Efficacy of ultraviolet radiation as an alternative technology to inactivate microorganisms in grape juices and wines

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Abstract
Since sulphur dioxide (SO2) is associated with health risks, the wine industry endeavours to reduce SO2 levels in wines with new innovative techniques. The aim of this study was, therefore, to investigate the efficacy of ultraviolet radiation (UV)-C (254 nm) as an alternative technology to inactivate microorganisms in grape juices and wines.

A pilot-scale UV-C technology (SurePure, South Africa) consisting of an UV-C germicidal lamp (100 W output; 30 W UV-C output) was used to apply UV-C dosages ranging from 0 to 3672 J l-1, at a constant flow rate of 4000 l h-1 (Re > 7500). Yeasts, lactic and acetic acid bacteria were singly and co-inoculated into 20 l batches of Chenin blanc juice, Shiraz juice, Chardonnay wine and Pinotage wine, respectively. A dosage of 3672 J l-1 resulted in an average log10 microbial reduction of 4.97 and 4.89 in Chardonnay and Pinotage, respectively. In Chenin blanc and Shiraz juice, an average log10 reduction of 4.48 and 4.25 was obtained, respectively.

UV-C efficacy may be influenced by liquid properties such as colour and turbidity. These results had clearly indicated significant (p < 0.05) germicidal effect against wine-specific microorganisms; hence, UV-C radiation may stabilize grape juice and wine microbiologically in conjunction with reduced SO2 levels.

1. Introduction
Although microorganisms play an imperative role in wine production (Liu, 2002; Cappello et al., 2004; Jolly et al., 2006; Bayman and Viljoen-Bloom, 2006; M aurilio et al., 2009; Ruiz et al., 2010), certain species of yeasts and bacteria can cause spoilage defects which commonly diminish the quality and acceptability of the final product (Sponholz, 1993; du Toit and Pretorius, 1994). These spoilage defects are usually recognized by hazy formation, increase in acetic acid or volatile acidity, ethanol concentration, volatile phenols, volatile sulphur and viscosity of wine (Jackson, 1994; Fleet, 1998; Fugelsang, 1997; du Toit and Pretorius, 2000; Bauer and Dicks, 2004; Delaherche et al., 2004; Dol and Longvaud-Funel, 2005; du Toit et al., 2006; Renouf et al., 2006; Fugelsang and Edwards, 2007; Bartowsky and Henschke, 2008; García-Ruiz et al., 2008; Romano et al., 2009). As a precaution, the activities of the microorganisms should be monitored and controlled continuously throughout wine production.

To date, the addition of SO2 has always been an effective means of stabilising grape juice and wine microbiologically (Ribéreau-Gayon et al., 2006a). But ever since SO2 had been associated with possible health risks, its use is being reviewed in the food, as well as the beverage industry (Jackson, 1994). In addition to this, the wine industry is challenged to meet consumers' demands of reducing the levels of SO2 used in wine production (du Toit and Pretorius, 2000). Other techniques such as filtration and fining are also efficient in controlling microbial growth, but unfortunately these techniques have detrimental effects on the sensorial properties of the wine (López et al., 2001; Gergely et al., 2003; Armada and Falqué, 2007; Suárez et al., 2007). Hence, the wine industry is seeking alternative strategies to either substitute or enhance the effect of reduced SO2 levels without modifying the chemical and sensorial properties of the wine.

