Food Chemistry 243 (2018) 222–230

Contents lists available at ScienceDirect

Food Chemistry

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

Micro-oxygenation does not eliminate hydrogen sulfide and mercaptans from wine; it simply shifts redox and complex-related equilibria to reversible oxidized species and complexed forms

Eduardo Vela, Purificación Hernandez-Orte, Ernesto Franco-Luesma, Vicente Ferreira*

Laboratory for Flavor Analysis and Enology (LAAE), Department of Analytical Chemistry, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), c/ Pedro Cerbuna 12, 50009 Zaragoza, Spain

ARTICLE INFO

Keywords:
Methanethiol
Dimethylsulfide
Copper
Disulfides
Persulfides
Reduction

ABSTRACT

This work seeks to assess the effects of micro-oxygenation (MOX) on the present and potential levels of Volatile Sulfur Compounds (VSCs) of wine. With such purpose, three red wines with a tendency to develop sulfury off-odors were subjected to three different MOX conditions (4.4–20 mg/L delivered at 0.05 or 0.2 mg/L/day). Samples were further subjected to Accelerated Reductive aging (AR) and analyzed for free and Brine Releasable (BR) VSCs and redox potential. Although MOX induced strong decreases in the levels of all free VSCs, hardly affected the ability of the wine to release back hydrogen sulfide and other mercaptans during AR-aging. During aging BR-levels of MOX samples became in most cases similar or higher than non-oxygenated controls. BR-levels and the fractions free/BR follow characteristic sigmoid plots when represented versus redox potential suggesting that all changes are the result of reversible equilibria between free, metal-complexed and oxidized forms of VSCs.

1. Introduction

Reductive or sulfur-like off-odors are responsible for an important proportion of faulty wines with potentially large economic losses (Goode, 2014). Such a problem is mostly caused by the development of low molecular weight Volatile Sulfur Compounds (VSCs) of which hydrogen sulfide (H_2S) is the most frequently found above its odor threshold followed by MeSH (Franco-Luesma & Ferreira, 2016a, 2016b; Siebert, Solomon, Pollnitz, & Jeffery, 2010; Ugliano, Kolouchova, & Henschke, 2011). The major source of both components seems to be alcoholic fermentation. H_2S is a by-product of the Sulfur (S) metabolism of yeast (Jiranek, Langridge, & Henschke, 1995; Schutz & Kunkee, 1977) and together with methanethiol (MeSH) it is involved in the yeast methionine and cysteine metabolisms (Barbosa, Mendes-Faia, & Mendes-Ferreira, 2012; Moreira et al., 2002; Spiropoulos, Tanaka, Flerianos, & Bisson, 2000). Factors affecting the levels of H_2S are the yeast sulfite reductase activity (Linderholm, Dietzel, Hirst, & Bisson, 2010), level of oxygen during fermentation (Bekker, Day, Holt, Wilkes, & Smith, 2016) and yeast assimilable nitrogen (Jiranek et al., 1995). The widespread use of reductive winemaking techniques in which the contact of oxygen is minimized throughout the winemaking process has increased the frequency of occurrence of reductive off-odors (Bekker et al., 2016). If these compounds are formed, winemakers try to decrease their levels by copper finning, aeration or addition of lees (Clark, Grant-Preece, Cleghorn, & Scollary, 2015; Ugliano et al., 2009; Vela, Hernández-Orte, Franco-Luesma, & Ferreira, 2017; Viviers, Smith, Wilkes, & Smith, 2013).

The effects of aeration and of micro-oxygenation (MOX) on VSCs are not completely understood. In the first stages of alcoholic fermentation the addition of oxygen is known to have a potentially positive effect on the yeast tolerance to ethanol (Valero, Millán, & Ortega, 2001) and in its efficiency to assimilate nitrogen, decreasing the risk of stuck fermentations and the production of sulfur compounds (Gomez-Plaza & Cano-Lopez, 2011). The effects of oxygen treatment during fermentation on the development of VSCs during wine maturation have been recently addressed by Bekker et al. (2016). These authors found increases of H_2S in O_2-treated wines is some periods during wine maturation, but in the long-term, oxygenated wines showed smaller levels of VSCs. Similarly, when oxygen is applied post-fermentation, there is agreement in the fact that MOX reduces effectively H_2S, MeSH and ethanethiol (EtSH) below their odor thresholds (McCord, 2003). Some authors, however, have warned about the possibility that O_2 will oxidize H_2S to elemental sulfur and mercaptans to disulfides (Zoecklein, 2007), species which could yield back the sulfury off-odors by reduction. Those reductive processes have not been clearly documented, and

