking process called "*estufagem*", where it is placed into large coated vats and the temperature is slowly increased at about 5 °C per day and is then maintained at 45–50 °C for 3 months. The wine is then allowed to undergo a maturation process in oak casks for a minimum of 3 years. Finally, some Madeira wines are submitted to an aging process for a minimum of 3–20 years or longer, where they are stored in cellars at 30–35 °C under the influence of the sun and at a humidity degree > 70% (Pereira, Reis, Saraiva, & Marques, 2010; Perestrelo, Barros, Câmara, & Rocha, 2011). During *estufagem* complex reactions occur as a result of several underlying physical, chemical and biochemical mechanisms promoted through heating, which are all essential for the development of the typical aroma, taste and colour of Madeira wines (Pereira, Albuquerque, Ferreira, Cacho, & Marques, 2011; Perestrelo, Silva, Pereira, & Câmara, 2016).

It is well reported that thermal processes cause important changes in terms of flavour, colour and nutritional properties in food products. To date, the most recent studies that focus on accelerated oxidative aging promoted by thermal processes have shown an increase in wine aroma complexity. In sweet Pedro Ximénez wines for example, application of a thermal process of 65 °C for up to 30 days resulted in an increase in VOCs related to Maillard reactions, such as 5-hydroxymethyl-2-furfural, furaneol, among others (de Lerma, Peinado, Moreno, & Peinado, 2010). The volatile profile of Chardonnay white wines obtained by accelerated aging (50 °C during 7 days) was compared with conventional storage (18 °C) and it was found that some VOCs belonging to the acetal and norisoprenoid chemical families were developed, while others (e.g. alcohols, terpenic and furanic compounds) dissipated with accelerated aging (Cejudo-Bastante, Hermosín-Gutiérrez, & Pérez-Coello, 2013). More recently, a study reporting the volatile profile of young Madeira wines submitted to the *estufagem* process (45 °C for 3 months) with

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https://doi.org/10.1016/j.foodres.2018.04.034
Received 7 November 2017; Received in revised form 13 April 2018; Accepted 15 April 2018
Available online 17 April 2018
0963-9969/

overheating conditions (70 °C for 1 month) showed that the concentration of furanic derivatives and esters increased, while the concentration of alcohols, terpenic components, acetates and acids decreased (Pereira, Cacho, & Marques, 2014).

In the current study, the influence of storage time (control (day 0), 30 and 120 days) and temperature (30, 45 and 55 °C) on the global volatomic signature of Madeira wines obtained from Tinta Negra grapes was evaluated. The VOCs were extracted using liquid-liquid microextraction and were analyzed using a gas chromatography ion trap mass spectrometry detector (LLME/GC-$_{IT}$MS). Tinta Negra is a versatile red grape that is responsible for the production of sweet, medium sweet, dry and medium dry Madeira wine and it represents > 80% of the total production in Madeira Island. Nevertheless, according to some winemakers this variety does not match the excellent quality of the other noble varieties, such as Malvasia, Bual, Sercial and Verdelho. The results obtained in the current study may be used to valorize the Tinta Negra wines in terms of the enrichment of their volatomic signature through storage time and temperature.

2. Material and methods

2.1. Reagents and standards

All chemicals used in this study were of analytical quality. Sodium chloride (NaCl, 99.5%) and sodium sulphate (Na_2SO_4, 99.5%) were purchased from Panreac (Barcelone, Spain). Standards of VOCs used for identification of target compounds, such as2-methylpropan-1-ol (99.5% purity), butan-1-ol (≥99.4%), 2-ethylhexan-1-ol (99%), benzyl alcohol (99.8%), 2-phenylethyl alcohol (> 98%), 3-methylbutan-1-ol (98%), hexan-1-ol (> 98%), isoamyl acetate (99%), ethyl hexanoate (99%), ethyl lactate (≥98%), ethyl octanoate (99.5%), ethyl decanoate (99%), diethyl succinate (99%), ethyl 3-hydroxyhexanoate (99%), acetic acid (≥99%), butanoic acid (≥99%), benzoic acid (≥99.5%), 3-methylbutanoic acid (99%), hexanoic acid (≥99%), octanoic acid (99%), decanoic acid (99%), dodecanoic acid (98%), 2-methylbutanal (98%), hexanal (> 98%), decanal (≥98%), benzaldehyde (≥99.5%), phenylacetaldehyde (≥98%), γ-octalactone (98%), γ-decalactone (≥ 98%), 2-furfural (99%), 5-methyl-2-furfural (98%), ethyl 2-furoate (99%), 5-hydroxymethyl-2-furfural (98%), vanillin (99%), methionol (98%) were purchased from Sigma-Aldrich (Madrid, Spain), whereas 2-phenylethyl acetate (≥99%), γ-hexalactone (≥ 97%) and eugenol (≥99%) from Fluka (Buchs, Switzerland).The individual stock solutions were prepared in ethanol at a concentration of 500 mg/L and were stored at 4 °C. Helium of purity 5.0 (Air Liquide, Portugal) was utilized as the GC carrier gas. A C_8 to C_{20} straight-chain *n*-alkane series (concentration of 40 mg/L in *n*-hexane) supplied from Fluka (Buchs, Switzerland) was used to determine the kovat index (KI), while 3-octanol obtained from Sigma-Aldrich (Madrid, Spain) was used as an internal standard (IS) to perform the semi-quantification.