Today, ultraviolet (UV-C) radiation along with pulsed electric fields (PEF) and high hydrostatic pressure systems are projected as emerging techniques to inactivate microorganisms in liquid food products without causing major changes to the sensorial properties of the product (Sizer and Balasubramaniam, 1999; Puértolas et al., 2009). Microbial inactivation caused by UV-C (254 nm) radiation is based on the rearrangement of the microorganism’s nucleic acid.
which directly interferes with the ability of microorganisms to reproduce (Bintsis et al., 2000; Thompson, 2003; Tran and Farid, 2004; Gabriel and Nakano, 2009). Since penetration of UV-C light is approximately 1 mm in fruit juices and even less than 0.1 mm in milk, the use of UV-C radiation as a pasteurisation method was, however limited in coloured and turbid liquid foods (Sizer and Basubramaniam, 1999; López-Malo and Palou, 2005; Guerrero-Beltrán and Barbosa-Cánovas, 2006). The penetration ability and ultimate efficacy of UV-C radiation depends, therefore on appearance and characteristics of the product such as colour, absorbance, density and dissolved and suspended solids (Koutchma et al., 2009), which can prevent UV-C light from reaching microorganisms in the liquid. Nevertheless, the use of UV-C radiation as a pasteurisation technology has been studied in a range of products including apple cider (Hanes et al., 2002; Koutchma et al., 2004; Panz et al., 2009), orange juice (Tran and Farid, 2004), apple juice (Guerrero-Beltrán and Barbosa-Cánovas, 2005), strawberry nectar (Keyer et al., 2008) and mango nectar (Guerrero-Beltrán and Barbosa-Cánovas, 2006).

UV-C sensitivity also differs among microorganisms, species, strains and growth stage of the culture (Guerrero-Beltrán and Barbosa-Cánovas, 2005; López-Malo and Palou, 2005; Koutchma et al., 2009). Recently, the effect of UV-C radiation to inactivate Saccharomyces cerevisiae in grape juice and beer has been investigated (Guerrero-Beltrán et al., 2009; Lu et al., 2010). The potential of UV-C radiation to inactivate other wine-related microorganisms in grape juice and wine is still unexplored. Thus, the aim of the study was to investigate the efficacy of UV-C radiation to reduce lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeasts in white and red grape juice and wine.

2. Materials and methods

2.1. Cultivation of cultures

Pure cultures were obtained from the Institute for Wine Biotechnology, Stellenbosch University, South Africa and cultivated on mother culture agar plates. Lactobacillus plantarum 130 and Pediococcus acidilactici were cultivated on 50 g l⁻¹ Mann Rogosa Sharpe (MRS) agar (Biolab, Merck, South Africa), whereas Oenococcus oeni 48 was grown on MRS agar supplemented with 10% (v/v) tomato juice (MRST) at a final pH range of 4.80 ± 0.20. The plates of L. plantarum 130 and P. acidilactici were incubated at 30 °C for 2 ± 3 days. Since the growth of O. oeni 48 was slightly slower than the other LAB, the incubation time at 30 °C were extended to 6 days. Acetobacter aceti DSM 3509 was cultivated for 6 days on yeast peptone mannitol (YPM) agar consisting of 25 g l⁻¹ mannitol (Merck, Saarchem), 5 g l⁻¹ yeast extract (Merck, Biolab) and 3 g l⁻¹ peptone (Merck, Biolab) with a final pH of 5.5. Brettanomyces bruxellensis ISA 1649 and S. cerevisiae VIN13 were grown on yeast peptone dextrose (YPD) medium comprising of 10 g l⁻¹ yeast extract (Merck, Biolab), 20 g l⁻¹ peptone (Merck, Biolab) and 20 g l⁻¹ glucose (Merck, Saarchem). The final pH of YPD was 6.5. S. cerevisiae VIN13 and B. bruxellensis ISA 1649 was incubated at 30 °C for 3 and 5 days, respectively. All the media were sterilized in an autoclave at 121 °C for 15 min. The desired concentration of each microorganism to be used as the inoculum was propagated in two steps. The microorganisms were obtained from the mother culture agar plates. For the subculture, a colony of the mother culture was suspended in broth and incubated according to the time indicated in Table 1. Subsequently, an aliquot of the subculture was transferred to a bigger volume of media in a 1% (v/v) ratio to reach the desired final concentration of ≥ 10⁸ colony-forming units per millilitre (cfu ml⁻¹). This culture was then used as the inoculum for grape juices and wines.

2.2. Stock cultures

The pure cultures were singularly inoculated in the respective liquid media and incubated overnight at 30 °C. A 500 ml aliquot of each overnight culture was mixed with 500 ml of sterile 50% (v/v) glycerol solution. These glycerol stocks were stored at 20 °C, until required.