* Corresponding author.
E-mail address: vferre@unizar.es (V. Ferreira).

http://dx.doi.org/10.1016/j.foodchem.2017.09.122
Received 21 April 2017; Received in revised form 22 September 2017; Accepted 25 September 2017
Available online 28 September 2017
0308-8146/

its existence would imply that thiols do not react with the quinones formed during wine oxidation, even if the high reactivity thiol-quinone has been experimentally demonstrated (Nikolantonaki, Chichuc, Teissedre, & Darriet, 2010; Nikolantonaki & Waterhouse, 2012; Waterhouse & Laurie, 2006).

The copper catalyzed oxidation of H_2S and other wine mercaptans has been recently addressed by Kreitman, Danilewicz, Jeffery, & Elias, 2016a). These authors have demonstrated that Cu(II) quickly coordinates to two mercaptans to form a complex (RS-Cu-SR) which most likely dimerizes to form a 4-atom ring alternating copper and S atoms in which Cu(II) is reduced to Cu(I) and the two S atoms are oxidized to S (-I). This structure could release a disulfide (R-S-S-R) and two (Cu(I)-SR) species. These species could subsequently aggregate forming tridimensional clusters to stabilize Cu(I) or, in the presence of O_2, could give Cu(II) back and a second disulfide. More recently, these authors have demonstrated for the first time the formation of nonvolatile mixed disulfides between glutathione and MeSH and polysulfanes formed by further oxidation of H_2S which could act as reservoirs of VSCs (Kreitman, Danilewicz, Jeffery, & Elias, 2017). Nevertheless, these authors used unrealistic concentrations of both MeSH (above 100 μg/L in all experiments) and copper (3 mg/L) and, moreover, they were not able to recover H_2S back from model wines containing disulfides and polysulfanes which questions whether these specific compounds are the precursors of H_2S. The existence of oxidized forms of H_2S able to form this molecule after reduction, is further supported by recent results showing that in a recently micro-oxygenated wine there was an important pool of H_2S non-detectable by the brine dilution method (BR) which became detectable after reductive aging (Vela et al., 2017).

Moreover, H_2S and mercaptans have been found to form stable non-volatile complexes with cation metals (Franco-Luesma & Ferreira, 2014) which can cleave by unclear reasons during reductive aging (Franco-Luesma & Ferreira, 2016a, 2016b). All this implies that any study addressing the real fate of VSCs during oxidation should take into account, not only the present levels of VSCs, but the existence of different species able to produce or release the VSCs during wine aging. This can be done by using the Accelerated Reductive aging (AR) assay (Franco-Luesma & Ferreira, 2016a, 2016b) including determinations for free and BR-forms of VSCs. The levels of VSCs formed or released in this assay were reasonably well correlated with the levels found after bottle aging (Franco-Luesma & Ferreira, 2016a, 2016b). Another alternative would be the assay recently proposed combining a chemical reducing agent and a strong Cu(I) chelator (Kreitman et al., 2017). The present paper seeks to determine the effects of MOX carried out at different doses and different O_2 exposure levels on the levels of free and BR-forms of VSCs, and also their effects on the ability of the wine to further form and release VSCs.

2. Materials and methods

2.1. Solvents and chemical standards

Ethanol was purchased from Merck (Darmstadt, Germany). Water with resistance of 18.2 MΩ·cm at 25 °C was purified in a Milli-Q system from Millipore (Bedford, Germany), sodium hydroxide and tartaric acid was purchased from PanReac AppliChem (Barcelona, Spain). Pure standards (> 95%) for VSCs calibration: H_2S, MeSH and EtSH were produced by addition of a water solution of sodium sulfide Na_2S, sodium methanethiolate, CH_3SNa and sodium ethanethiolate, CH_3CH_2SNa (all supplied by Sigma–Aldrich, St. Louis, MO, USA) at pH 9.6. This solution was daily prepared and was kept in the anoxic chamber. The Na_2S standard is stored under argon (Ar) in a desiccator to avoid hydration. Dimethylsulfide (DMS) was obtained from Merck (Darmstadt, Germany), ethylmethylsulfide (EMS), 1-propanethiol (PrSH) and thiophene were provided by Sigma-Aldrich (Steinheim, Germany). Stock solutions of DMS, EMS, PrSH and thiophene (ca. 2 g/L) were prepared in iso-octane in amber vials and were stored at −25 °C. These solutions were controlled by direct injection in the gas chromatography with pulsed flame photometric detection (GC-pFPD) system.

