Food Chemistry 214 (2017) 686–693

Contents lists available at ScienceDirect

Food Chemistry

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

A fast and environment-friendly MEPS$_{PEP}$/UHPLC-PDA methodology to assess 3-hydroxy-4,5-dimethyl-2(5H)-furanone in fortified wines

Jorge Freitas [a], Rosa Perestrelo [a], Rubina Cassaca [a], Mariangie Castillo [a], Mariana Santos [a], Jorge Pereira [a], José S. Câmara [a,b,*]

[a] CQM – Centro de Química da Madeira, NPRG, Campus Universitário da Penteada, 9000-390 Funchal, Portugal
[b] Faculdade de Ciências Exatas e da Engenharia da Universidade da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal

ARTICLE INFO

Article history:
Received 4 January 2016
Received in revised form 18 July 2016
Accepted 18 July 2016
Available online 25 July 2016

Keywords:
Sotolon
Wine
Oxidative aging
Premox
Microextraction by packed sorbent (MEPS)

ABSTRACT

Sotolon is widely associated with the quality of fortified aged wines, and has also been linked to premature oxidative aging (*premox*). Here we developed a single, fast and environmental-friendly microextraction by packed sorbent ultra-high pressure liquid chromatography analysis (MEPS/UHPLC-PDA) for sotolon quantification in different wines. The best extraction conditions (loading three times 250 μL samples through the MEPS$_{PEP}$ sorbent and elution with 100 μL of 50% MeOH) were combined with a fast UHPLC separation (5 min separation using acidified 10% MeOH isocratic flow in a CORTECS C18 column) to allow unparalleled minimum sample and solvents volumes usage. The validated methodology showed good linearity (r^2 > 0.993) and precision (<5.6%); high recovery (>81%) and detection limits (0.45–2.51 μg/L) far below sotolon odor threshold for any type of wine. The methodology was successfully applied to selected white table and Madeira wines, encompassing therefore a wide range of alcohol and sugar contents. Furthermore, as far we may know, this is the first time a single methodology can be used to assess both wine aging or *premox* according to the type of wine.

1. Introduction

Sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone) is a chiral lactone that confers a characteristic spicy odor to wines, particularly to dry sweet wines oxidatively aged (Du Toit, Marais, Pretorius, & Du Toit, 2006; Lavigne, Pons, Darriet, & Dubourdieu, 2008; Picard, Tempere, de Revel, & Marchand, 2015). Its presence correlates with the quality of these wines, notably Port, Madeira and Sherry (Camara, Marques, Alves, & Silva Ferreira, 2004; Castro, Martins, Teixeira, & Silva Ferreira, 2014; Collin, Nizet, Claeys Bouuaert, & Despatures, 2012; Guichard, Pham, & Etievant, 1993; Martin, Etievant, Lequere, & Schlich, 1992; Silva Ferreira, Barbe, & Bertrand, 2003). In Port and Madeira wines involving oxidative aging, sotolon was shown to positively correlate with sugar content and aging time, up to the milligrams per litre level in very old wines (>25 years) (Camara et al., 2004; Silva Ferreira et al., 2003). In turn, in the Sherry-type white wine *Fino*, which involves a peculiar biological aging winemaking procedure using the flor yeasts, sotolon was shown to accurately assess the degree (duration) of the biological aging, allowing a better control of the quality and uniformity of the commercial product regardless of the cellars environmental factors and sherry winemaking conditions used (Moreno, Zea, Moyano, & Medina, 2005). Sotolon has been also identified in Garnacha Tintorera-based sweet wines (Figueiredo-González, Regueiro, Cancho-Grande, & Simal-Gándara, 2014), as well as in *Marsala*, a wine exclusively produced in Sicily and considered one of the four most important dessert wines together with Madeira, Sherry and Port (Dugo et al., 2014). Sotolon, however, can be also generated in table wines, being desirable while its concentration does not exceed its odor threshold (only 10 μg/L in white wines (Guichard et al., 1993)), otherwise sotolon will impart to these wines flavors usually associated with aged sweet wines, overlapping the freshness that characterize

Abbreviations: ACN, acetonitrile; BIN, barrel insert and needle; D/MD, dry/medium dry Madeira wines; DCM, diclorometane; FA, formic acid; GC-MS, gas chromatography – mass spectrometry; LC-PDA, liquid chromatography with photodiode detection; LLE, liquid-liquid extraction; LOD, limit of detection; LOQ, limit of quantification; MeOH, methanol; MEPS$_{PEP}$, microextraction by packed sorbent using the PEP sorbent; PS/DVB, porous polystyrene–divinylbenzene polymer; *premox*, premature oxidative wine aging; S/MS, sweet/medium sweet Madeira wines; SPE, solid-phase extraction; UHPLC-MS, ultra-high pressure liquid chromatography-mass spectrometry.

