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Characterization and suitability of polyphenols-based formulas to replace sulfur dioxide for storage of sparkling white wine

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ABSTRACT

The sparkling wine protection against air is of interest for maintaining its sensorial profile and it is achieved through the use of antioxidants while disgorging. Sulfur dioxide (SO_2) is commonly added, but its amount should be limited due to human health problems. The suitability of three polyphenols-based commercial formulas containing plant gallic and ellagic acids extracted from grape (*Vitis vinifera* L.) (AO1), plant ellagic acid and gum arabic (AO2), and plant gallic, ellagic acids and *Saccharomyces cerevisiae* cell-wall fractions (AO3) was evaluated after 7 months storage (at 15 °C and 25 °C) of disgorged sparkling white wine. The phenolic composition of these formulas was investigated through spectrophotometric measurements. Moreover, the phenols were characterized and quantified by HPLC-MS analyses. The sotolon concentration and the absorbance values at 420 nm were determined in wines. The HPLC-MS analysis showed that the formula AO1 mainly contained gallotannins, ellagic tannins and flavan-3-ols, while AO2 had high levels of flavan-3-ols and gallotannins. Flavan-3-ols were the only phenols found in AO3. The addition of these formulas increased the yellow hue. Sotolon was higher than the perception threshold in the samples with AO2 and at trace amount in the samples with both AO1 and AO3 only stored at 25 °C. The tested antioxidant formulas seemed to be less effective of SO_2 for the storage of sparkling white wine. However, the investigation of phenolics in antioxidant formulas could be helpful for the proper choice of a potential substitute of SO_2 due to increase interest in sulfur-free wine production.

1. Introduction

Disgorging and corking are critical steps in sparkling wine production because the wine can be easily exposed to the air which leads to oxygen dissolution. Oxygen can worsen the sensorial properties of sparkling wine and shorten the shelf life because it can degrade some aromatic esters and terpenes (Roussis, Lambropoulos, & Tzimas, 2007) and it can speed up the formation of compounds with oxidized off-odor such as sotolon (4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one) (Lavigne, Pons, Darriet, & Dubourdieu, 2008).

Sotolon odor is perceived as a defect in young dry white wine since it decreases the intensity of the fruity and flowery notes as well as the expected freshness character (Silva Ferreira, Barbe, & Bertrand, 2003). Sotolon can arise from the aldol condensation of 2-ketobutyric acid and ethanal (Cutzach, Chatonnet, & Dubourdieu, 1999; Kobayashi, 1989; König et al. 1999), as well as from the Maillard reaction (Pons, Lavigne, Landais, Darriet, & Dubourdieu, 2010) and the oxidative degradation of ascorbic acid in a hydro-alcoholic solution (König et al. 1999). These pathways are quantitatively favored as the concentrations of oxygen and reducing sugars increase (Camara, Marques, Alves, & Silva Ferreira, 2004; Cutzach et al. 1999; Lavigne et al. 2008). Its perception threshold in white wine was reported to be 7–8 μg/l (Guichard, Pham, & Etievant, 1993) and sotolon might be adopted as a chemical marker of oxidative aging.

In order to avoid oxidation of aromatic compounds and the formation of oxidized off-flavors, sulfur dioxide (SO_2) is commonly added to sparkling white wine while disgorging since this compound is rapidly oxidized to sulfate by an oxidation/reduction cycle of hydroxycinnamoyl tartaric acids (Danilewicz, 2003). As a consequence, the dissolved oxygen can be consumed quicker in

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presence of this antioxidant (Danilewicz, 2011). Though SO_2 is useful to limit the oxidative damage of white wine, its amount should be limited because of the detrimental effect on human health and the intolerance shown by a number of wine consumers, mainly asthmatics (Lester, 1995; Pozo-Bayón, Monagas, Bartolomé, & Moreno-Arribas, 2012; Vally & Thompson, 2001). Therefore, other antioxidant compounds safer to human health should be considered and tested in winemaking. Ascorbic acid could be effective to this aim (Marks & Morris, 1993) due to its low redox potential (Danilewicz, 2003), but its oxidation gives rise to both hydrogen peroxide (Riberau-Gayon, Glories, Maujean, & Dubourdieu, 2006, chap. 5) and 2-ketobutyric acid (Pons et al., 2010). Glutathione (GSH) showed to be effective in decreasing sotolon formation in the oxidative aging of barreled white wine (Lavigne & Dubourdieu, 2004). Nevertheless, high concentrations of GSH might need to be effective, but its average amounts in wine hardly exceed few milligrams per liter (Cassol & Adams, 1995; Du Toit, Lisjak, Stander, & Prevoo, 2007; Fracassetti & Tirelli, 2015). Oxygen in wine can also be consumed by polyphenols due to their low redox potential. Polyphenols containing trihydroxyphenyl groups (i.e. galloylated phenols) have a lower redox potential than polyphenols containing dihydroxyphenyl groups and they can completely deplete oxygen from wine (Danilewicz, 2011, 2012). White wine usually contains negligible amounts of trihydroxyl substituted phenyl compounds and the addition of mixtures containing phenols into the wine might limit the oxidative reactions in sparkling white wine during shelf life. Recently, the use of plant phenolics extract was shown to be effective as an alternative to SO_2 in white wine aged in barrels (Gonzáles-Rompinelli et al., 2013). The addition of gallotannins showed to play a positive role in the maintenance of esters in white wine after 1 year storage (Sonni, Chinnici, Natali, & Riponi, 2011). However, it is known that astringency and bitterness are affected to high concentration of tannins, but their perception is strictly dependent to the phenols concentration (Robichaud & Noble, 1990). The effectiveness of polyphenols-based preparation needs to be elucidate since no data are available related to their phenolic content and the nature of the single phenols. The knowledge of the phenols composition can be helpful for better comprehend the effect of these antioxidant preparation in wine. The investigation of the consequences on oxidative damage of sparkling white wine in comparison to SO_2 is also required.

On this purpose, this study was aimed to investigate the addition of three different antioxidant formulas added to an Italian sparkling white wine (*Champenoise* method) while disgorging as potential substitutes of SO_2. The phenolic composition of these antioxidant formulas was attentively characterized by spectrophotometric and HPLC-MS analysis. The latter allowed the identification and quantification of the single phenolic compounds. The levels of sotolon and GSH, and the changes of color were also evaluated. To the best of our knowledge, the phenolic composition of industrially-produced antioxidant formulas for oenological purpose has never been investigated as well as their effect throughout sparkling wine storage.

2. Material and methods

2.1. Chemicals

All the chemicals were of analytical grade. 3-Mercaptopropionic acid (3MPA) and p-benzoquinone (pBQ) were purchased from Fluka (Switzerland). Glutathione (GSH), cysteine (Cys), sotolon, ascorbic acid (AA), dehydroascorbic acid (DHA), 1,2-phenylenediamine dihydrochloride (OPDA), dichloromethane (DCM), $FeSO_4.7H_2O$, sodium chloride (NaCl), anhydrous sodium sulfate and trifluoroacetic acid (TFA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Polyvinylpolypyrrolidone (PVPP) was purchased from Dal Cin (Sesto San Giovanni, Milan, Italy). Citric acid was purchased from J. T. Baker (Phillipsburg, NJ, US); HPLC grade methanol was from Panreac (Barcelona, Spain), and HPLC grade water was obtained by a Milli-Q system (Millipore Filter Corp., Bedford, MA, USA). The synthetic wine solution contained 5 g/l tartaric acid in 12% ethanol/water solution (v/v), adjusted to pH 3.5 with 12 M sodium hydroxide (Sigma–Aldrich). Three commercial powders containing phenolics as antioxidant purpose for the winemaking use were purchased on the market. These formulas were labeled as mixtures of plant gallic and ellagic acids extracted from grape (*Vitis vinifera* L.) (sample coded as AO1), plant ellagic acid and gum arabic (sample coded as AO2), and plant gallic, ellagic acids and *Saccharomyces cerevisiae* cell-wall fractions (samples coded as AO3).