2.3. White and red grape juice

Grape varieties such as Chenin blanc and Shiraz were immediately crushed after harvest according to standard winemaking procedures. Both grape juices were pressed and therefore did not contain seeds and skins. These unclarified grape juices were obtained from a local cellar in the Western Cape. The Chenin blanc juice consisted of 22.5 Brix, pH 3.5 and 5.0 g l⁻¹ titratable acidity (TA), whilst 21 Brix, pH 3.73 and 4.51 g l⁻¹ TA was obtained for Shiraz juice. The chemical analyses of the juices were performed by the wineries.

2.4. White and red wine

Chardonnay 2008 and Pinotage 2009 were also supplied by a commercial wine cellar. The chemical composition of Chardonnay 2008 was as follows: 12.43% (v/v) alcohol, pH 3.45, 6.67 g l⁻¹ TA, 20 mg l⁻¹ free molecular SO₂ and 50 mg l⁻¹ total SO₂. Pinotage 2009 consisted of 14.06% (v/v) alcohol, pH 3.84, 4.71 g l⁻¹ TA, 13.1 nephelometric turbidity units (NTU), 24 mg l⁻¹ free molecular SO₂ and 34 mg l⁻¹ total SO₂.

2.5. Pilot-scale UV-C reactor system

A company named SurePure, based in Milnerton, South Africa, has designed and manufactured a novel pilot-scale UV-C reactor system. The configuration of the pilot-scale UV-C reactor in this study is similar to the one that was used by Keyer et al. (2008), and therefore the same theoretical calculations were applicable. Initially, the holding tank of the UV-C system was filled with 20 l of

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Mother culture agar plates</th>
<th>Sub-culture broth</th>
<th>Culture broth (Inoculum)</th>
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<tbody>
<tr>
<td>Lactobacillus plantarum 130</td>
<td>MRS; pH 6.5; 72 h</td>
<td>MRS; pH 6.5; 36 h</td>
<td>MRS; 24 h</td>
</tr>
<tr>
<td>Pediococcus acidilactici</td>
<td>MRS; pH 6.5; 72 h</td>
<td>MRS; pH 6.5; 36 h</td>
<td>MRS; 24 h</td>
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<tr>
<td>Oenococcus oeni 48</td>
<td>MRST; pH 4.8±5.2; 144 h</td>
<td>MRST; pH 4.8±5.2; 72 h</td>
<td>MRST; pH 4.8±5.2; 72 h</td>
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<tr>
<td>Acetobacter aceti DSM 3509</td>
<td>YPM; pH 5.6; 120 h</td>
<td>YPM; pH 5.6; 72 h</td>
<td>YPM; pH 5.6; 72 h</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae VIN13</td>
<td>YPD; pH 6.5; 72 h</td>
<td>YPD; pH 6.5; 24 h</td>
<td>YPD; 36 h</td>
</tr>
<tr>
<td>Brettanomyces bruxellensis ISA 1649</td>
<td>YPD; pH 6.5; 168 h</td>
<td>YPD; pH 6.5; 72 h</td>
<td>YPD; 56 h</td>
</tr>
</tbody>
</table>

MRS: Mann Rogosa Sharpe media. MRST: Mann Rogosa Sharpe media supplemented with 10% tomato juice. YPM: Yeast Peptone Mannitol media. YPD: Yeast Peptone Dextrose media.
treat by the UV-C energy penetrates into liquid flow medium instead of area, it was therefore more relevant to work with UV-C dosage per volume of the liquid. Hence, UV-C dosage will be expressed as J l⁻¹ in this particular study. The operation time of the UV-C treatment is based on the quantity of product to be treated and the flow rate of the product feed. Only 18 s is required for the 20 litre (l) product to pass through the system once at a flow rate of 4000 l h⁻¹, thus one pass of the product through the system is equivalent to a UV-C dose of 22.95 J l⁻¹ or 23.40 mJ cm⁻².

Manual sampling was done aseptically and the grape juice or wine was extracted directly from the flow stream. The UV-C light was switched off after each consecutive dosage, to avoid excessive exposure of the product in the UV-C reactor while sampling occurred. The temperature was continuously regulated by a thermometer that is installed adjacent to the lamps. Since the temperature had remained constant subsequent to each UV-C treatment, UV-C radiation can be classified as an adiabatic process. A standard "Cleaning In Place" (CIP) process as described by Keyser et al. (2008) was implemented prior to and following each UV treatment to avoid microbial contamination.

