REDUCTION OF THE SULPHITE LEVEL IN WINE USING A NEW POLYMER-SUPPORTED CARBONYL COMPOUNDS SCAVENGER

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Sulfur dioxide is now considered as a toxic chemical by most of the world health authorities. However, it remains an irreplaceable additive in enology for wine conservation, combining antioxidant and antibacterial properties. Wines often present high SO_2 levels due to their high sulfur dioxide binding power. The objective of this work was to develop a new method for reducing this binding power by partially eliminating the carbonyl compounds naturally present in these wines that are responsible for this phenomenon. A selective liquid-solid removal technique was developed. Acetaldehyde, pyruvic acid, and 2-oxoglutaric acid are some of the main carbonyl compounds responsible for the SO_2 binding power of wines. A hydrazine scavenging function was grafted on a porous polymer support and its efficiency on the selective removal of these carbonyl compounds was evaluated in wines. The results obtained showed that the method was efficient for removing carbonyl compounds and for significantly reducing the binding power of the wines. Hydrazine traces analysis in treated wines revealed a very low level of contamination. Sensory analysis revealed that this process did not deteriorate their organoleptic qualities.
Keywords: carbonyl compound, sulfur dioxide in wines, selective liquid-solid extraction, sensory analysis

Reduktion der Sulfitgehalte in Weinen mittels eines neuen polymergestützten Scavengers für Carbonylverbindungen. Schwefeldioxid wird derzeit von den meisten Gesundheitsbehörden der Welt als eine giftige Chemikalie betrachtet. Es bleibt jedoch ein unersetzliches Additiv in der Önologie für die Weinkonservierung, da es antioxidative mit antibakteriellen Eigenschaften kombiniert. Weine weisen aufgrund ihrer hohen Schwefeldioxid-Bindungskraft oft hohe SO_2-Werte auf. Das Ziel dieser Arbeit war es, ein neues Verfahren zur Verringerung dieser Bindungskraft zu entwickeln durch die teilweise Eliminierung der in diesen Weinen natürlich vorkommenden Carbonylverbindungen, die für dieses Phänomen verantwortlich sind. Dafür wurde eine selektive Flüssig-Fest-Extraktionstechnik entwickelt. Acetaldehyd, Brenztraubensäure und 2-Oxoglutarsäure sind einige der wichtigsten Carbonylverbindungen, die für die SO_2-Bindungskraft der Weine verantwortlich sind. Ein Hydrazin-Scavenger-Molekül wurde auf einen porösen polymeren Träger gepfropft und seine Effizienz bezüglich der selektiven Entfernung dieser Carbonylverbindungen in Weinen evaluiert. Die Ergebnisse zeigten, dass das Verfahren zur Entfernung von Carbonylverbindungen effizient war und die Bindungskraft des Weines erheblich reduziert wurde. Hydrazinspurenanalysen der behandelten Weine ergaben eine nur sehr geringe Hydrazin-Kontamination. Die sensorische Analyse zeigte, dass dieser Prozess die organoleptischen Eigenschaften der Weine nicht verschlechterte.
Schlagwörter: Carbonylverbindungen, Schwefeldioxid in Wein, selektive Flüssig-Fest-Extraktion, sensorische Analyse

Sulphur dioxide (SO_2) remains an indispensable winemaking additive, combining antioxidant and antibacterial properties (Bennet and Hammond, 1992). When sulphur dioxide is added to wine, a balance is established between the various forms of this compound. At pH-values of wines (3 to 5), most of the free SO_2 is present in bisulphite (HSO_3^-) form. The SO_2 that reacts with carbonyl compounds naturally present in the wine to produce carbonyl bisulphite is known as "bound" SO_2 (Divol and Duckitt, 2012). As a rule, several mmol/l of total SO_2 are required to obtain 0.20 to 0.50 mmol/l of active (free) SO_2, the concentration necessary to avoid any further yeast fermentations and bacterial alterations. SO_2, under the $H_2SO_3^-$ form (active SO_2), rapidly enters cells (about 2 minutes), disrupts their development, growth, multiplication and finally causes cell death. (Jackowetz and Mira de Orduna, 2013). The toxicity of sulphur dioxide and carbonyl bisulphites has been studied since the early 20th century (Ingram, 1978; Vally and Thompson, 2001), leading authorities to regulate the quantity of sulphur dioxide permitted in wine for health reasons (EC Regulations). Reducing the total quantity of sulphur dioxide in wine is a challenge that has not yet been reached.