2.2. Sparkling wine samples

The sparkling white wine was industrial-scale produced by a cellar located in the Franciacorta area (Lombardy, Italy) in the 2010 vintage from Chardonnay grape. The rational winemaking procedures usually adopted in the winery for the manufacture of *Champenoise* sparkling wine were followed and no addition of SO_2 was carried out. Base wine (10 hl) was bottled, the second fermentation was performed and the sparkling wine was maintained 12 months on the yeast lees before the disgorging.

2.3. Experimental design

Sulfur dioxide (50 mg/l) and the three antioxidant formulas (20 mg/l and 40 mg/l) were separately added to bottled sparkling white wine samples after *à la glace* disgorging. The bottles were manually filled with 10 ml of the same sparkling white wine containing the antioxidant in order to reach the final volume of 750 ml and they were closed with crown cap. Control samples were disgorged, filled with sparkling white wine antioxidant-free and capped. The chemical parameters of both base wines (control and test) are reported in Table 1 and only negligible differences were found. All the bottles were stored for 7 months in two different rooms at 15 °C and 25 °C in the dark. For each treatment and temperature investigated, the content of GSH, sotolon, AA and DHA, and the absorbance values at 420 nm were evaluated. Each trial was performed in duplicate.

2.4. Determination of sotolon

Sotolon was measured in both sparkling wines and antioxidant formulas. The wine samples preparation was carried out as described by Gabrielli, Fracassetti, and Tirelli (2014). Briefly, 3 g of NaCl were dissolved in 30 ml wine in a 100 ml bottle then 40 ml of dichloromethane (DCM) were added. The bottle was hermetically closed and shaken for 10 min with a wrist action stirrer (Griffin

Table 1
Chemical composition of the base wines produced in triplicate fermentation.

Parameter	Wine control	Wine test
Ethanol (%)	12.4 ± 0.6	12.3 ± 0.4
Sugar (g/l)	<2	<2
pH	3.3 ± 0.1	3.2 ± 0.1
Total acidity (g tartaric acid/l)	6.6 ± 0.3	7.1 ± 0.5
Volatile acidity (g acetic acid/l)	0.43 ± 0.04	0.45 ± 0.02
Free sulfur dioxide (mg/l)	<5	<5
Total sulfur dioxide (mg/l)	30 ± 4	20 ± 3

Flask Shaker). The mixture was centrifuged 5 min at 5000 × g and the DCM was separated by a separatory funnel and recovered. This solvent extraction procedure was carried out for 3 times. The organic solvent fractions were jointly collected and 2 g of anhydrous sodium sulfate were added. The DCM was evaporated under-vacuum and the dry material was dissolved with 2 ml of methanol 5% which was purified by a PVPP 50 mg SPE cartridge recovering the eluted solution.

For the antioxidant formulas, 200 mg of powder were dissolved in 50 ml of the synthetic wine solution. The liquid/liquid extraction of sotolon was carried out as reported as above for the sparkling wine samples. Each wine sample and formula was analyzed in triplicate.

2.5. Determination of glutathione and free and adsorbed cysteine

Glutathione was evaluated in both sparkling wines and commercial formulas. For the sparkling wine samples, its content was determined as described by Fracassetti and Tirelli (2015). Briefly, the sparkling wine (2 ml) treated with PVPP and centrifuged was derivatised with pBQ followed by the addition of 3MPA. The reaction mix was filtered through 0.22 μm pore size PTFE membrane (Millipore, Billerica, MA, USA) and the HPLC analysis was performed. Glutathione in antioxidant formulas was measured as described by Tirelli, Fracassetti, and De Noni (2010). Briefly, the powders were suspended in citrate buffer 75 mmol/l at pH 5 for GSH and Cys determination and in citrate buffer 75 mmol/l at pH 5 where Cys (5 mg/l) for adsorbed Cys, derivatised with pBQ and 3MPA was added. The reaction mix was filtered through a 0.22 μm pore size PTFE membrane (Millipore) and submitted to the HPLC separation. The GSH and Cys content in the antioxidant formulas was directly quantified by the HPLC analysis, while the Cys absorbed by the powders was determined by difference with the response (peak area) obtained injecting Cys 5 mg/l dissolved in citrate buffer 75 mmol/l at pH 5. Each wine sample and powder was analyzed in triplicate.

2.6. Determination of ascorbic acid and dehydroascorbic acid

Quantification of AA and DHA was carried out as previously described by Zapata and Dufour (1992) with some modifications. Thirty milliliters of wine and 100 mg of the antioxidant formula dissolved in 100 ml of synthetic wine solution containing EDTA (0.03%). The samples were filtered through a 0.45 μm PVDF filter and purified on a C18 Sep-Pak cartridge (Waters, Mil-ford, MA, US). The HPLC analysis was carried out after derivatisation of DHA into the fluorophore 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with OPDA. Standard solutions of both AA and DHA ranged from 2 mg/l to 50 mg/l were prepared in synthetic wine solution. Reversed phase HPLC separation was performed with a Waters Alliance 2695 (Milford, MA, US) equipped with a photodiode array detector Waters 2996 and a C18 column (Nova-Pak 150 × 3.9 mm, 4 μm, Waters). The chromatographic separation was carried out with an isocratic elution running acetate buffer 50 mmol/l at pH 4.5/methanol 95/5 (v/v) for 15 min followed by column washing (100% methanol for 2 min) and column conditioning (4 min). The flow rate was 0.9 ml/min. Column temperature was 25 °C and the injection volume was 20 μl. Chromatographic data were registered from 230 nm to 500 nm and processed at 261 nm and 348 nm respectively for AA and DHA by Empower 2 software (Waters). Each formula was analyzed in triplicate.

2.7. Antioxidant capacity assays

The antioxidant capacity of the antioxidant formulas was carried out both DPPH and ABTS assays.

The free radical scavenging activity determined with DPPH assay followed the method of Brand-Williams, Cuvelier, and Berset (1995) with some modifications (Espín, Soler-Rivas, Wichers, & García-Viguera, 2000; Llorach, Tomás-Barberán, & Ferreres, 2004). The DPPH solution was diluted with methanol to obtain 1.00 ± 0.03 absorbance units at 515 nm. In a 96-wells micro plate (Nunc, Roskilde, Denmark), 250 μl of DPPH solution were placed in each well and 2 μl sample were added. The sample was dissolved in 70% methanol (20 g/l) and, after centrifugation, it was serially diluted. The ABTS method was performed as reported by Mena et al. (2011). The ABTS solution was diluted with water to obtain 1.00 ± 0.03 absorbance units at 414 nm. In a 96-wells micro plate (Nunc, Roskilde), 250 μl of ABTS solution were put in each well and 2 μl sample were added. The sample was dissolved in water (20 g/l) and, after centrifugation, it was serially diluted. For both assays, the reaction kinetic was monitored for 50 min at 25 °C by micro plate reader (Infinite® M200, Tecan, Grödig, Austria). A calibration curve was made by adding increasing concentration of Trolox ranged from 50 to 1000 μmol. Each concentration was assayed in quadruplicate, as well each sample. Results were expressed as mol Trolox per 100 g of powder.

2.8. Determination of phenolic compounds in the antioxidant formulas

2.8.1. Spectrophotometric analysis

The total phenols (TP) level of the antioxidant formulas was estimated colorimetrically by Folin–Ciocalteu method (Scalbert, Monties, & Janin, 1989). The formulas (1 g/l) were dissolved in methanol/water 50/50 (v/v) and diluted 2.5, 5 and 10 times in the same solvent. The Folin–Ciocalteu reagent was diluted 10 times in water (v/v) and 2.5 ml was added to 0.5 ml of sample. Two milliliters of 75 g/l sodium carbonate solution were added and the tubes were kept 1 h at room temperature in the dark. In the meanwhile, the calibration curve for gallic acid (5–100 mg/l) dissolved in methanol/water 50/50 (v/v) was achieved. The absorbance at 765 nm was measured and the results were expressed as g gallic acid/100 g powder. Each formula was analyzed in triplicate.

