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Effect of UV-C treatment on the microbial population of white and red wines, as revealed by conventional plating and PMA-qPCR methods

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ABSTRACT

Ultraviolet C (UV-C) irradiation is one of the emerging techniques for the inactivation of microorganisms in liquid food products, and it holds considerable promise also for treatment of wine. This application can be of particular interest to reduce or even eliminate the use of sulphur dioxide as a preservative in winemaking, given its potential health risks.

In this study, UV-C light treatment was applied to ten different white and red wines during winemaking, for the first time at industrial scale, using a commercial turbulent flow system. The effect of 1.0 kJ/l dosage treatment on the viability of the natural microbial population, i.e., total yeasts, lactic acid bacteria and acetic acid bacteria, was investigated both with conventional plating and optimized specific propidium monoazide (PMA)-qPCR. Results of the two methods were mainly concordant for control and UV-C treated samples, and, in some cases, PMA-qPCR was able to detect small amounts of viable cells, probably in the VBNC state. Remarkably, PMA-qPCR allowed to obtain reliable results much faster than conventional plating methods.

Data indicated that the UV-C irradiation was effective in reducing microbial counts for up to five log CFU/ml, depending on the wine (white or red) and on the microbial load of the sample. The treatment was much more effective in white wines: a statistically significant decrease was observed for putative spoilage-related bacteria, besides the most pronounced effect on yeast cells.

Outcomes strongly support the applicability of UV-C treatment for white wine production, thanks to its efficacy towards all the microbial groups considered.

1. Introduction

The addition of sulphur dioxide (SO_2) during different steps of the production process is a well-established practice in winemaking, due to its multiple and diverse properties (Ribèreau-Gayon, Dubourdie, Donèche, & Lonvaud, 2006). For example, SO_2 acts as microbiological control agent in musts and wines and as enzyme inhibitor to prevent must browning and wine oxidation. Moreover, it can be considered a clarification agent and it behaves as a solvent. However, allergies caused by SO_2-derived compounds, i.e. the sulfites, are becoming more frequent, causing several symptoms, such as headaches, gastric irritation, nausea, and difficulties for breathing in asthma patients (Santos, Nunes, Saraiva, & Coimbra, 2012). In this view, the International Organization of Vine and Wine (OIV) has been progressively reducing the maximum concentration authorized in wines, which is nowadays 150 mg/l for red wines and 200 mg/l for white wines (Regulation (EC) No 606/2009). In the same way, since November 25th 2005, is mandatory to declare the presence of sulfites in wines when the concentration of SO_2 exceeds 10 mg/l (Directive 2003/89/EC).

Besides the legislative rules, consumers are becoming much more health-conscious and, as a result, prefer healthy products free of chemical additives. Thus, researchers and the wine industry are looking for innovative methods that can reduce or even eliminate the use of SO_2 as preservative without significantly changing the quality attributes of wine. To this aim, the application of ultraviolet C light (UV-C, 200–280 nm) is one of the emerging techniques that was successfully used to inactivate microorganisms in water and, more recently, in various types of liquid foods and beverages, such as fruit juices, soft drinks, beer and wine (Franz, Specht, Cho, Graef, & Stahl, 2009; Fredericks, Du Toit, & Krügel, 2011; Gibbs, 2000; Koutchma, 2009; Lu et al., 2010). The germicidal effect of UV-C is due to the rearrangement of the nucleic acid bonds, which block DNA transcription and replication, and eventually cause cell death

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(Guerrero-Beltrán & Barbosa-Cánovas, 2004). Fredericks et al. (2011) have shown that, in artificially inoculated grape juices and wine, UV-C irradiation was capable of destroying a broad spectrum of common wine-associated microorganisms, such as *Acetobacter aceti*, *Brettanomyces bruxellensis*, *Pediococcus acidilactici*, *Oenococcus oeni*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae*. UV-C efficacy was largely affected by dosage, turbidity and colour of the liquid product, and initial microbial load.

To determine the effectiveness of a treatment to reduce the bacterial load, a fast and reliable detection and quantification technique is needed, also considering that wine-associated microorganisms can often be underestimated using the conventional cultivation-based methods, due to variable growing rates of the different microorganisms in culture media or to the presence of viable but non-cultivable (VBNC) microorganisms (Andorrà, Esteve-Zarzoso, Guillamón, & Mas, 2010). Cell viability is a key concept, and it has been discussed in the literature, as it could be defined in different ways, but it is mainly based on the presence of some metabolic activities or of an intact membrane, the latter being the most conservative criterion (Nocker & Camper, 2009). Intercalating dyes, such as propidium monoazide (PMA) or ethidium monoazide (EMA), are able to penetrate in membrane-compromised cells or dead cells and can covalently bind to nucleic acid after photoactivation. Since DNA that is covalently bound to these dyes cannot be PCR amplified, only DNA from viable cells, including those in the VBNC state, can be detected and the application of quantitative PCR (qPCR) could specifically reveal viable cells. This approach has been effectively evaluated to detect and quantify different microorganisms associated with various types of foods (as reviewed by Elizaquìvel, Aznar and Sánchez, 2014) and in wine (Andorrà et al., 2010; Shi et al., 2012; Vendrame, Iacumin, Manzano, & Comi, 2013). Also, recently, Fittipaldi, Nocker, and Codony (2012) concluded that PMA and EMA behave nearly identically as intercalating stains, but they differ in the permeation ability through cell membranes, with PMA more effective than EMA in terms of live–dead discrimination.