We postulated that this goal may be achieved by removing part of the carbonyl compounds naturally present in wine and combined with SO_2 by using a selective scavenging molecule grafted onto an insoluble support. It was expected that the amount of bound SO_2 will be therefore reduced by equilibrium displacement, allowing to obtain a higher level of free SO_2 for a given amount of total SO_2 initially injected. By this treatment, a lower amount of total SO_2 will be required in order to reach the same level of free, active sulphur dioxide.

Acetaldehyde, pyruvic acid and 2-oxoglutaric acid contribute to more than 90 % of bound SO_2 in white and red wines (Jackowetz and Mira de Orduna, 2013).

In previous papers, we have established that extraction of the major part of these compounds with polymer-bound sulfonylhydrazide was an efficient method to reduce the total amount of SO_2 needed in wine for its conservation (Bakai et al., 2014; Saidane et al., 2013a; Saidane et al., 2013b; Saidane et al., 2010; Blasi et al., 2008; Blasi et al., 2007). This process has been patented (US Patent, 2010). Unfortunately, hydrazine traces analysis in treated wines indicated contamination levels incompatible with the development of this method.

In the present work, we developed a new insoluble polymer support where the extracting function is covalently attached to the support by a C-$NHNH_2$ bond expected to be more chemically stable at the pH of wine than the S(O_2)-$NHNH_2$ bond previously used.

MATERIALS AND METHODS

CHEMICALS

Glycidyl methacrylate (GMA), ethylene glycol dimethacrylate (EGDMA), divinylbenzene (DVB) (80 %), cyclohexanol, acacia gum, azobisisobutyronitrile (AIBN), hydrazine monohydrate (50 to 60 % aqueous solution), acetaldehyde, pyruvic acid, 2-oxoglutaric acid, L-(+)-tartaric acid and analytical kits for pyruvic acid determination were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Boehringer-Mannheim kits for acetaldehyde determination, glutamate dehydrogenase and NADH were purchased from R-Biopharm (Saint Didier au Mont d'Or, France). Solvents were used without further purification. The wines studied were two differently bottled (i.e. sulphited) samples. Baron de Lestac (white wine, Bordeaux area, France, vintage 2013) and Château Cavalier (rosé wine, Côtes de Provence, France, vintage 2013). The initial total and free SO_2 concentrations were 2.26 mmol/l and 0.55 mmol/l in the white wine, and 1.36 mmol/l and 0.25 mmol/l in the rosé wine.

PREPARATION AND CHARACTERIZATION OF THE SUPPORTS

The suspension polymerization was performed in a 1 l-parallel-sided glass reactor designed according to the recommendations of literature (Hodge and Sherrington, 1980). In a typical experiment, the organic phase was prepared by mixing GMA (22 g, 154 mmol), EGDMA (15 g, 76 mmol), DVB (6 g, 46 mmol), AIBN (1 g, 5.9 mmol) as the initiator, and cyclohexanol (86g, 90 ml) as the porogen. The mixture was suspended in wa-

ter (400 ml) containing a suspension stabilizer (acacia gum, 20 g) and sodium chloride (4 g) and stirred at 300 rpm. The polymerization was performed at 80 °C for 3 h under constant stirring. After cooling to room temperature, the beads were filtered, thoroughly washed with ethanol in a Soxhlet apparatus for 48 h, and finally dried under vacuum at 50 °C until constant weight to give 38.7 g of white beads with a diameter between 400 and 800 µm. (Y = 90 %). Polymer beads (30 g) were refluxed in a 250 ml flask equipped with a condenser and a mechanical stirrer with a solution of hydrazine hydrate (70 ml, 1.75 mol) for 3 h. After cooling to room temperature, the beads were filtered, thoroughly washed with a water/ethanol mixture (50:50) in a Soxhlet apparatus for 48 h, and finally dried under vacuum at 50 °C until constant weight to give 29 g of yellowish beads. (Y = 97 %).

Support epoxide and hydrazide functions groups loading was estimated using methods reported in literature (Kling and Ploehm, 1995; Smith and Wilcox, 1942).