In order to investigate deeply on the phenols in these formulas, the total flavonoids (TF) and non-flavonoids (NF) contents of the antioxidant formulas were also determined in accordance with Di Stefano, Cravero, and Gentilini (1989). The formulas (1 g/l) were dissolved in synthetic wine solution, diluted in chloridric-ethanol solution (ethanol/water/chloridric acid 70/30/1 v/v/v) and the absorbance at 280 nm was measured. The TF concentration was expressed as mg gallic acid/g powder obtained through a calibration curve of gallic acid dissolved in the chloridric-ethanol solution (50–200 mg/l). Each formula was analyzed in triplicate. The NF concentration was estimated by subtracting to the absorbance value from TF the absorbance value found for the proanthocyanidins (see below) corrected at 280 nm. It was expressed as g gallic acid/100 g powder.

2.8.2. Determination of proanthocyanidins

Proanthocyanidins were assessed as described by Bate-Smith (1981). The antioxidant formulas (1 g/l) were dissolved in the synthetic wine solution. In two separate test tubes (reaction tube and blank tube) 2 ml of sample, 10.5 ml of ethanol and 12.5 ml of hydrochloric acid 37% (v/v) containing 300 mg/l of $FeSO_4.7H_2O$ were added. The reaction tube was placed in a water bath at 100 °C for 50 min, while the blank tube was left to stand in the dark in ice. After 50 min, the reaction tubes were cooled in ice for 10 min. The absorbance was measured at 550 nm. The concentration of proanthocyanidins was calculated multiplying the absorbance

difference among the reaction tube and the blank tube by the factor 1162.5 and results were expressed as g cyanidin/100 g powder (Di Stefano et al., 1989). The determination was carried out in triplicate.

2.8.3. Reactivity to sulfur dioxide

The reactivity to SO_2 of powders was determined by spectrophotometric analysis in order to assess the oxidized phenols which higher concentrations lead to an increase of absorbance in presence of SO_2 (Di Stefano & Cravero, 1991). The formulas (1 g/l) were dissolved in the synthetic wine solution and the absorbance at 280 nm was measured before and after the addition of SO_2 (0.3%). Water was used as blank. The difference of absorbance values between the readings carried out before and after the addition of SO_2 was expressed as g gallic acid reactive to SO_2 per 100 g of powder through a calibration curve of gallic acid dissolved in the synthetic wine solution (50–500 mg/l). The determination was carried out in triplicate.

2.8.4. Determination of o-dihydroxyl and o-trihydroxyl phenols

The *o*-dihydroxyl and *o*-trihydroxyl phenols were spectrometrically determined, as described by Riberau-Gayon (1968), chap. 3. The method took into account the different absorbance response at 545 nm of *o*-dihydroxyl (pyrocathecol) and *o*-trihydroxyl phenols (pyrogallol) dissolved in reaction buffer (sodium and potassium tartrate 5 g/l, $FeSO_4$ 1 g/l) after addition of borate buffer (12.37 g/l boric acid, 14.91 g/l potassium chloride, pH 8.1–8.3 adjusted with NaOH 1 N) or acetate buffer (10% ammonium acetate, pH 8.1–8.3 adjusted with ammonium hydroxide 10%). The content of *o*-dihydroxyl and *o*-trihydroxyl phenols was expressed as percentage. The determination was carried out in triplicate.

2.8.5. LC-MS analysis

The phenols characterization was carried out on the three antioxidant formulas added to the sparkling wine. The phenolic compounds were identified and quantified as reported by Fracassetti, Costa, Moulay, and Tomás-Barberán (2013). The extraction of phenolics was performed as follows: 1 g of the formulas was added to 25 ml of methanol/water 50/50 acidified with 1% formic acid. The formulas were vortexed for 2 min, sonicated for 15 min (Sonicator Branson 5510, Emerson, Danbury, CT, US) and centrifuged at 5000 × g for 15 min at 4 °C (Centrifuge 5804 R, Eppendorf, Hamburg, Germany). The supernatants were recovered, freeze-dried under vacuum, suspended in 2 ml of the corresponding extraction solvent, then filtered with a PVDF filter 0.22 μm (Millipore) and injected in LC-MS. The identification and quantification of phenols were performed using an Agilent 1100 Series equipment (Agilent, Santa Clara, CA, USA) equipped with G1312A binary pump, G1313A autosampler, G1315B photodiode array detector, and G1322A degasser controlled by the Agilent software v. A08.03. HPLC was coupled with a detector MSD Trap 1100 Series (Agilent) with an electrospray ionisation system (ESI), with the following conditions: the heated capillary was 350 °C and 3–3.5 kV voltage, mass scan (MS) and MS/MS were measured from 100 to 1500 *m/z*. Collision induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 75%. Mass spectrometry data were acquired in the negative ionisation mode. A column Pursuit XRs C18 250 × 40 mm from Varian (Agilent) was used and a flow rate of 0.8 ml/min. The used solvents were 1% formic acid in water (A) and acetonitrile (B) which was in the following separation gradient: 1% B in A at 0 min, 9% B at 10 min, 35% B at 48 min, and 95% B at 52 min, following by washing and conditioning steps. Data were registered from 250 nm to 700 nm and the phenolic compounds were quantified at 280 mn, 360 mn, and 520 nm, depending on the type of phenolic compound. Integrations were performed by Agilent ChemStation for LC 3D, Rev. B.01.03 SR1. MS trap control was carried out Bruker Daltonic version 5.2. Quantification of gallic acid, ellagic acid, myricetin and their derivatives, and ellagitannins was carried out with the calibration curves obtained for gallic acid (1–300 mg/l), ellagic acid (1–300 mg/l), rutin (1–300 mg/l), and vescalagin (0.1–100 mg/l), respectively, at the appropriate wavelengths. All the samples and standards were injected in triplicate.

2.9. Total phenols in sparkling wine

The total phenols concentration in sparkling wine samples was assessed through spectrophotometric analysis recording the absorbance at 280 nm (Di Stefano et al., 1989). The data were expressed as mg gallic acid/L obtained through a calibration curve of gallic acid dissolved in synthetic wine solution (50–200 mg/l). The analyses were carried out in triplicate.

2.10. Statistical analyses

The one-way ANOVA was performed using STATISTICA 9 software (Statsoft Inc., Tulsa, OK, US). Significant differences were judged to using a 5% significance level ($p < 0.05$). The correlation coefficients between GSH, GRP, sotolon and the absorbance at 420 nm were computed through the Pearson correlation.

3. Results and discussion

3.1. Characterization of the commercial antioxidant formulas

The phenolics of the antioxidant formulas were characterized in order to achieve more detailed composition of them. The TP concentrations determined by the Folin–Ciocalteu reagent corresponded to 58% and 51% for the formulas AO1 and AO2, respectively (Table 2). Lower amount of TP was detected in AO3 (14.2%). The presence of polymeric (as proanthocyanidins) and monomeric phenols was evaluated. Flavan-3-ol polymers were most abundant in AO2 (19.0%) and not detectable in AO3. The formula AO1 showed highest amounts of both TF (39.5%) and non-flavonoids (37.5%).

