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Analytical Methods

A new chemical criteria for white wine: The glutathione equivalent capacity

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ABSTRACT

The present work aimed at showing the interest in applying liquid chromatography with amperometric detection at a silver electrode (LC-EC-Ag) in order to record a chromatogram highly selective to non volatile aminothiols present in white wines. By integrating and summing the peak area of the aminothiols and by normalizing with respect to the peak area of an injected standard solution of glutathione (1 μM) a new quantitative criteria for white wine characterization is proposed namely, the glutathione equivalent capacity (GEC). The LC setup uses a C18 column in isocratic mode and the analysis takes less than 4 min. The wine sample needs no sample treatment other than dilution with the mobile phase. This new methodology and concept is illustrated by the LC-EC-Ag analysis of several white wines of different origins especially Alsace Riesling wines and Riesling grape juice. It is anticipated that in addition to the determination of the GEC, the developed method may be of interest for establishing a white wine "signature" based on the chromatographic profile.

1. Introduction

Glutathione (GSH) and related soluble aminothiol compounds such as cysteine (CYS) have been shown to be important biomarkers involved in aroma precursor development in musts and in protecting white wines from browning and from fruity aroma and flavor loss both due to oxidative processes (Coetzee & Du Toit, 2012; Kritzinger, Bauer, & du Toit, 2013; Roussis, Lambropoulos, & Papadopoulou, 2005) Pena-Gallego et al., 2012. GSH protects white wine from oxidative browning by interfering with the polyphenol polymerization processes (Cejudo-Bastante, Perez Coello, & Hermosin-Gutierrez, 2010; Recamales, Sayago, Gonzalez-Miret, & Hernanz, 2006; Singleton, Salgues, Zaya, & Trousdale, 1985). In grape must, the biocatalytic oxidation of polyphenols such as caftaric (or coutaric) acid by means of grape polyphenol oxidase (PPO) produces quinones which tend to polymerize to brown colored polymers. This polymerization process is inhibited by GSH with formation of a colorless grape reaction product (GRP). The PPO itself is inhibited by ethanol and sulfur dioxide but the enzyme laccase which is present in botrytized grapes can further oxidize the GRP with formation of brown polymers (Cejudo-Bastante et al., 2010; Li, Guo, & Wang, 2008). It was shown, recently, that cysteine exerts a synergistic effect on the antioxidant activity of polyphenols (Fujimoto, Inai, & Masuda, 2013). Another recent study on a model white wine, however, pointed out that GSH in the presence of polyphenols may induce coloration due to oxidation by molecular oxygen, with formation of some polymeric pigments depending on GSH concentration (Sonni, Clark, Prenzier, Riponi, & Scollary, 2011). After fermentation, the PPO activity decreases and oxidative browning is mainly attributed to polyphenol chemical oxidation eventually accelerated by temperature fluctuations, by light and by diffusion of molecular oxygen through the cork of the bottled wine (Kilmartin, 2009; Recamales et al., 2006). During wine aging, the protective effect against loss of some wine aroma volatiles has been reported to be due to the thiol moiety of aminothiols acting as an antioxidant functional group (Roussis, Papadopoulou, & Sakarellos-Daitsiotis, 2009). Soluble aminothiols and the volatile thiols responsible for positive fragrances disappear in white wines during aging due to oxidation by molecular oxygen and to addition reactions with o-quinones generated during oxidation of polyphenols (Nikolantonaki, Chichuc, Teissedre, & Darriet, 2010). Numerous methods have been developed for the analysis of wines at all stages of the winemaking process in order to better understand the chemistry underlying wine formation and ultimately for quality improvement (de Villiers, Alberts, Tredoux, & Nieuwoudt, 2012). The determination of polyphenol antioxidants in white wines can be accomplished by several methods such as the classical Folin–Ciocalteu colorimetry, liquid chromatography or by electroanalytical methods (Aguirre et al., 2010; Airado-Rodriguez, Galeano-Diaz, & Duran-Meras, 2010; Makhotkina & Kilmartin, 2010; Makhotkina & Kilmartin, 2012; Rebelo, Rego, Ferreira, & Oliveira, 2013; Sanchez-Arribas, Martinez-Fernandez, Moreno, Bermejo, &

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Zapardiel, 2013). The determination of aminothiol antioxidant markers such as GSH, CYS and homocysteine (HCYS) in white wine has been performed by liquid chromatography (LC) and capillary electrophoresis. Both methods used preferably a fluorimetric detector (FD) since the studied aminothiols are present in white wines in the low micromolar concentration and since they exhibit low UV absorbance. The LC–FD analyses consisted to perform pre or post-column derivatization of the aminothiols (Andujar-Ortiz, Pozo-Bayon, Moreno-Arribas, Martin-Alvarez, & Rodriguez-Bencomo, 2012; Marchand & de Revel, 2010; Park, Boulton, & Noble, 2000). More recent LC methods exploit mass spectrometry detection for the sensitive and selective analysis of simple and complex aminothiols in wines (du Toit, Lisiak, Stander, & Prevoo, 2007; Fracassetti et al., 2011; Inoue et al., 2013; Mattivi et al., 2012). Our laboratory has recently developed an isocratic LC method for the determination of aminothiols and N-acetylcysteine (Sarakbi et al., 2013). An amperometric detector with a silver electrode polarized at a potential close to 0.0 V vs Ag/AgCl, KCl 3 M, was shown to be suitable for the LC-amperometric (LC-EC-Ag) determination of a mixture of aminothiols and N-acetylcysteine in white wines. The detector response was related to the interaction of the aminothiol with silver ions at the electrode/solution interface. Advantages of the developed method were that the LC set up used an isocratic mode with a classical C18 column and the detector showed good selectivity towards soluble thiol species in the submicromolar concentration range. The analyzed wine samples needed no pretreatment other than dilution with the mobile phase and the chromatographic run took less than 4 min. The method permitted the quantification of micromolar concentrations of GSH, CYS, HCYS, and N-acetylcysteine (NAC) in white wines. The detector needed cleaning by manual smoothing on a polishing cloth once a month and the developed method permitted the use of the LC column for periods longer than 10 months (more than 3000 injections) including the assay of white wines. The fact that the detector was sensitive towards soluble thiols and "blind" towards the vast amount of molecules present in wine was of particular interest. Chloride, sulfide and sulfite ions present in white wines eluted close to the solvent front with no interference on the subsequently eluted aminothiols (Sarakbi et al., 2013). Numerous other species present in white wines such as polyphenols, ethanol, tartaric acid were not detected. Volatile thiols present in white wines are hydrophobic (eluted at longer retention times) and are present at too low concentrations (nM) to be detected.