The effective porosity and the pore size distribution of beads were determined by mercury intrusion porosimetry using a Micromeritics Autopore IV 9500 porosimeter with the following parameters: contact angle = 130°, mercury surface tension = 485 mN/m, maximum intrusion pressure = 124 MPa.

The specific surface area was determined by N2 adsorption measurements performed on a Micromeritics ASAP 2010. The collected data were subjected to the BET (Brunauer, Emmet and Teller)-theory (Brunauer et al., 1938).

Optical microscopy images of dry beads were obtained with a 5 Mpixels digital camera to acquire greyscale photographs in TIFF format. Care was taken to obtain high-quality digital images with high contrast and resolution. Each image was taken from randomly chosen independent samples of each polymer beads batch to give a reliable representation of the size distribution (at least 100 beads were present in the image). The greyscale picture files were then processed with ImageJ software (National Institutes of Health, USA) into binary files. The software identifies objects within the image by the difference in pixel intensity and calculates their perimeter. The results were then filtered to exclude any particle with a perimeter <100 µm and aggregates and the data were exported to a spreadsheet for statistical analyses.

CARBONYL COMPOUND REMOVAL FROM WINES

Extraction experiments using different amounts of support (0.25 g; 0.50 g) were performed on wine samples (100 ml) at room temperature without any agitation with a contact time of 24 h. Acetaldehyde and pyruvic acid concentrations remaining in solution were determined, using commercial enzymatic kits. The enzymatic method described by Blouin (Peynau et al., 1966) was used for the determination of 2-oxoglutaric acid concentration. Absorbances for enzymatic determination were carried out at λ = 340 nm on a Spectronic 20 Genesys spectrophotometer (Thermo Fisher Scientific Instruments, Massachusetts, USA).

Free and total sulphur dioxide levels were measured using the international official method (Frantz-Paul method) (O.I.V., 2008). Estimation of hydrazine contamination was performed on wines after treatment by a spectrophotometric method using copper (II) neocuproine (Besada, 1988).

The sensory evaluation is very important for the evaluation of the viability of our process. Sensory triangular tests were performed to evaluate the organoleptic impact of the process and look for exogenous notes (Meilgaard et al., 1999). The sensory panel consisted of eighteen persons who attended a weekly training session. The test was performed in individual booths at controlled room temperature of 20 °C, using covered AFNOR (French Standard Association) glasses, containing 40 ml of wine. Just after heterogeneous extraction, free SO_2 concentrations were adjusted by sodium metabisulfite addition to obtain a value of 40 mg/l in all samples. Two triangular tests were performed: In the first round, one glass contained the treated wine and the two others the original wine and the presentation was reversed in the second round.

RESULTS AND DISCUSSION

SYNTHESIS OF THE SCAVENGING SUPPORT

The support synthesised was a glycidyl methacrylate-co-ethylene glycol dimethacrylate-co-divinylbenzene copolymer (56:27:17 %mol.) prepared in bead shape by suspension polymerisation (Hodge and Sherring-

TON, 1980). A good site isolation of the grafted moieties was insured by the preparation of highly crosslinked macroporous beads in order to avoid hydrazine moieties condensation. A precipitant porogen (cyclohexanol) was used to obtain a specific surface area value in the medium range. The general route followed for the synthesis of the polymer support and its functionalization is represented in Figure 1.

Epoxide group loading was estimated as 3.60 ± 0.15 mmol/g by a reported method (KLING and PLOEHN, 1995). The hydrazine function was then grafted onto the beads by reaction with an excess of hydrazine hydrate (BICAK et al., 2002). The hydrazine content of the resulting support was estimated as 1.70 ± 0.10 mmol/g (Y = 47 %) using a published procedure (SMITH and WILCOX, 1942). The particle size distribution was estimated to 600 ± 200 µm. Specific surface area (BET) was 102 ± 1 m2/g, total porosity was estimated to 39 ± 2 % with an average pore size of 30 ± 5 nm.

REMOVING CARBONYL COMPOUNDS FROM WINES

Polymer-supported hydrazine was then tested in the extraction of acetaldehyde, pyruvic acid and 2-oxoglutaric acid from two wines. The different extraction experiments performed are E_{L1} and E_{L2} (Baron de Lestac); E_{C1}, and E_{C2} (Château Cavalier) wines with molar concentrations of supported scavenging functions of 4.25 mmol/l and 8.50 mmol/l, respectively. The efficiency of carbonyl compounds removal from wine and the evolution of sulphur dioxide concentrations are reported in Table 1.

