

Inactivation of microorganisms by UV-treatment of must and wine

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Abstract. The objective was to investigate the applicability of UV-C technology to inactivate yeasts and bacteria in must and wine. Experiments were carried out in vintage 2016 with Riesling musts of different quality containing their natural microflora. Yeasts were tested more resistant to UV-C energy than bacteria. *Saccharomyces cerevisiae* showed higher tolerance against UV-C irradiation than *Hanseniaspora uvarum* facilitating new opportunities to control spontaneous fermentations. However, inactivation efficacy was strongly dependent on turbidity of musts and the initial degree of contamination suggesting a shadowing effect of individual germs. Compared with thermal pasteurization, UV-C treatment of must with 1 kJ/L showed similar effects in germ-reduction. While thermal pasteurization significantly decreased aroma precursors in musts, UV-C treatment did not change concentrations of glycosidically-bound C6-alcohols, monoterpenes and C13-norisoprenoids as shown by GC-MS analysis. Applying UV-C technology in wines, it was possible to irreversibly stop ongoing alcoholic fermentation indicating that UV-C treatment is capable to replace SO₂ addition to produce wines with residual sugar. Besides inactivation power, UV-C is known for its ability to form powerful off-flavours such as methional or methanethiol. Sensory analysis revealed that the application of UV-C at doses < 2 kJ/L in must is uncritical. However, applying UV-C after alcoholic fermentation can result in rising concentrations of mercaptans already at doses < 1 kJ/L. In this context, compounds such as caftaric acid, riboflavin and dissolved oxygen are thought to positively contribute to the UV-induced formation of off-flavours in wine.

1. Introduction

Warm temperatures due to climate change decrease acidity in grapes and allow new allochthonous parasites to establish. Higher pH values limit the microbicide effect of SO₂ and enable good living conditions for harmful organisms, which may empower the formation of SO₂ binding metabolites such as pyruvate and acetaldehyde, further reducing the microbicide effect of SO₂. Various physical sterilization processes like UV-C treatment are considered as techniques for the inactivation of microorganisms. While UV-C treatment is a well-established method in other industries such as water purification, only a few publications focused on the effect of UV-C on microorganism in must and wine [1,2]. UV-C induces photochemical reactions such as strand breakage of the DNA. As a result, damaged cells are not able to reproduce. For the inactivation of microorganisms, wavelengths between 250 and 260 nm are particularly suitable, since the DNA absorbs very well in this range. The effect of UV-C in liquids generally depends on the penetration depth of the light waves [3], which are either trapped by the absorption of ingredients or scattered by colloids in the liquid [4]. However, this problem can be circumvented by choosing an appropriate technique such

as shown in several UV-C reactors [5]. Application fields for the UV-C technology in wineries are the treatment of musts with microbial contamination, the treatment of young wines to stop fermentation, and treatment of wines for the purpose of stabilization, e.g. prior to bulk wine shipping. The technique might be considered as an alternative for thermal pasteurization. In any case, treated products must not suffer from UV-C irradiation. However, UV-C is known for its ability to form powerful off-favours such as methional or methanethiol [6]. Accordingly, the objective of this study was to strive for the minimal UVC energy to inactivate microorganisms without generating detrimental light-struck flavour.

2. Materials and methods

Vitis vinifera grapes of the variety Riesling were harvested in 2016 from the vineyards of the DLR Rheinpfalz, Neustadt, Germany. Harvesting by hand was conducted in three consecutive weeks on same sites obtaining grapes of different quality and different microflora. Immediately after harvesting, grapes were crushed and pressed obtaining musts which were further processed by means of sedimentation for clarification reasons (< 50 NTU). In order to investigate UV-C efficiency in turbid musts, grape lees were blended into the clarified