In order to achieve a deeper knowledge of the antioxidant formulas employed for this research, the low-molecular weight phenols were characterized by LC-MS as shown in Table 3 and Fig. 1. All the compounds were characterized by their UV spectra and their molecular ion and fragments obtained with an ESI-MS/MS detector (Table 3) and comparison, wherever possible, was carried out with standard compounds. Flavonols, ellagic acid conjugates, ellagitannins and proanthocyanidins were the most represented polyphenols. Quercetin (3,5,7,3′,4’-pentahydroxyflavone) (56) and its 3-O-glycoside (53) were detected, the latter in AO3 only. They showed the characteristic UV spectra of flavonols with a free hydroxyl group at position 3 for quercetin (UV band I maximum at 370 nm), as well as its glycosylated form at position 3 (UV band I maximum at 356 nm) (Table 3). The pseudomolecular ions recorded with the HPLC-ESI MS and the fragments obtained confirmed these structures with the characteristic losses of a glycosyl residue respectively leading to the quercetin aglycone fragment at *m/z* 301. Kaempferol (3,4′,5,7-tetrahydroxyflavone) (57) and its 3-*O*-glycosyl derivative (54) were revealed, the latter only in AO3. This compound showed *m/z* 755 and it is probably a hexoxyl-rhamnosyl-hexoside derivative of kaempferol. In addition, myricetin (3,5,7,3′,4′,5’-hexahydroxyflavone) (55) was detected in AO3 only. The isomeric ellagitannin *C*-glucosides vescalagin (2) and castalagin (4) were characterized by both the pseudomolecular ion at *m/z* 933 and the characteristic fragments that did not include the ellagic acid fragment at *m/z* 301 as they were C-glycosides. These two

Table 2
Content of the phenolic fractions spectrophotometrically determined in the antioxidant formulas. Data are reported as mean values (n = 3) ±standard deviation; n.d.: not detected.

Formula	Total phenols index	Total flavonoids	Non-flavonoids	Phenols reactive to SO_2	Proanthocyanidins	*O*-dihydroxyl phenols	*O*-trihydroxyl phenols
	g gallic acid/100 g powder	*g gallic acid/100 g powder*	*g gallic acid/100 g powder*	*g gallic acid/100 g powder*	*g cyanidin/100 g powder*	*%*	*%*
AO1	57.8 ± 3.2	39.48 ± 0.11	37.54 ± 0.11	2.53 ± 0.31	7.84 ± 0.01	9	91
AO2	50.9 ± 9.5	23.10 ± 0.60	17.69 ± 0.59	3.59 ± 0.55	19.00 ± 3.97	51.2	48.8
AO3	14.2 ± 3.3	4.86 ± 0.08	4.86 ± 0.08	2.29 ± 0.31	n.d.	0	100

Table 3
Low molecular weight phenols detected by HPLC-DAD-ESI-MS-MS in the antioxidant formulas.

Number	Compound	Retention time (min)	$[M-H]^-$	λ max (nm)	MS fragments
Flavonols					
53	Quercetin 3-*O*-glucoside	32.2	463	256, 356	301, 151
54	Kaempferol 3-*O*-hexosyl-rhamnosyl-hexoside	32.4	755	264, 350	284
55	Myricetin	38.1	317	256, 374	179, 151
56	Quercetin	45.5	301	256, 370	301, 179, 151
57	Kaempferol	52.4	285	254, 370	285, 151
Ellagitannins and ellagic acid					
2	Vescalagin	11.6	933	242	915, 631
4	Castalagin	14	933	242	915, 631
5	Hexahydroxy-diphenoyl-galloyl-glucose	14.5	633	254, 376	301
6	Hexahydroxy-diphenoyl-galloyl-glucose	16.6	633	254, 376	301
33	Ellagic acid	31.5	301	254, 374	301
Gallotannins					
7	Digalloyl quinic acid	17.8	495	236,274	343, 269, 169
9	Digalloyl quinic dimer	18.8	991	236, 276	495, 343, 169, 125
12	Trigalloyl quinic acid	23.1	647	238, 276	495, 343, 169
13	Trigalloyl quinic acid	23.9	647	238, 276	495, 343, 169
15	Trigalloyl quinic acid	24.6	647	238, 276	495, 343, 169
16	Trigalloyl quinic acid	25.2	647	238, 276	495, 343, 169
18	Digalloyl quinic acid	25.9	495	236, 274	343, 269, 169
19	Tetragalloyl quinic acid	27.1	799	236, 274	647, 495
20	Tetragalloyl quinic acid	27.8	799	236, 274	647, 495
21	Trigalloyl quinic acid	27.9	647	238, 276	495, 343, 169
22	Tetragalloyl quinic acid	28.1	799	236, 274	647, 495
23	Tetragalloyl quinic acid	28.5	799	236, 274	647, 495
24	Trigalloyl quinic acid	28.6	647	238, 276	495, 343, 169
25	Tetragalloyl quinic acid	29.0	799	236, 274	647, 495
26	Tetragalloyl quinic acid	29.1	799	236, 274	647, 495
27	Trigalloyl-mono(digalloyl) quinic acid	29.2	951	236, 274	799, 647, 495
28	Tetragalloyl quinic acid	29.6	799	236, 274	647, 495
29	Trigalloyl quinic acid	29.8	647	238, 276	495, 343, 169
30	Tetragalloyl quinic acid	30.0	799	236, 274	647, 495
31	Tetragalloyl quinic acid	30.1	799	236, 274	647, 495
32	Trigalloyl quinic acid	30.1	647	238, 276	495, 343, 169
34	Tetragalloyl quinic acid	30.7	799	236, 274	647, 495
36	Trigalloyl-mono(digalloyl) quinic acid	31.2	951	236, 274	799, 647, 495
37	Trigalloyl-mono(digalloyl) quinic acid	31.5	951	236, 274	799, 647, 495
38	Trigalloyl-mono(digalloyl) quinic acid	32.1	951	236, 274	799, 647, 495
39	Trigalloyl-mono(digalloyl) quinic acid	32.5	951	236, 274	799, 647, 495
40	Trigalloyl-mono(digalloyl) quinic acid	32.7	951	236, 274	799, 647, 495
41	Trigalloyl-mono(digalloyl) quinic acid	33.0	951	236, 274	799, 647, 495
42	Digalloyl-di(digalloyl) quinic acid	33.4	1103	238, 274	951, 799, 647
43	Digalloyl-di(digalloyl) quinic acid	33.7	1103	238, 274	951, 799, 647
44	Digalloyl-di(digalloyl) quinic acid	33.9	1103	238, 274	951, 799, 647
45	Digalloyl-di(digalloyl) quinic acid	34.1	1103	238, 274	951, 799, 647
46	Digalloyl-di(digalloyl) quinic acid	35.0	1103	238, 274	951, 799, 647
47	Digalloyl-di(digalloyl) quinic acid	35.1	1103	238, 274	951, 799, 647
48	Digalloyl-di(digalloyl) quinic acid	35.5	1103	238, 274	951, 799, 647
49	Digalloyl-di(digalloyl) quinic acid	35.7	1103	238, 274	951, 799, 647
50	Digalloyl-di(digalloyl) quinic acid	36.1	1103	238, 274	951, 799, 647
51	Galloyl-tri(digalloyl) quinic acid	37.1	1255	256, 279	1103, 951, 799, 647
52	Galloyl-tri(digalloyl) quinic acid	37.4	1255	256, 279	1103, 951, 799, 647
Gallic acid derivatives					
1	Gallic acid	9.1	169	272	169, 125
Proanthocyanidins					
3	Gallocatechin	13.7	305	268	261, 219, 179, 125
8	Gallocatechin-dimer	18.2	611	240, 272	547, 305, 219
10	Catechin-dimer	20.7	577	238, 274	559, 451, 425, 289, 245
11	Catechin-dimer	21.6	577	238, 274	559, 451, 425, 289, 245
14	Gallocatechin-gallate-dimer	23.9	915	240, 274	457, 305
17	Gallocatechin-gallate	25.8	457	238, 276	331, 305, 169
35	Catechin-gallate-dimer	30.8	883	238, 278	441, 289