* Corresponding author at: CQM – Centro de Química da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal.
E-mail address: jsc@uma.pt (J.S. Câmara).

http://dx.doi.org/10.1016/j.foodchem.2016.07.107
0308-8146/

young wines (Du Toit et al., 2006). This premature oxidative aging, known as *premox* (Pons, Lavigne, Darriet, & Dubourdieu, 2013; Thibon et al., 2015), affects more extensively white wines due to their lower concentration in antioxidants, namely polyphenols (Silva, Pereira, Wouter, Giro, & Camara, 2011) and it is a problem with important economic consequences as, depending on the storage conditions, a white wine with only two years of bottle aging may be already affected (Pérez-Coello, González-Viñas, García-Romero, Díaz-Maroto, & Cabezudo, 2003). Therefore, sotolon is a valuable compound to assess white wine quality, being often known as an important marker of *premox* in these wines (Gabrielli, Fracassetti, & Tirelli, 2014; Pons, Lavigne, Landais, Darriet, & Dubourdieu, 2010). Despite its relevance, the mechanisms leading to sotolon generation in wine are not yet fully elucidated. Nevertheless, different processes, such as thermal degradation of Maillard intermediates (Blank, Lin, Fumeaux, Welti, & Fay, 1996; Guerra & Yaylayan, 2011; Hofmann & Schieberle, 1997), aldol condensation between α-keto butyric acid and acetaldehyde (Cutzach, Chatonnet, & Dubourdieu, 1999; Guichard et al., 1993; Thuy, Elisabeth, Pascal, & Claudine, 1995) or reaction between ethanol and ascorbic acid (Konig et al., 1999) have been shown to be involved in formation of this compound. Similarly, the winemaking conditions also seem to have a relevant role in sotolon generation, particularly the oxygenation levels (Cutzach et al., 1999; Lavigne et al., 2008; Silva Ferreira, Avila, & Guedes de Pinho, 2005), sugar concentration (Camara et al., 2004) and storage time and temperature (Cutzach et al., 1999; Silva Ferreira et al., 2005). Overall, it is consensual that wine oxidation is a hallmark in the formation of sotolon in wine (Cutzach et al., 1999; Escudero, Cacho, & Ferreira, 2000; Moreno et al., 2005; Silva Ferreira, de Pinho, Rodrigues, & Hogg, 2002; Silva Ferreira et al., 2003; Thuy et al., 1995). Sotolon is a volatile compound and so its quantification has been reported in the literature using gas chromatography (GC) (Camara et al., 2004; Collin et al., 2012; Escudero et al., 2000; Ferreira, Jarauta, Lopez, & Cacho, 2003; Lavigne et al., 2008; Silva Ferreira et al., 2005). However, the analytical approach involving liquid-liquid extraction (LLE) followed by liquid chromatography (LC) is often required (Gabrielli et al., 2014, 2015; Guichard et al., 1993). In all of these reports the sophistication of instrumentation using in sotolon analysis was evolved, however the sample preparation step has been completely underappreciated in the whole experimental layout, involving laborious procedures and significant sample and solvents volumes usage (Gabrielli et al., 2014, 2015). Microextraction has been successfully applied to different fields of research, from environment, to pharmaceutical, forensic, biomedical applications (reviewed in Pereira, Camara, Colmsjo, and Abdel-Rehim (2014), Pereira, Gonçalves, Alves, and Câmara (2013), Pereira, Silva, Perestrelo, Goncalves, Alves, and Camara (2014)), presenting several advantages, as the drastic reduction in the sample and solvents requirements, simpler and more user-friendly experimental layouts less prone to experimental errors, and several high-throughput solutions. Microextraction by packed sorbent (MEPS) is a form of micro solid phase extraction (SPE) in which the sorbent is tightly packed in a small cylinder (barrel insert and needle – BIN), through which the samples and solvents are easily loaded using a syringe (details of MEPS using eVol® can be found elsewhere (Pereira, Gonçalves, Alves, & Câmara, 2013)). There is a wide range of sorbents available for MEPS allowing its application to the microextraction of analytes with broad chemical properties and in different matrices, as biological fluids, food matrices, drinks, waste waters, etc. (reviewed in Pereira, Camara et al. (2014), Pereira et al. (2013), Pereira, Silva et al. (2014)). Madeira wine is a fortified wine produced in Madeira Island for more than five centuries using a unique baking process, *estufagem,* in which wine is maturated during 90 days at 45–50 °C (Camara et al., 2004). It usually presents an alcohol content between 18% and 20% (v/v) and a wide range of sugar concentrations, from residual up to 110 g/L, corresponding to the dry (D, less than 25 g/L of sugar), medium dry (MD, up to 65 g/L of sugar), medium sweet (MS, up to 90 g/L of sugar) and sweet styles (S, usually up to 110 g/L of sugar, although higher levels can be obtained) (Câmara, Alves, & Marques, 2006). Here we report the use of $MEPS_{PEP}$-UHPLC-PDA methodology for the fast, selective and sensitive quantification of sotolon in fortified wines, using a very low amount of sample (750 μL of wine) and less than 1 mL of methanol in the whole procedure. Moreover, the methodology has been also validated for table white wines presenting lower alcohol and sugar contents, showing its suitability to quantify sotolon in a broad range of wines in terms of alcohol and sugar content. As far as we know this is the first time that a single methodology ($MEPS_{PEP}$/UPLC-PDA) is used for sotolon quantification in any type of wine, regardless its alcohol and sugar contents.