The data reported clearly shows that carbonyl compounds are effectively removed from wine using the polymeric scavenger newly developed. In the case of Baron de Lestac wine (white wine), extraction efficiency increased significantly with the amount of extracting agent used and can reach up to 73 % of the total amount of carbonyl compounds present. Extraction efficiency varied according to the type of carbonyl compound involved. Acetaldehyde and pyruvic acid present the best efficiency of removal (up to 91 % and 69 %, resp.). Extraction of 2-oxoglutaric acid appeared to be less efficient: only 44 % of extraction in the best case. The extraction behaviour is rather similar in the case of Château Cavalier wine (rosé wine) with somewhat lower values.

The total and free SO_2 concentrations were measured before and after processing (O.I.V., 2008), bound SO_2 being estimated by difference. The results showed that carbonyl compound removal is accompanied by a decrease in the bound SO_2 concentration, the level of free SO_2 remaining almost constant. This suggests that, when free carbonyl compounds react with grafted hydrazine and are extracted from the wine, some of the corresponding carbonyl bisulphites dissociate to form free carbonyl compounds and HSO_3^-, in agreement with the mass action law (Fig. 2). Total SO_2 concentrations decrease simultaneously as the amount of scavenging agent involved increases. Some of the SO_2 reduction may be due to wine oxidation, but the largest part must be attributed to another mechanism. The formation of adducts between sulphur dioxide and hydrazine has been reported (BUDKULEY, 1992). We can suppose that a fraction of the grafted $-N_2H_3$ moieties, protonated in the acidic medium, react with the HSO_3^- ion, and form a grafted $-N_2H_4HSO_3$ neutral adduct (Fig. 2).

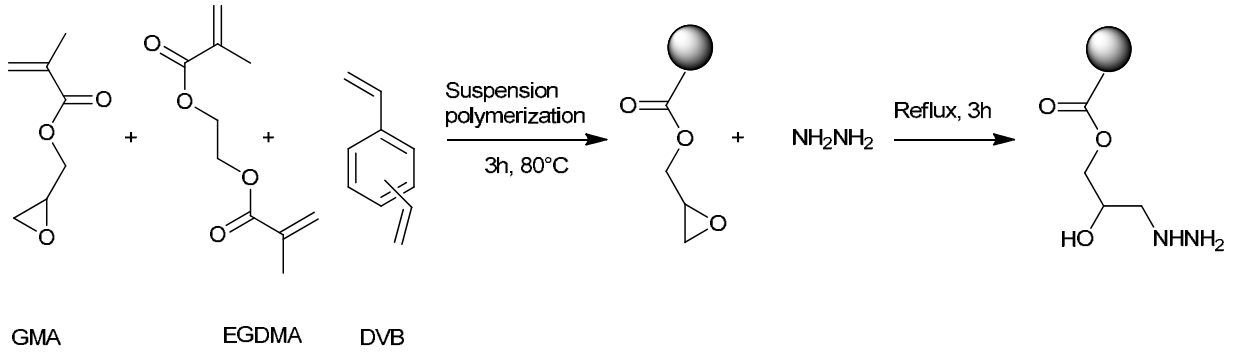

Fig. 1: Preparation of polymer-supported hydrazine beads

Tab.1 Carbonyl compounds extraction and SO_2 concentration evolution in wines

	Experiments	Baron de Lestac			Château Cavalier		
		E_{L0} (blank)	E_{L1}	E_{L2}	E_{C0} (blank)	E_{C1}	E_{C2}
Extraction function used (mmol/l)		-	4.25	8.50	-	4.25	8.50
Acetaldehyde	Concentration in solution (mmol/l)	1.53	0.57	0.14	0.89	0.29	0.10
	Extraction efficiency (%mol.)	-	62.7	90.8	-	67.4	88.7
Pyruvic acid	Concentration in solution (mmol/l)	0.29	0.13	0.09	0.21	0.09	0.07
	Extraction efficiency (%mol.)	-	55.1	68.9	-	57.1	66.6
2-oxoglutaric acid	Concentration in solution (mmol/l)	0.59	0.37	0.33	0.43	0.33	0.31
	Extraction efficiency (%mol.)	-	37.2	44.0	-	23.2	27.9
Total	Concentration in solution (mmol/l)	2.11	1.07	0.56	1.53	0.71	0.48
	Extraction efficiency (%mol.)	-	49.2	73.4	-	53.5	68.6
Total SO_2 (mmol/l)		2.26	1.91	1.69	1.36	1.22	0.99
Free SO_2 (mmol/l)		0.55	0.49	0.40	0.25	0.22	0.19
Bound SO2 (mmol/l)		1.71	1.42	1.29	1.11	1.00	0.80