In the present work, the LC-EC-Ag method was applied to study the chromatographic profile of several white wines. The methodology consisted to first inject GSH (1 μM) then the diluted wine sample. The area of the peaks attributed to aminothiols were normalized against the GSH peak, this permitted to define a GSH equivalent capacity (GEC) of the analyzed wine. Comparison of the chromatographic profile of different white wines has been realized.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical reagent grade. CYS, HCYS, cysteinylglycine (CYSGLY), GSH, NAC, sodium sulfite, sodium sulfide were purchased from Fluka (Steinheim, Germany). Formic acid and sodium nitrate were obtained from Merck (Darmstadt, Germany). Methanol, ethanol, glucose, fructose, tartaric acid, citric acid, sodium chloride and $EDTANa_2H_2$ were purchased from VWR and BDH Prolabo (Leuven, Belgium). Sodium hydroxide was from Janssen (Geel, Belgium). The mobile phase was prepared by dissolving 4.250 g sodium nitrate, 0.375 g $EDTANa_2H_2$ and 9.20 g formic acid (98%) in 1.5 L of water then the pH was adjusted to 4.5 with a 2.0 M sodium hydroxide solution and the volume was made up to 2 L with water after addition of 20.0 mL of methanol. Stock solutions of the aminothiols (1×10^{-2} M), prepared in the formate mobile phase pH 4.5 and kept at 4 °C, were found to be stable for more than 10 days. Standard solutions were prepared on a daily basis by appropriate dilution of the stock solution with the mobile phase.

2.2. Materials

The LC experiments were performed using a Dionex ICS-5000 LC system, equipped with an autosampler controlled by Chromeleon software. Separation was performed in isocratic mode on an Atlantis® dC18 column (3 μm, 4.6 × 100 mm) from Waters. A silver disk (1 mm diameter) working electrode housed in a flow-through electrochemical cell was coupled to the LC system. The auxiliary electrode was in titanium and the reference electrode was made of Ag/AgCl, 3 M KCl. The cell volume of the detector was 0.2 μL. Cleaning of the working electrode by manual surface smoothing on a polishing cloth in the presence of alumina powder was realized once a month. The autosampler and the entire LC setup were thermostated at 25.0 ± 0.2 °C. HPLC grade water was obtained from a Milli-Q filtration station (Millipore filter corp., Bedford, MA, USA). The mobile phase was filtered using a 0.2 μm GH Polypro hydrophilic polypropylene membrane fixed in a SolVac™ filter holder (Pall, WVR Belgium). The pH of the mobile phase was determined using a 744 pH meter (Metrohm, Belgium). Prior to LC injection, standard solutions and diluted wine samples were filtrated through a 0.45 μm membrane (PAL Acrodisc® LCPVDF, VWR-Belgium).

Intensity of wine colour (yellow to pale brown) was determined by measuring the absorbance at 420 nm using a Genesys 20 (Thermoscientific-Belgium) spectrophotometer.

2.3. Gold nanoparticles (GNP)s

The gold nanoparticles solution was prepared by vigorous boiling and stirring an aqueous solution (50 mL) of $HAuCl_4$ (0.0105 wt.%) in a 100 mL beaker. Then 400 μL of trisodium citrate (1 wt.%) was rapidly added to the chloroauric solution. The solution was boiled for 3 more minutes during which its color changed from pale yellow to red. The absorbance of the resulting solution gave a maximum at 520 nm. It was inferred from the literature that under the applied experimental conditions the size of the obtained nanoparticles was of the order of 20–30 nm (Long et al., 2009). The solution was analyzed by using a Genesys 10 spectrophotometer (Thermoscientific-Belgium). The wine sample (Chenin blanc 2009) spiked with the colloidal gold solution was stirred by a vortex IKA Genius 3 (Staufen, Germany) for reaction completion before LC-EC-Ag analysis.

2.4. Preparation of grape juice

Fresh grapes of Riesling Saering were collected mid of Octobre 2013 and stored 10 days at 5 °C before analysis. Forty intact berries were carefully sorted and snipped with scissors at the torus. The berries were transferred into a porcelain mortar and gently crushed manually with a porcelain pestle during less than 3 min. Then 1 mL of grape juice was transferred to a tube containing 99 mL of mobile phase. After shaking, the samples were filtered through a 0.45 μm filter (PAL Acrodisc® LCPVDF, VWR-Belgium) and immediately analyzed by LC-EC-Ag. Experiments were repeated 5 times ($N = 5$).