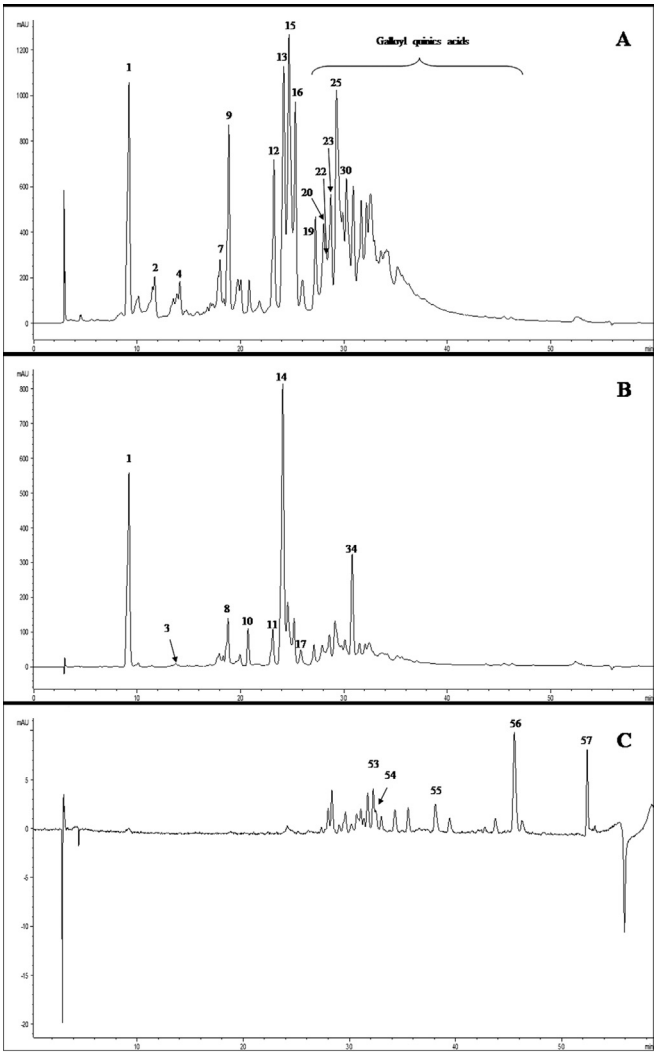

Fig. 1. HPLC analyses of phenolic compounds from AO1 at 280 nm (A) and AO3 at 360 nm (B) and at 280 nm (C). For compounds characterization see Table 3.

phenols were confirmed by chromatographic comparisons with their respective standards. Ellagic acid (33) and ellagitannins hexahydroxy-diphenoyl-galloyl-glucose isomers (5, 6) were detected (Table 3) only in AO1 as they showed the characteristic UV spectrum of ellagic acid and ellagitannins. Among the latter two phenols, the main one was free ellagic acid that showed a pseudomolecular ion at *m/z* 301 and it overlapped chromatographically with an authentic standard of this phenol. Several hydrolysable tannins, mainly gallotannins, were particularly abundant in AO1, some of these compounds were also revealed in AO2, while none of them was found in AO3. Most of the hydrolysable tannins were recognized as galloyl derivatives of quinic acid through the comparison of the molecular weight with both parent and daughter ions and UV spectra (Clifford, Stoupi, & Kuhnert, 2007). Gallic acid (1) was also detected and it was confirmed by the chromatographic analysis of the standard compound. Flavan-3-ols were also present in these antioxidant formulas with some differences among them (Table 3). Gallocatechin (3) a dimer of catechin (11) and gallocatechin gallate (17) were revealed only in AO3. A dimer of catechin gallate (35) was found in AO1, AO2 and AO3. A dimer of gallocatechin (8) was detected in AO2 and AO3. The three antioxidant formulas contained a dimer of catechin (10) and a dimer of gallocatechin gallate (14).

The antioxidant formula AO1 contained the highest level of low molecular weight phenols (15.8 g/100 g powder) and the gallotannins were the most abundant compounds (70%) (Table 4). Ellagic acid and ellagitannins were detected only in such formula (1.50 g/100 g powder). Gallic acid (1.15 g/100 g powder) and proanthocyanidins (2.11 g/100 g powder) were also found as well as flavonols (0.04 g/100 g powder), namely quercetin and kaempferol. The data obtained by LC-MS confirm the high level of hydrolyzable tannins in AO1 mainly represented by trihydroxyl phenols (Table 4) as found also spectrophotometrically (Table 2). The high concentration of phenolic compounds in AO1 could ease an effective consumption of oxygen (Danilewicz, 2011). The antioxidant activity values of the formulas tested (Table 5) seemed to confirm this hypothesis and were proportional with the TP levels. The ratio value DPPH/TP (Table 5) showed a poor antioxidant ability of AO2 polyphenols, in spite of the gallotannins presence (Table 4), when it was compared to same value of AO3 formula which did not contain gallotannins (Table 4). This could indicate the presence of oxidized phenols in AO2 as also suggested by the spectrophotometric analysis showing a higher level of phenols reactive to SO_2 in comparison to AO1 and AO3 (Table 2). The presence of *o*-quinones could be indicated by the presence of absorbed Cys revealed in AO2 (Table 5) since these compounds have a strong reactivity with the thiols (Riberau-Gayon et al. 2006, chap. 5). Cys was absorbed even by AO3 (Table 5) and the ability of binding the Cys could partly explain the low ratio values DPPH index/TP and ABTS/TP found for both AO2 and AO3 (Table 5). The levels of GSH and AA were evaluated in order to assess the presence of non-phenolic antioxidants. No antioxidant formula contained AA, whereas GSH was detected only in AO3 (5.8 g/100 g powder). This is in accordance with the presence of yeast cell-wall fractions (Tirelli et al. 2010) as declared by the supplier.

3.2. *Influence of antioxidant formulas on sparkling white wines*

The addition of antioxidant formulas potentially replacing SO_2 was evaluated in sparkling white wine. The use of SO_2 should be minimized owing to its problems for human health (Pozo-Bayón et al., 2012). This compound should be replaced in wine with suitable antioxidant mixtures. The proper amount of the polyphenols-based antioxidant formulas in sparkling white wine was chosen taking into account both technological and sensory factors since AO3 can be responsible for wine haze due to the yeast cell-wall fractions it contained. Antioxidant formulas AO1 and AO2 were mainly constituted with polyphenols which could confer astringency if added in high concentrations (Robichaud & Noble, 1990). Moreover, tannins could react with the wine proteins which lead to haziness and worsen the foaming properties (Coelho, Rocha, & Coimbra, 2011; Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Magariño, 2015). Therefore, additions up to 20 mg/l and 40 mg/l of each tested antioxidant formula were carried out, as also suggested by the supplier. These additions did not affect the wine astringency since the highest concentration of phenols added was about 23.2 mg/l which was lower than the amount of tannin causing its perception (Bertand et al., 2000; Robichaud & Noble, 1990). The total phenols content ranged from 118.5 mg/l to 147.4 mg/l in wine samples. Significant differences were found due to the addition of both AO1 and AO2 in comparison to control wine samples and those samples where SO_2 and AO3 were added (Table 6). This could be due to the own high concentration of total phenols of these formulas (Table 2). The oxidation of phenols to quinones due to air entrance in the bottle could be expected especially in the sparkling wine samples containing the formulas which showed lower concentration of *o*-trihydroxyl phenols (Danilewicz, 2011).

Table 4
Content of low molecular weight phenols in the antioxidant formulas. Data are reported as mean values (n = 2) ±standard deviation; n.d.: not detected.