2. Material and methods

2.1. Reagents and samples

Sotolon (>98%), formic acid (FA, >98%), acetonitrile (ACN, 99.5%, gradient grade), methanol (MeOH, 99.5%, gradient grade), glycerol (99.5%) and glucose (99.5%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Tartaric acid (foodstuff grade) and sodium hydroxide (NaOH) were obtained from Riedel-de-Haen (Madrid, Spain). All these chemicals were analytical gradient grade and used as received. Ultrapure water was obtained with a Milli-Q ultrapure water system from Millipore (Milford, MA, USA). All solvents and samples were filtered through 0.22 μm membrane filters from Millipore (Milford, MA, USA), before use. Seven different synthetic wine (SW) solutions were obtained by dissolving 5 g/L tartaric acid in different ethanol/water solutions (v/v) (0, 12 or 18%), adjusted to pH 3.3 with 1 M sodium hydroxide (Sigma-Aldrich) and adding two levels of glucose concentrations to simulate dry/medium dry (D/MD, 50 g/L) and sweet/medium sweet Madeira wines (S/MS, 90 g/L) (Perestrelo, Petronilho, Camara, & Rocha, 2010). The dealcoholized and 12% alcohol D/MD and S/MS wines were obtained by total or partial dealcoholization of D/MD and S/MS Madeira wines under vacuum at 40 °C, up to 1/4 of initial volume as previously reported (Perestrelo, Silva, & Camara, 2015). The volume of dealcoholized extracts was adjusted to initial sample volume with the SW solution without ethanol. Sotolon quantification was performed in ten table white wines acquired in the local markets (aged up to two years and representative of the main demarcated Portuguese regions, namely Verde, Douro, Alentejo and Madeira) and nine Madeira wine samples from dry (*Sercial*) and sweet (*Malvasia*) styles with different aging times (3, 5, 10, 15 and 20 years). The Madeira wine certified samples were provided by the Madeira wine company Henriques & Henriques – Vinhos, S.A.

2.2. MEPS optimization

MEPS optimization followed a univariate experimental design as previously described for other wine compounds (Goncalves & Camara, 2011; Perestrelo et al., 2015) and matrices (Magiera & Baranowska, 2014; Mendes, Silva, Mendonca, Pereira, & Camara, 2013) and it was carried out using a semi-automatic MEPS apparatus (eVol®, SGE Analytical Science, Melbourne, Australia), equipped with a 250-μL gas-tight syringe and removable needles containing the commercially available sorbents BINs (C2 (ethyl-silica), C8 (octyl-silica), C18 (octadecyl-silica), SIL (unmodified silica) and M1 (a mixed mode sorbent containing 80% C8 and 20% strong cationic exchange (SCX)) (SGE Analytical Science, Melbourne, Australia) and PGC (HyperSep Hypercarb porous graphitized