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juice yielding musts with 100, 500 and 900 NTU. UV-C treatment of musts was achieved by a pilot-scale coiled tube reactor designed and assembled at the Max-Rubner-Institut (Karlsruhe, Germany). The main component is a module which consists of a helically wound quartz glass tube with a diameter of 3.0 mm which is assembled around a 30 W low pressure mercury lamp with maximum peak radiation at 254 nm (UVN 30, UV Technik Speziallampen GmbH, Wümbach, Germany). Between lamp and coiled quartz glass, a water cooling system allows for stable must temperatures during treatment. Musts were pumped through the device at a flow rate of 25 L/h by a peristaltic pump (Pump drive Pd 5206, Heidolph, Schwabach, Germany). Flow was adjusted to 25 L/h after preliminary studies taking into account that turbulent flow is necessary to maximize UV-C effect [7]. In this study, various UV C doses were obtained by recirculating must and wine through the device for multiple times. UV C dose in J/L was determined using a potassium iodide/iodate chemical actinometry method previously described [8]. All experiments were conducted in triplicate.

Microbial analysis was carried out in 10⁻⁵-dilutions (in 0.9% NaCl) using YPD medium (with chloramphenicol and kanamycin) for yeasts and LB medium (with cycloheximide) for bacteria. Agar plates were incubated at 30 °C for 24h, then colonies were counted. GC-MS analysis of aroma precursors in Riesling must was carried out after SPE purification and enzymatic hydrolysis using a 30 m × 0.25 mm i.D. fused silica capillary column ZB-Wax (PEG) as described by Schober [9]. Photometric analysis was conducted according to OIV-MA-AS2-11 procedure using a Varian Cary100 Spectrophotometer. In order to investigate the sensory effect of UV-C treatment, musts were processed by means of a standardized winemaking protocol. Microvinification of UV-C treated and control musts was carried out in 15 L batches at 18 °C using a dry active yeast strain of *Saccharomyces cerevisiae*. After alcoholic fermentation wines were racked off the lees and stored for two months at 15 °C for clarification reasons. Stabilization was achieved by using a commercial solution of ammonium hydrogensulphite yielding 30 mg/L free SO₂. Sensory analysis was performed by 21 judges examining the wines by triangle test according to DIN 10954. Statistical calculations were performed using analysis of variance and Tukey-Kramer post-hoc test, significance level was set at p ≤ 0.05.

3. Results and discussion

3.1. UV-C inactivation of natural yeast flora in grape must

Harvesting the Riesling on Sep. 24th, Oct. 6th and Oct. 17th yielded musts with total yeast counts of 6.8 × 10⁴, 3.2 × 10⁵ and 2.0 × 10⁷ cfu/ml, respectively. Right after pressing, four yeasts could be clearly identified in the fresh grape musts, in detail *Saccharomyces cerevisiae*, *Hanseniaspora uvarum*, *Schizosaccharomyces pombe* and *Pichia fermentans*. When applying UV-C treatment, cultivable yeast cells decreased immediately in the musts. Regardless of the type of yeast, the inactivation success was strongly depended on the initial cell count. Table 1 shows the inactivation power of UV-C with 2 kJ/l for

Tab. 1. Inactivation power of UV-C treatment with 2 kJ/L for *Schizosaccharomyces pompe* in clarified Riesling grape must. The yeast was inoculated in definite concentrations at after must pasteurization.

Initial cell count (cfu/ml)	Reduction of cells (log ₁₀ cfu/ml)
10 ⁷	1
10 ⁶	1.5
10 ⁵	2
10 ⁴	2.5

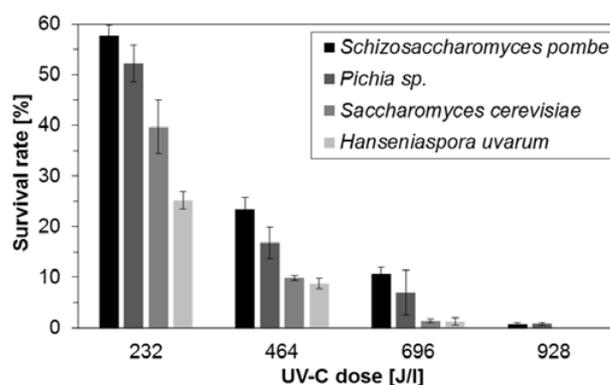


Fig. 1. Survival rates of naturally occurring yeasts in clarified Riesling must after UV-C treatment with different doses. The initial number of total yeast germs counted on YPD medium was 3.2 × 10⁵ cfu/ml.