Number	Compound	AO1	AO2 (mg/100 g powder)	AO3
Flavonols				
53	Quercetin 3-*O*-glucoside	n.d.	n.d.	12.55 ± 0.96
54	Kaempferol 3-*O*-hexosyl-rhamnosil-hexose	n.d.	n.d.	2.70 ± 0.20
55	Myricetin	n.d.	n.d.	16.77 ± 0.40
56	Quercetin	29.09 ± 0.64	9.77 ± 0.75	98.81 ± 0.18
57	Kaempferol	10.91 ± 0.92	0.22 ± 0.12	49.47 ± 1.06
	Total	40.00 ± 1.56	9.99 ± 0.89	180.30 ± 2.92
Ellagitannins and ellagic acid				
2	Vescalagin	689.45 ± 2.87	n.d.	n.d.
4	Castalagin	636.23 ± 4.1	n.d.	n.d.
5	Hexahydroxy-diphenoyl-galloyl-glucose	42.72 ± 1.52	n.d.	n.d.
6	Hexahydroxy-diphenoyl-galloyl-glucose	40.44 ± 1.28	n.d.	n.d.
33	Ellagic acid	95.22 ± 1.91	n.d.	n.d.
	Total	1504.06 ± 11.93	–	–
Gallotannins				
7	Digalloyl quinic acid	283.73 ± 17.76	n.d.	n.d.
9	Digalloyl quinic dimer	827.95 ± 10.28	176.34 ± 8.51	n.d.
12	Trigalloyl quinic acid	773.02 ± 17.16	134.71 ± 1.45	n.d.
13	Trigalloyl quinic acid	1893.72 ± 68.63	326.79 ± 2.71	n.d.
15	Trigalloyl quinic acid	1032.30 ± 13.85	167.58 ± 1.78	n.d.
16	Trigalloyl quinic acid	n.d.	67.18 ± 2.31	n.d.
18	Digalloyl quinic acid	456.95 ± 1.84	78.88 ± 1.60	n.d.
19	Tetragalloyl quinic acid	382.10 ± 18.13	n.d.	n.d.
20	Tetragalloyl quinic acid	n.d.	52.72 ± 0.83	n.d.
21	Trigalloyl quinic acid	180.00 ± 8.71	n.d.	n.d.
22	Tetragalloyl quinic acid	618.35 ± 5.82	n.d.	n.d.
23	Tetragalloyl quinic acid	n.d.	92.16 ± 2.41	n.d.
24	Trigalloyl quinic acid	797.50 ± 21.63	n.d.	n.d.
25	Tetragalloyl quinic acid	548.06 ± 10.70	n.d.	n.d.
26	Tetragalloyl quinic acid	n.d.	173.94 ± 1.84	n.d.
27	Trigalloyl-mono(digalloyl) quinic acid	472.76 ± 3.50	n.d.	n.d.
28	Tetragalloyl quinic acid	n.d.	20.14 ± 0.73	n.d.
29	Trigalloyl quinic acid	562.67 ± 20.21	n.d.	n.d.
30	Tetragalloyl quinic acid	269.70 ± 7.31	n.d.	n.d.
31	Tetragalloyl quinic acid	n.d.	26.30 ± 0.80	n.d.
32	Trigalloyl quinic acid	139.49 ± 4.72	n.d.	n.d.
37	Trigalloyl-mono(digalloyl) quinic acid	247.36 ± 12.66	26.96 ± 2.05	n.d.
38	Trigalloyl-mono(digalloyl) quinic acid	246.99 ± 3.30	23.02 ± 0.47	n.d.
39	Trigalloyl-mono(digalloyl) quinic acid	521.57 ± 5.5	59.33 ± 2.15	n.d.
40	Trigalloyl-mono(digalloyl) quinic acid	129.19 ± 9.77	n.d.	n.d.
41	Trigalloyl-mono(digalloyl) quinic acid	26.13 ± 1.41	n.d.	n.d.
42	Digalloyl-di(digalloyl) quinic acid	62.52 ± 1.48	n.d.	n.d.
43	Digalloyl-di(digalloyl) quinic acid	54.74 ± 1.24	n.d.	n.d.
44	Digalloyl-di(digalloyl) quinic acid	45.87 ± 0.84	n.d.	n.d.
45	Digalloyl-di(digalloyl) quinic acid	42.02 ± 2.89	n.d.	n.d.
46	Digalloyl-di(digalloyl) quinic acid	114.07 ± 6.48	n.d.	n.d.
47	Digalloyl-di(digalloyl) quinic acid	61.43 ± 5.39	n.d.	n.d.
48	Digalloyl-di(digalloyl) quinic acid	52.93 ± 2.53	n.d.	n.d.
49	Digalloyl-di(digalloyl) quinic acid	34.01 ± 4.11	n.d.	n.d.
50	Digalloyl-di(digalloyl) quinic acid	20.73 ± 2.8	n.d.	n.d.
51	Galloyl-tri(digalloyl) quinic acid	72.71 ± 3.53	n.d.	n.d.
52	Galloyl-tri(digalloyl) quinic acid	35.61 ± 1.17	n.d.	n.d.
	Total	11,006.18 ± 184.09	1426.05 ± 14.18	–
Gallic acid derivatives				
1	Gallic acid	1147.05 ± 14.87	727.47 ± 2.68	2121.62 ± 21.08
Proanthocyanidins				
3	Gallocatechin	n.d.	n.d.	49.53 ± 5.4
8	Gallocatechin-dimer	n.d.	55.88 ± 2.03	190.34 ± 1.40
10	Catechin-dimer	128.29 ± 3.43	155.87 ± 2.30	582.06 ± 10.11
11	Catechin-dimer	n.d.	n.d.	121.68 ± 5.92
14	Gallocatechin-gallate-dimer	1255.60 ± 72.97	1714.41 ± 7.96	4570.72 ± 77.15
17	Gallocatechin-gallate	n.d.	n.d.	328.62 ± 4.95
35	Catechin-gallate-dimer	721.53 ± 17.24	499.97 ± 1.56	1918.53 ± 61.34
	Total	2105.42 ± 52.3	2426.13 ± 9.25	7761.48 ± 20.08
	Total phenols	**15,802.51 ± 378.73**	**4589.64 ± 30.17**	**10,063.4 ± 23.12**

The addition of SO_2 was the most effective in protecting wine against the oxidation since sotolon was not found and the lowest absorbance values at 420 nm were observed (Table 6). Higher absorbance values were revealed in the sparkling wine samples where the three antioxidant formulas were added, particularly those supplemented with AO2. Sotolon in concentration close to (6.41 μg/l) or higher than (13.37 μg/l) the perception threshold was detected in the wine samples where 40 mg/l of AO2 were added and they were stored at 15 °C and 25 °C, respectively. This finding was not expected since the sparkling white wine samples containing lower amount of polyphenols-based formulas were supposed to consume oxygen at lower rate leading to a lower sotolon

Table 5
Content of sotolon, glutathione, free and adsorbed cysteine, ascorbic acid, dehydroascorbic acid and antioxidant capacity of the antioxidant formulas. Data are reported as mean values (n = 3) ±standard deviation; n.d.: not detected.