2.2. Wine samples

Ten monovarietal Tinta Negra fortified Madeira wines, five sweet (96.1 to 150 g glucose per L) and five dry (49.1 to 64.8 g glucose per L) were considered in this study. The samples were kindly provided by Madeira Wine Company, the most representative producer of Madeira wine. Commercial 750 mL glass bottles were transported to the laboratory and were then stored in the dark at 4 °C until further analysis. The ethanol content of the Madeira wines under study ranged from 18 to 21% (v/v).

Later, 50 mL samples of wine were put into 100 mL brown bottles (7 bottles in total for assay: 3 temperatures × 2 sampling times +1 control) and were tightly capped. Samples were then incubated under controlled temperature at 30, 45 and 55 °C up to a maximum of 4 months (120 days). Each assay was performed in triplicate.

2.2.1. Liquid-liquid microextraction (LLME) procedure

The LLME procedure was adopted from a previous study performed in our laboratory (R. Perestrelo, Silva, Silva, & Câmara, 2017). For LLME assays, 5 mL of sample, 10 µL of 3-octanol (IS, 250 µg/L) and 0.5 g of NaCl were added into a 50 mL flask. The solution was stirred during 10 min at 500 rpm and extracted twice with 500 µL of dichloromethane. Prior to analysis, both of the obtained organic phases were blended and dried over addition of anhydrous Na_2SO_4. The organic extract was concentrated to 50 µL under a gentle stream of nitrogen. Each sample was analyzed, at least, in triplicate and was kept at −20 °C until further analysis. One µL of extract was injected into the GC-$_{ITD}$MS system.

2.3. Gas chromatography-mass spectrometry conditions

Separation of VOCs was performed with a Varian Star 3400 Cx Series II gas chromatograph coupled to an ion trap mass spectrometer Varian Saturn 3 equipped with a DB–Waxetr fused silica capillary column (30 m × 0.25 mm i.d. ×0.5 µm film thickness) using a stationary phase of polyethylene glycol. Splitless injection mode was used. The carrier gas used was helium at a flow rate of 1 mL/min. The oven temperature programme was maintained at 40 °C for 1 min, raised to 220 °C at 2 °C/min and was finally held at this temperature for 10 min. The ion trap detector was set as follows: transfer line temperature 220 °C; manifold and trap temperatures 180 °C. The mass range was *m/z* 30–300, the emission current 15 µA and the electron multiplier was set in the relative mode to the auto tune procedures. All mass spectra were acquired in the electron impact (EI) mode (E_i = 70 eV, source temperature, 180 °C).

Identification was achieved by comparison of the mass spectra obtained for the samples with those obtained for the pure VOC standards injected under the same conditions, as well as by comparison with the mass spectra present in the NIST and Wiley MS library database, and/or by comparison, when available, with the kovat retention index (KI) values determined according to the van den Dool and Kratz equation (van Den Dool & Dec Kratz, 1963) with the values reported in the literature for similar columns (Lee & Noble, 2003; Pereira et al., 2014; Perestrelo, Barros, Rocha, & Câmara, 2011; Spínola, Perestrelo, Câmara, & Castilho, 2015; Varming, Petersen, & Poll, 2004).

The VOCs concentration was estimated, semi quantitatively, using the added amount of 3-octanol (IS) according to the following equation: VOCs concentration = (VOC GC peak area/IS GC peak area) × IS concentration. However, our main aim was related to the evolution of VOCs under different storage conditions. This approach was already performed in a previous scientific study (Pereira et al., 2014).

2.4. Statistical analysis

The obtained data were analyzed for statistical significance using *one-way* ANOVA followed by Tukey's test for post-hoc multiple comparisons of means. Confidence intervals of 95% or significant level of $\alpha = 0.05$ were used. Principal component analysis (PCA) was applied to the autoscaled areas of the 65 VOCs identified in Tinta Negra wines, each with three independent replicates. All statistical determinations were performed using IBM SPSS Statistics 23.0 software (LEAD Technologies Inc., Chicago, USA).

3. Results and discussion

3.1. Impact of storage time and temperature on volatomic signature of Tinta Negra wines

This study is focused on the chemical changes that take place in the volatomic signature of young Tinta Negra wines during storage in order to establish the general pattern of changes and to determine whether any link between the wine storage conditions or the wine type (sweet