Schizosaccharomyces pompe in clarified Riesling grape must.

Data in Table 1 clearly reveals that inactivation efficacy of UV-C is dependent on the initial degree of contamination suggesting a shadowing effect of individual germs during treatment. Another factor influencing the inactivation efficacy of UV-C is the type of yeasts present in the grape must. The broken-down view on the four yeast types named earlier allows for detailed assessment of the UV-C sensitivity of different yeasts occurring on grapes. Figure 1 shows the survival rates of naturally occurring yeasts in clarified Riesling must after UV-C treatment with different doses.

Schizosaccharomyces pompe was found to least sensitive to UV-C treatment, followed by *Pichia fermentans*, *Saccharomyces cerevisiae* and *Hanseniaspora uvarum*. While *Saccharomyces* and *Hanseniaspora* were completely killed at doses >1 kJ/l, *Schizosaccharomyces* and *Pichia* still remained in small cell counts. However, at higher doses <1 kJ/l, all occurring yeasts could be inactivated. The technical option to completely or partially inactivate yeasts in grape musts facilitate new opportunities to control spontaneous fermentations.

3.2. UV-C inactivation kinetics of yeast and bacteria in grape must

While facilitating the targeted inactivation of yeasts with UV-C treatment, the question arises as to whether a similar attempt is possible with parallel existence of bacteria and yeast in must and wine. Therefore, UV-C inactivation kinetics have been investigated in clarified Riesling must, which was co-inoculated with *Schizosaccharomyces pompe*, *Brettanomyces bruxellensis* and *Lactobacillus*

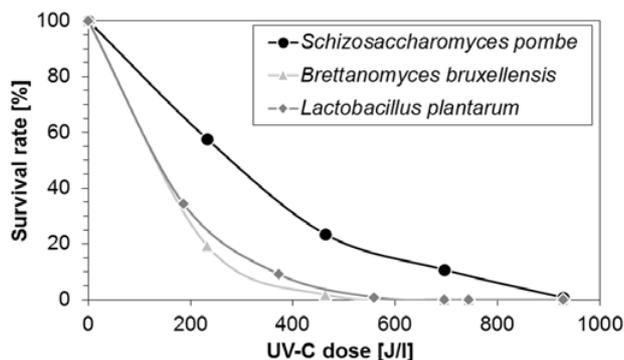


Fig. 2. UV-C inactivation kinetics for *Schizosaccharomyces pompe*, *Brettanomyces bruxellensis* and *Lactobacillus plantarum* in clarified Riesling must. All microorganisms were inoculated at 10^5 cfu/mL after must pasteurization.

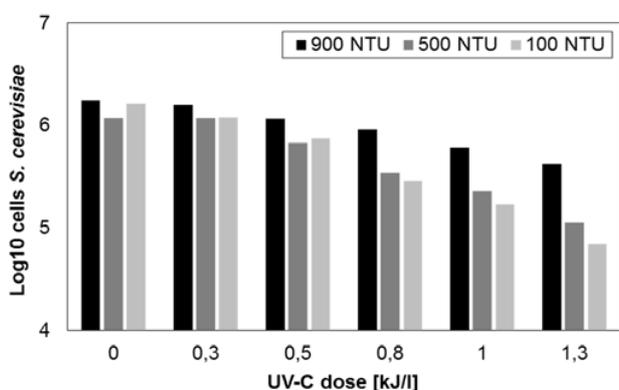


Fig. 3. Influence of turbidity levels on UV-C inactivation of *Saccharomyces cerevisiae* in Riesling must.

plantarum, each at 10^5 cfu/mL, after must pasteurization (Fig. 2).