Although UV-C treatment does not directly target cell membrane, membrane damage is the ultimate result of severe damage of UV-treated cell. Therefore, the aim of this study was to evaluate the effect of UV-C irradiation on the viability of the microbial population associated with different white and red wines at industrial scale. The levels of yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) were analyzed in wine samples before and after the UV-C treatment by conventional counting in selective media and PMA-qPCR, as a rapid molecular tool for investigating the UV-C efficiency in the inactivation of wine microorganism cells.

2. Materials and methods

2.1. White and red wines

A total of ten wines produced in wineries located at different Italian regions were considered in this study. Five of them were white wines: Pinot grigio (Trentino region, sample W1), Chardonnay (Trentino, W2), Trebbiano (Romagna, W3) and two Garganega (Veneto, W4 and W5) wines. They were taken within few weeks after the end of alcoholic fermentation from stainless steel tanks kept at 10–12 °C. The other five are red wines: Barbera (Lombardia region, sample R1), Sangiovese (Toscana, R2), Aglianico (Puglia, R3) and two Red wines (Romagna, R4 and Veneto, R5). They were taken immediately after malolactic fermentation (MLF), except for R5 that, after MLF, was maintained for four months in a stainless steel tank at 14 °C. All these wines were selected because low level of sulfite was used during the vinification process (<10 mg/l free molecular SO_2, as determined by the official method OIV-MA-AS323-04B).

2.2. Application of UV-C to wine

The ten selected wines were treated at the different wineries using the commercial UV-C reactor system designed and manufactured by SurePure (Milnerton, South Africa). The reactor is constituted by 40 pipes containing UV lamps that emit light at the specific wavelength of 254 nm, comprised in the range C of the UV rays (UV-C). The tubes are designed to generate a turbulent flow which increase the intimate contact of the liquid with the radiation. The liquid is circulated within the system at a flow rate of 4000 l/h, and to completely fill the inner space 1000 l are required. As a result, the first treated sample comes out from the installation after 15 min. The applied dosage at one pass of the product through the system was of about 1.0 kJ/l (Keyser, Müller, Cilliers, Nel, & Gouws, 2008). The unit was cleaned after every treatment using standard 'Cleaning In Place' (CIP) processes. Wine samples (50 ml) were collected aseptically with an in line sampler from the flow stream without stopping the treatment process, and stored at 4 °C until the microbiological analysis were performed.

2.3. Counting of microorganisms on selective media

To improve cell recovery, the wine samples were centrifuged at 8000 × g for 10 min, re-suspended in recovery medium (2% glucose, 2% peptone and 1% yeast extract, w/v) and stored for 2 h at 13 °C. The microbial counts were determined by plating serial 10-fold dilutions of the samples on the corresponding solid medium: Wallerstein Laboratory (WL) nutrient agar (Fluka, Milan, Italy) supplemented with 100 ppm chloramphenicol for yeasts (at 27 °C for 5 days), WL (Fluka) Differential (WLD) nutrient agar with 100 ppm cycloheximide for AAB (at 27 °C for 5 days), and MRS agar (Fluka) with 100 ppm cycloheximide, 0.5% fructose, 0.1% malic acid and 0.01% Tween 80 for LAB (at 27 °C in anaerobiosis for 7 days). To detect lower amounts of cells, the wines were concentrated 10-fold by centrifugation. Colony counts were performed in duplicate.

2.4. Counting of microorganisms by PMA-qPCR

2.4.1. Microbial strains and culture conditions

The target strains used to set up PMA-qPCR assays were: *S. cerevisiae* CBS 1171^T, *B. bruxellensis* MUCL 27700^T, *O. oeni* PSU-1 (Mills, Rawsthorne, Parker, Tamir, & Makarova, 2005), *L. plantarum* ATCC 14917^T, and *Acetobacter pasteurianus* LMG 1272^T. Yeasts were cultured in YPD (1% yeast extract, 2% peptone and 2% glucose, w/v) at 27 °C for 1–3 days. LAB were cultured in MRS (Fluka) added with 0.5% fructose, 0.1% malic acid and 0.1% Tween 80 (w/v), pH 4.8, at 27 °C for 2–5 days. AAB were cultured in ACE (5% glucose, 0.5% yeast extract, w/v, pH 6.5) at 30 °C for 4–5 days.

2.4.2. Propidium monoazide (PMA) treatment of the samples

Total yeasts, AAB and LAB were enumerated in the ten analyzed wines by the PMA-qPCR method. Before PMA treatment, cells were incubated in recovery medium as described above. This step was crucial to obtain more reproducible results, as it allows partially damaged cells to recover and repair membranes without increasing cell number, in accordance with the protocols described by Andorrà et al. (2010) and Shi et al. (2012).

PMA (Sic – Società Italiana Chimici, Roma, Italy) was re-suspended in 20% dimethyl sulfoxide (DMSO) to obtain a stock concentration of 1 mM, and stored at −20 °C in the dark. PMA solution was added to 1 ml of sample to yield a final concentration of 100 μM (Fittipaldi et al., 2012) and an incubation of the samples for 5 min in the dark at room temperature was performed. This