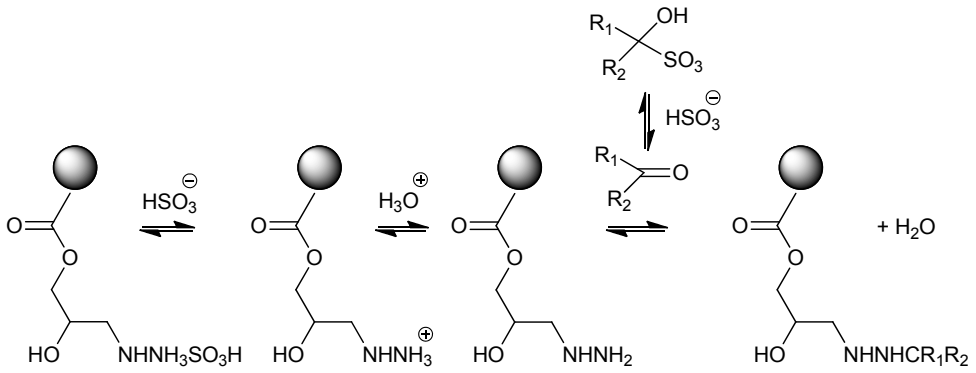

Fig. 2: Postulated equilibrium occurring in wine in the presence of sulphur dioxide and scavenger

This hypothesis was confirmed by observing a decrease of total SO_2 when adding increasing quantities of support in an acidified hydroalcoholic solution containing only sulphur dioxide in the absence of carbonyl compounds. Finally, free SO_2 which represented 24.3 % and 18.3 % of the total SO_2 in bottled wines before proceeding presents similar values of 25.6 % and 18.0 % after using a slight excess of extracting agent (experiments E_{L1} and E_{C1}, resp.), whereas the total SO_2 has decreased simultaneously of 15.4 % and 10.2 %, respectively. When using a high excess of extracting agents (experiments E_{L2} and E_{C2}, resp.), the corresponding figures become 23.3 % and 19.1 %; 25.2 % and 27.2 %.

Finally, estimation of hydrazine contamination was performed on wines after treatment using a spectrophotometric method (Besada, 1988). Results found on treated wines (in triplicate) indicated an average value under the detection level of the method (<2 ppm).

Sensory analysis of the different wines treated showed that the treatment with the support did not give any exogenous odor to the wine and did not modify its aromatic qualities. Triangular tests to evaluate the organoleptic impact of extraction with the support showed that it was difficult for the panel to recognize the treated wine. In the worst case, in the two-sample presentation, only six persons identified the different glass whereas 12 failed. According to the null hypothesis, the number of correct assessments was binomial with parameters n = 18 and p = ⅓. There was no significant difference at the threshold of 0.1 % between treated and sample wines (Martin et al., 1999).

CONCLUSIONS

A new polymer-supported scavenger based on glycidyl methacrylate-*co*-ethylene glycol dimethacrylate-*co*-divinylbenzene copolymer post-modified by hydrazine was developed to extract selectively carbonyl compounds from wine. The extraction efficiency of the support was tested on bottled wines. Acetaldehyde, pyruvic acid and 2-oxoglutaric acid were efficiently extracted, resulting in a decrease of bound sulphur dioxide without significant variation of the free SO_2 level. Therefore, the total SO_2 content of the wine after treatment can be reduced from 10 % to almost 30 % of its initial value without significantly reducing the free SO_2 level. No contamination of treated wines by hydrazine could be detected. Furthermore, sensory analysis revealed that this process did not deteriorate their organoleptic qualities. This process thus appears promising in view to reduce the total amount of sulphur dioxide (sulphites) necessary in wine to insure its proper conservation.

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