Formula	Sotolon *μg/100 g powder*	Glutathione *g/100 g powder*	Cysteine		Ascorbic acid	Dehydroascorbic acid	Antioxidant capacity		DPPH/TP ratio	ABTS/TP ratio
			Free	Absorbed			DPPH	ABTS		
			mg/100 g powder				*M Trolox/100 g powder*			
AO1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8776 ± 650	1660 ± 109	151.8	28.7
AO2	n.d.	n.d.	n.d.	9.0 ± 0.28	n.d.	n.d.	5990 ± 443	1338 ± 98	117.7	26.3
AO3	n.d.	5.77 ± 0.18	n.d.	64.5 ± 2.0	n.d.	n.d.	1768 ± 131	133 ± 10	124.5	9.4

concentration. However, AO2 showed the highest level of dihydroxyl phenols (Table 2) which are responsible for a lower rate of oxygen consumption in comparison to trihydroxyl phenols (Danilewicz, 2011). This suggests that oxygen could participate to other oxidative phenomena. Trace amount of sotolon was detected in the wine samples containing 20 mg/l of AO2 as well as in the wine samples supplemented with AO1 and AO3 only stored at 25 °C. This finding was in accordance to the research carried out by Cutzach, Chatonnet, and Dubourdieu (2000) who reported that high storage temperature (up to 33 °C) can promote the formation of sotolon in *Vins doux Naturels*. High storage temperatures can also promote the Maillard reaction which is included among the pathways affecting the sotolon formation (Hofmann & Schieberle, 1996; Pons et al. 2010). The phenols content did not seem to affect since trace level of sotolon was found in wine samples where both AO1 and AO3 were added. However, qualitative difference in the phenolics could favor the sotolon formation and further investigations could elucidate the compound(s) majorly affecting the sotolon increase during the storage. The storage temperature seems to play a strong role since significant differences were found in the absorbance values ($p = 0.0080$), GSH ($p = 0.0002$) and GRP ($p = 0.0003$). It also appeared that oxidative phenomena took place in the wine samples treated with the antioxidant formulas which seemed to have a negative impact on wine in comparison to SO_2, in terms of off-flavor formation. In fact, minor differences in the absorbance values at 420 nm were noticed in the control wine sample and in the wine samples containing AO3 whose phenols concentration was about 4 folds lower than that of AO1 and AO2 (Table 2).

Besides the formation of brown polymers, a decreased content of GSH was also expected (Salgues, Cheynier, Gunata, & Wylde, 1986). Slight differences were found in the GSH content among the sparkling white wine samples (Table 6). The addition of both SO_2 and the antioxidant formulas did not affect the GSH content in wine. The GSH concentration in wine samples showed an unexpected trend since higher levels were detected in the samples stored at 25 °C. Moreover, the GSH content was higher in the samples where higher amounts of antioxidant formulas were added, including those supplemented with AO1 and AO2 which did not contain GSH, in comparison to the antioxidants-free wine samples. The rationale behind the increased GSH content is not clear. As hypothesis, GSH could arise from the glutathionyl-phenols adducts since GRP decreased over the storage and lower concentration of this compound was found at 25 °C in comparison to 15 °C (Table 6). A positive correlation was found between increased GSH content and decreased GRP content for the different temperatures of storage ($p = 0.46$ at 15 °C; $p = 0.40$ at 25 °C). The antioxidant formula AO3 containing GSH (5.8 g/100 g) did not lead to a higher concentration of GSH in the wine samples where it was added in comparison to the samples supplemented with AO1 and AO2.

4. Conclusions

Our results highlight that the knowledge of the phenolic composition of antioxidant formulas can be helpful for the choice of an appropriate antioxidant mixture in sparkling white wine production. However, the tested polyphenols-based antioxidants were unsuitable to avoid the use of SO_2 as antioxidant in sparkling wine. These formulas seemed to have a detrimental role into the oxidative decay of sparkling white wine whose shelf-life was shorten if compared to sparkling white wine without antioxidant. Our data are not enough to support a correlation between the phenols amount and the oxidative damage though the appearance of the atypical aging was detected into the wine containing the formulas tested. Further investigations will need to find an effective

Table 6
Concentration of glutathione, 2-S-glutathionyl caftaric acid and sotolon, and absorbance values in sparkling white wine samples stored under different conditions. Data are reported as mean values (n = 3) ±standard deviation; n.d.: not detected. Different letters mean significant difference ($p < 0.05$).

Antioxidant added	Dosage (mg/l)	Storage temperature (°C)	Total phenols (mg gallic acid/l)	Absorbance 420 nm	Glutathione (mg/l)	2-S-glutathionyl caftaric acid (mg/l)	Sotolon (μg/l)
No addition	–	15	118.5 ± 9.5^a	0.142 ± 0.001^a	1.33 ± 0.040^a	0.57 ± 0.018^a	n.d.
No addition	–	25	124.5 ± 10.0^a	0.150 ± 0.001^a	2.66 ± 0.082^a	0.41 ± 0.013^a	n.d.
SO_2	50	15	119.7 ± 9.6^a	0.099 ± 0.001^a	0.93 ± 0.029^a	0.50 ± 0.016^a	n.d.
SO_2	50	25	122.8 ± 9.8^a	0.122 ± 0.002^a	2.25 ± 0.068^a	0.47 ± 0.015^a	n.d.
AO1	20	15	131.4 ± 10.5^b	0.154 ± 0.001^b	1.80 ± 0.054^a	0.54 ± 0.017^a	n.d.
AO1	20	25	136.1 ± 10.8^b	0.158 ± 0.000^b	3.58 ± 0.11^a	0.41 ± 0.013^a	<2
AO1	40	15	143.1 ± 11.4^b	0.156 ± 0.000^b	2.13 ± 0.066^a	0.70 ± 0.021^a	n.d.
AO1	40	25	147.4 ± 11.8^b	0.160 ± 0.000^b	3.38 ± 0.10^a	0.61 ± 0.020^a	<2
AO2	20	15	131.4 ± 10.5^b	0.157 ± 0.000^b	1.31 ± 0.041^a	0.72 ± 0.022^a	<2
AO2	20	25	132.5 ± 10.6^b	0.172 ± 0.000^b	2.37 ± 0.073^a	0.53 ± 0.017^a	<2
AO2	40	15	133.9 ± 10.6^b	0.170 ± 0.001^b	1.39 ± 0.043^a	0.57 ± 0.017^a	6.41 ± 0.11
AO2	40	25	145.8 ± 11.2^b	0.181 ± 0.002^b	4.26 ± 0.076^a	0.35 ± 0.011^a	13.37 ± 0.22
AO3	20	15	119.2 ± 9.5^a	0.147 ± 0.001^a	1.57 ± 0.049^a	0.56 ± 0.016^a	n.d.
AO3	20	25	121.2 ± 9.7^a	0.147 ± 0.000^a	2.51 ± 0.079^a	0.45 ± 0.014^a	<2
AO3	40	15	127.2 ± 9.9^a	0.141 ± 0.000^a	1.58 ± 0.049^a	0.58 ± 0.017^a	n.d.
AO3	40	25	125.1 ± 10.0^a	0.155 ± 0.000^a	3.78 ± 0.12^a	0.45 ± 0.014^a	<2

antioxidant formula substituting SO_2 while disgorging the sparkling wine allowing the production of sulfur-free wine which has been assuming increasing interest.

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References

Bate-Smith, E. C. (1981). Astringent tannins of the leaves of *Geranium* species. *Phytochemistry, 20*, 211–216.

Bertand, A., Canal-Llaubères, R. M., Feuillat, M., Hardy, G., Lamadon, F., Lanvaud-Funel, A., et al. (2000). *Produits de traitement and auxiliares d'élaboration des moûts et de vins*. Bordeaux: Edition Féret.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology, 28*, 25–30.

Camara, J. S., Marques, J. C., Alves, M. A., & Silva Ferreira, A. C. (2004). 3-Hydroxy-4,5-dimethyl-2(5H)-furanone levels in fortified Madeira wines: relationship to sugar content. *Journal of Agricultural and Food Chemistry, 52*, 6765–6769.

Cassol, T., & Adams, D. O. (1995). Detection of glutathione in white wines using an enzymatic analytical method. *American Journal of Enology and Viticulture, 46*, 410.

Clifford, M. N., Stoupi, S., & Kuhnert, N. (2007). Profiling and characterization by LC-MSn of the galloylquinic acid of green tea, tara tannin, and tannic acid. *Journal of Agricultural and Food Chemistry, 55*, 2797–2807.

Coelho, E., Rocha, S.,M., & Coimbra, M. A. (2011). Foamability and foam stability of molecular reconstituted model sparkling wines. *Journal of Agricultural and Food Chemistry, 59*, 8770–8778.