Brettanomyces bruxellensis and *Lactobacillus plantarum* revealed similar survival rates due to UV-C treatment and were found to be much more sensitive compared to *Schizosaccharomyces pompe*. This finding suggests that targeted inactivation is also possible for mixtures of bacteria and yeasts in must or wine allowing for new possibilities in controlling harmful growth of bacteria, e.g. at the end of alcoholic fermentation. The relatively high sensitivity of *Brettanomyces bruxellensis* towards UV-C might be also considered as an important hint for cellars with *Brettanomyces* problems. However, it was already noted that UV-C efficacy is influenced by anthocyanins and other phenolics, which play a major role in red wines [1].

3.3. Influence of turbidity on UV-C inactivation of yeast in grape must

Turbidity in must and wine is known to reduce UV-C efficacy [1]. Several technical optimization strategies have been proposed to overcome restrictions in UV-C effect in turbid liquids [5]. When employing state-of-the-art UV-C technology, efficacy of the germicidal effect still depended on the turbidity as shown in Fig. 3.

Figure 3 reveals that grape lees attenuate UV-C efficacy with an increasing UV-C dose. At 1 kJ/l, UV-C treatment

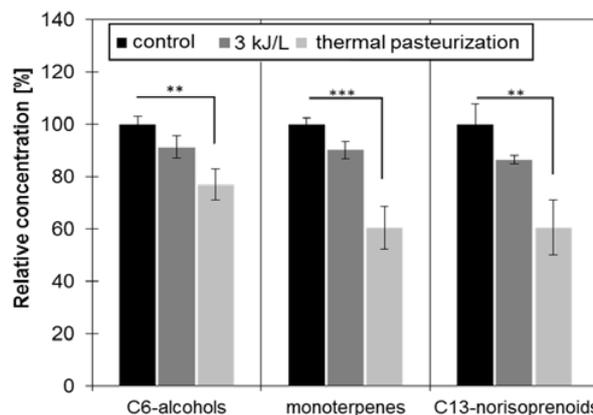


Fig. 4. Influence of UV-C treatment (3 kJ/l) and thermal pasteurization (80 °C, 120 s) on glycosidically bound aroma precursors in Riesling must. The substance classes represent the sum of 2–6 aroma precursor compounds within each class.

resulted in an average \log_{10} microbial reduction of 0.49, 0.72 and 1.37 for musts with 900, 500 and 100 NTU respectively. While 500 and 900 NTU are unrealistically high turbidity values for fermenting grape musts, these findings indicate that existing UV-C technology can still be improved towards higher efficacy.

3.4. Influence of UV-C treatment on aroma precursors in grape must

The efficacy of UV-C is undisputedly a capable technology to inactivate microorganisms in grape juices. Accordingly, the technology has to be considered as an alternative for thermal pasteurization. Therefore, the effects of UV-C treatment and thermal pasteurization on glycosidically-bound aroma precursors in Riesling must were compared with each other (Fig. 4).

Both, UV-C treatment with 3 kJ/l and thermal pasteurization at 80 °C for 120 s yielded sterile musts. While heat resulted in significantly lower concentrations of glycosidically bound C6-alcohols, monoterpenes and C13-norisoprenoids, UV-C did not cause major losses of aroma precursors. It may be concluded, that UV-C with 3 kJ/l is more gently than heating at 80 °C for 120 s.

3.5. Use of UV-C treatment to stop alcoholic fermentation

To obtain natural residual sugar the alcoholic fermentation of wines is typically stopped by the addition of high concentrations of SO_2 . In order to investigate the efficacy of UV-C to stop alcoholic fermentation, a fermenting Riesling at 17 g/L residual sugar was treated either with UV-C or 100 mg/L SO_2 . The \log_{10} microbial reduction after these treatments is displayed in Fig. 5.