Intermediate methanolic solutions were stored at −25 °C in amber vials with Mini-inert valves (Supelco, CA, USA). All these solutions were manipulated in the anoxic chamber.

Brine containing 350 g/L of sodium chloride, NaCl, (Panreac, Barcelona, Spain) in Milli-Q water. Synthetic wine was a pure water solution containing 5 g/L of tartaric acid, 12% v/v ethanol and pH 3.4 adjusted with diluted NaOH (0.1 M).

2.2. Wines

Three red wines with tendency to develop sulfur-like off-odors were used in the study. Wine W1 was from 2014 made with Mencía in Bierzo (León, Spain). Wines W2 and W3 were from La Mancha (Spain), from 2015 vintage and were made with Syrah and with Tempranillo, respectively. All wines were directly taken from the cellar without filtration treatments. W1 contained 109.5 and 1347 μg/L of copper and iron, W2 93.0 and 737, and W3 18.9 and 1342, respectively. The metal content was determined by inductively coupled plasma mass spectrometry as described in the reference (Vela et al., 2017).

2.3. Laboratory scale micro-oxygenation

The micro-oxygenation systems were sterile 650 mL bottles hermetically closed and made with materials of different O_2 permeabilities. The most permeable bottles were made from polycarbonate (blue capped) and the least permeable (green capped) were from PETG (polyethylene glycol terephthalate). Both bottles were supplied by VWR North American Cat., (Radnor, Pennsylvania). The constant and reproducible O_2 permeability of the systems was assessed in previous studies and was additionally rechecked in the experiment with the introduction of reference samples. Fifteen polycarbonate and 8 PETG bottles, each one containing a PSt6 O_2 sensor were used in the experiment. The bottles and the wine models (12% ethanol, 5 g/L tartaric acid pH fixed 3.5) were introduced in the anoxic glove chamber four days before the experiment in order to eliminate any O_2 contained in the walls, caps or liquid.

Five 750 mL bottles of each wine were then introduced in the chamber and were mixed in order to avoid bottle effects. Sample aliquots for the analysis of initial free and BR-VSCs and redox potential, and for the AR-aging assays were then taken. The wines were distributed in the plastic bottles. Four polycarbonate and two PETG bottles were filled with each wine. Polycarbonate bottles were filled up to 630 mL allowing a little 30-mL headspace; PETG bottles were filled just with 450 mL allowing nearly 210 mL of headspace. Additionally, 3 polycarbonate and 2 PETG bottles were similarly filled with the wine model and used to determine the mass of O_2 penetrating in the systems. All the plastic bottles were capped and the caps were further sealed with glue to ensure complete airtightness. Three days inside the chamber were further allowed to ensure the glue runs dry. The bottles were then taken out of the chamber and let in an incubator (ClimasLab, Barcelona, Spain) at 20 °C different times. The level of O_2 was daily monitored in all bottles using an Oxygen analyzer from Nomacorc (Thimister-Clermont, Belgium). The MOX experiment was finished by introducing the bottles in the glove chamber. Two polycarbonate bottles per wine were introduced back in the glove chamber after 4 weeks, where they were kept, by schedule reasons, just 1 more week, during which dissolved O_2 levels dropped to 0.02 mg/L (from around 0.1 mg/L). These bottles correspond to the MOX2 experiment in which the wines consumed 7.7–8.0 mg/L of O_2. The two other polycarbonate bottles per wine were introduced in the glove chamber after 3 months (MOX3), and were left there for 5–7 additional weeks to ensure final dissolved O_2 levels below 0.01 mg/L (from nearly 3 mg/L). These MOX3 bottles consumed 19.0–20.3 mg/L of O_2. PETG bottles were also