Cutzach, I., Chatonnet, P., & Dubourdieu, D. (1999). Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *Journal of Agricultural and Food Chemistry, 47*, 2837–2846.

Cutzach, I., Chatonnet, P., & Dubourdieu, D. (2000). Infuence of storage conditions on the formation of some volatile compounds in white fortified wines (*vins doux naturels*) during the aging process. *Journal of Agricultural and Food Chemistry, 48*, 2340–2345.

Danilewicz, J. C. (2003). Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: central role of iron and copper. *American Journal of Enology and Viticulture, 54*, 73–85.

Danilewicz, J. C. (2011). Mechanisms of autoxidation of polyphenols and participation of sulfite in wine: key role of iron. *American Journal of Enology and Viticulture, 62*, 319–328.

Danilewicz, J. C. (2012). Review of oxidative processes in wine and value of reduction potentials in enology. *American Journal of Enology and Viticulture, 63*, 1–10.

Di Stefano, R., & Cravero, M. C. (1991). Metodo per lo studio dei polifenoli dell'uva. *Rivista di Viticultura ed Enologia, 2*, 37–45.

Di Stefano, R., Cravero, M. C., & Gentilini, N. (1989). Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico, 5*, 83–89.

Du Toit, W. J., Lisjak, K., Stander, M., & Prevoo, D. (2007). Using LC-MSMS to asses glutathione levels in South African white grape juices and wines made with different levels of oxygen. *Journal of Agricultural and Food Chemistry, 55*, 2765–2769.

Espín, J. C., Soler-Rivas, C., Wichers, H. J., & García-Viguera, C. (2000). Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. *Journal of Agricultural and Food Chemistry, 48*, 1588–1592.

Fracassetti, D., Costa, C., Moulay, L., & Tomás-Barberán, F. A. (2013). Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (*Myrciaria dubia*). *Food Chemistry, 139*, 578–588.

Fracassetti, D., & Tirelli, A. (2015). Monitoring of glutathione concentration during winemaking by a reliable high-performance liquid chromatography analytical method. *Australian Journal of Grape and Wine Research*. http://dx.doi.org/10.1111/ajgw.12139.

Gabrielli, M., Fracassetti, D., & Tirelli, A. (2014). UHPLC quantification of sotolon in white wine. *Journal of Agricultural and Food Chemistry, 62*, 4878–4883.

González-Rompinelli, E. M., Rodríguez-Bencomo, J. J., García-Ruiz, A., Sánchez-Patán, F., Martín-Álvarez, P. J., Bartolomé, B., et al. (2013). A winery-scale trial of the use of antimicrobial plant phenolic extracts as preservatives during wine ageing in barrels. *Food Control, 33*, 440–447.

Guichard, E., Pham, T. T., & Etievant, P. (1993). Quantitative determination of sotolon in wines by high-performance liquid chromatography. *Chromatographia, 37*, 539–542.

Hofmann, T., & Schieberle, P. (1996). Studies on intermediates generating the flavour compounds 2-methyl-3-furanthiol, 2-acetyl-2-thiazoline and sotolon by Maillard-type reactions. In *Flavour Science: Recent developments* (pp. 182–187). London: The Royal Society of Chemistry.

Kobayashi, A. (1989). Sotolon, identification, formation and effect on flavor. In *Flavor chemistry, trends and development. ACS Symposium Series 388*. Washington, DC: American Chemical Society.

König, T., Gutsche, B., Hartl, M., Hübscher, R., Schreier, P., & Schwab, W. (1999). 3-Hydroxy-4,5-dimethyl-2[5H]-furanone (sotolon) causing an off-flavor: elucidation of its formation pathways during storage of citrus soft drinks. *Journal of Agricultural and Food Chemistry, 47*, 3288–3291.

Lavigne, V., & Dubourdieu, D. (2004). Affinamento sulle fecce e freschezza dei vini bianchi. *Vigne Vini, 31*, 58–66.

Lavigne, V., Pons, A., Darriet, P., & Dubourdieu, D. (2008). Changes in the sotolon content of dry white wines during barrel and bottle aging. *Journal of Agricultural and Food Chemistry, 56*, 2688–2693.

Lester, M. R. (1995). Sulfite sensitivity: significance in human health. *Journal of the American College of Nutrition, 14*, 229–232.

Llorach, R., Tomás-Barberán, F. A., & Ferreres, F. (2004). Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. *Journal of Agricultural and Food Chemistry, 52*, 5109–5116.

Marks, A. C., & Morris, J. R. (1993). Ascorbic acid effects on the post-disgorgement oxidative stability of sparkling wine. *American Journal of Enology and Viticulture, 44*, 187–190.

Martínez-Lapuente, A., Guadalupe, Z., Ayestarán, B., & Pérez-Magariño, S. (2015). Role of major wine constituents in the foam properties of white and rosé sparkling wines. *Food Chemistry, 174*, 330–338.

Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D. A., Bartual, J., Saura, D., et al. (2011). Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *Journal of the Science of Food and Agriculture, 91*, 1893–1906.

Pons, A., Lavigne, V., Landais, Y., Darriet, P., & Dubourdieu, D. (2010). Identification of a sotolon pathway in dry white wines. *Journal of Agricultural and Food Chemistry, 58*, 7273–7279.

Pozo-Bayón, M. A., Monagas, M., Bartolomé, B., & Moreno-Arribas, M. V. (2012). Wine features related to safety and consumer health: an integrated perspective. *Critical Reviews in Food Science and Nutrition, 52*, 31–57.

Riberau-Gayon, P. (1968). *Les composes phénoliques des végétaux*. Paris: Dunod.

Riberau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). *Handbook of enology, the chemistry of wine stabilization and treatments* (2nd ed., Vol. 2). Chichester: John Wiley & Sons Ltd.

Robichaud, J. L., & Noble, A. C. (1990). Astringency and bitterness of selected phenolics of wine. *Journal of the Science of Food and Agriculture, 53*, 343–353.

Roussis, I. G., Lambropoulos, I., & Tzimas, P. (2007). Protection of volatiles in a wine with low sulfur dioxide by caffeic acid or glutathione. *American Journal of Enology and Viticulture, 58*, 274–278.

Salgues, M., Cheynier, V., Gunata, Z., & Wylde, R. (1986). Oxidation of grape juice 2-s-glutathionyl caffeoyl tartaric acid by *Botrytis cinerea* laccase and characterization of a new substance: 2,5-di-s-glutathionyl caffeoyl tartaric acid. *Journal of Food Science, 51*, 1191–1194.

Scalbert, A., Monties, B., & Janin, G. (1989). Tannins in wood: comparison of different estimation methods. *Journal of Agricultural and Food Chemistry, 37*, 1324–1329.

Silva Ferreira, A. C., Barbe, J. C., & Bertrand, A. (2003). 3-Hydroxy-4,5-dimethyl-2(5H)-furanone: a key odorant of the typical aroma of oxidative aged port wine. *Journal of Agricultural and Food Chemistry, 51*, 4356–4363.

Sonni, F., Chinnici, F., Natali, N., & Riponi, C. (2011). Pre-fermentative replacement of sulphur dioxide by lysozyme and oenological tannins: effect on the formation and evolution of volatile compounds during the bottle storage of white wines. *Food Chemistry, 129*, 1193–1200.

Tirelli, A., Fracassetti, D., & De Noni, I. (2010). Determination of reduced cysteine in oenological cell wall fractions of *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry, 58*, 4565–4570.

Vally, H., & Thompson, P. J. (2001). Role of sulfite additives in wine induced asthma: single dose and cumulative dose studies. *Thorax, 56*, 763–769.

Zapata, S., & Dufour, J. F. (1992). Ascorbic, dehydroascorbic and isoascorbic acid simultaneous determinations by reverse phase ion interaction HPLC. *Journal of Food Science, 57*, 506–511.