Although none of the treatments yielded absolute inactivation of *Saccharomyces cerevisiae*, alcoholic fermentation may be considered as interrupted when cells are $<10^2$ cfu/ml. UV-C with 1 kJ/l was not capable to reach that number, however, a treatment with > 2 kJ/l exceeded the effect of 100 mg/L SO_2 by 1.5 \log_{10} counts.

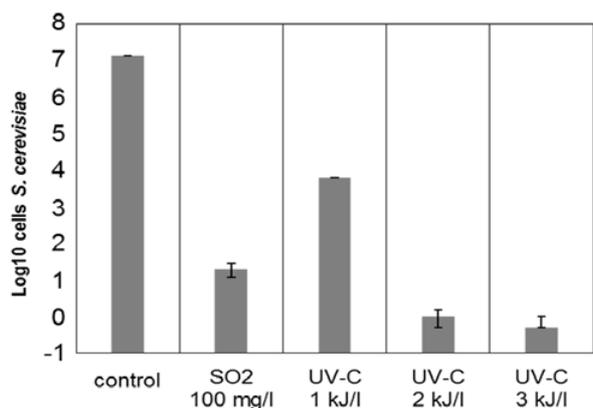


Fig. 5. Efficacy of UV-C treatment to stop alcoholic fermentation of Riesling at 17 g/L residual sugar compared to the use of 100 mg/L SO₂.

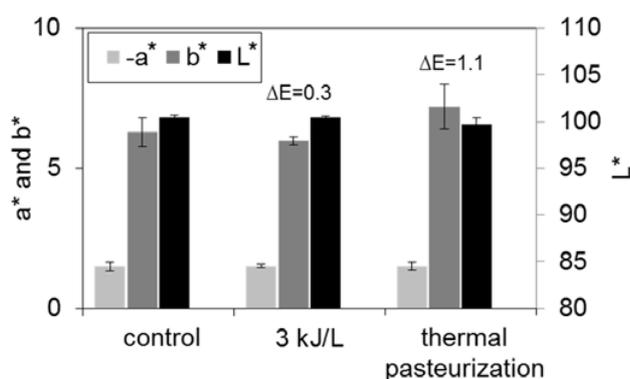


Fig. 6. Chromatic properties of wines made from Riesling musts which were either treated with UV-C (3 kJ/L) or thermal pasteurization (80 °C, 120 s).

3.6. Sensory and colour changes in wine due to UV-C treatment of grape must

UV-C light is known for its ability to form powerful off-flavours such as methional or methanethiol [6]. Increasing concentrations of mercaptans due to UV-C treatment of grape must with < 2 kJ/l could not be determined yet. Sensory triangle testing revealed no differences between wines from control and UV-C treated musts. However, higher energies than 2 kJ/l applied in must were tested significantly different in the wine stadium and even low energies <1 kJ/l applied in wines seem to develop off-flavours reminding of cooked cabbage, popcorn and

camphor. Other than aroma changes, chromatic properties of wines made from Riesling musts which were either treated with UV-C or thermal pasteurization have already been measured (Fig. 6). A slightly higher yellow colour was observed for wines obtained from heated musts but not due to UV-C treatment.

4. Conclusion and outlook

Outcomes in this ongoing research project support the applicability of UV-C treatment to inactivate microorganisms in must and wine. The efficacy towards different germs is much higher than anticipated and the fact, that UV-C is adjustable in its intensity, opens up new vistas in enology. Different sensitivities of yeasts and bacteria may be utilized to inactivate the unwanted and to let the beneficial ones survive. The current focus lies on the potential off-flavour formation by UV-C. GC-PFPD analysis towards low volatile sulphides and GC-MS analysis of 2-aminoacetophenone are about to be completed shortly. Since high concentrations of caftaric acid, riboflavin and dissolved oxygen seem to positively contribute to UV-C induced sensory defects in wines, this finding will be pursued.

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