2.6. UV-C treatment of white and red grape juice

*B. bruxellensis* ISA 130 and *S. cerevisiae* VIN13 were co-inoculated into 20 l Chardonnay wine with an initial microbial count of 10⁶ cfu ml⁻¹, respectively. Twenty litre of Shiraz juice was also inoculated with *B. bruxellensis* ISA 130, *S. cerevisiae* VIN13 and *L. plantarum* 130 to obtain initial microbial counts of 10⁷ cfu ml⁻¹, respectively. Duplicate 50 ml samples were taken of the juices before UV-C radiation and subsequent to UV-C dosages of 459, 918, 1377, 1836, 2295, 2754 and 3672 (J l⁻¹), after which microbiological analysis was performed. Three replicates of each experiment were performed.

2.7. UV-C treatment of white and red wine

Free SO₂ was removed from the Chardonnay and Pinotage wines through meticulous addition of 40 volume hydrogen peroxide (Zoecklein et al., 1995). The Chardonnay and Pinotage wines that contained 0 mg l⁻¹ free SO₂ were inoculated with *L. plantarum* 130, *P. acidilactici*, *O. oeni* 48, *B. bruxellensis* ISA 1649, *S. cerevisiae* VIN13 and *A. aceti* DSM 3509, respectively, to obtain an initial count of 10⁶ cfu ml⁻¹. Duplicate 50 ml sampling of the wines occurred before inoculation, after inoculation and subsequent to UV-C dosages of 459, 918, 1377, 1836, 2295, 2754 and 3672 (J l⁻¹), after which microbiological analysis was performed. Three replicates of each experiment were performed.

Microbiological analysis was performed on all the samples. Three replicates of each experiment were performed.

2.8. The enumeration of microorganisms from grape juices and wines

One millilitre of the grape juice and wine samples was transferred aseptically to 9 ml quarter strength Ringer’s solution (Merck) and vortexed thoroughly. A serial tenfold dilution range were prepared (10⁻¹ to 10⁻⁶) and 1 ml of each dilution was poured using the selective media to determine the viability of the microorganisms after UV-C treatment.

*L. plantarum* 130 and *P. acidilactici* were enumerated with MRS agar supplemented with 50 mg l⁻¹ Delvocid (DSM Food Specialities, The Netherlands) and 30 mg l⁻¹ kanamycin sulphate (Quantum Biotechnologies) to inhibit the growth of yeasts and AAB, respectively (Du Plessis et al., 2002). The plates were incubated for 2–3 days at 30 C. MRS agar supplemented with 10% (v/v) tomato juice was used to enumerate *O. oeni* 48. Prior to sterilization, the pH value of the media was adjusted using 1 M HCl (Merck, Saarchem) to a range of pH 4.8–5.2. Delvocid and kanamycin sulphate were also added to MRST agar for selective growth of *O. oeni* 48. The plates for *O. oeni* 48 were anaerobically incubated at 30 C for 7 days (Fleet, 1993) (Anaerojar, Quantum Biotechnologies). Wallenstein (WL) nutrient (Quantum Biotechnologies) agar supplemented with 30 mg l⁻¹ kanamycin sulphate, 50 mg l⁻¹ cycloheximide (Merck) and 50 mg l⁻¹ chloramphenicol (Sigma–Aldrich) were used to enumerate *B. bruxellensis* ISA 1649. These antibiotics prevent the proliferation of AAB, yeasts and bacteria (Du Plessis et al., 2002; Fugelsangs and Edwards, 2007; Oelofse et al., 2009). Growth of *B. bruxellensis* ISA 1649 was detected after an incubation period of 7 days at 30 C. Delvocid (50 mg l⁻¹) and chloramphenicol (50 mg l⁻¹) were added to YPM agar for the enumeration of *A. aceti* DSM 3509. The YPD agar supplemented with sodium metabisulphite (Sigma–Aldrich) at a final concentration of 0.02% and 12% (v/v) absolute ethanol (Merck, Saarchem) was used to enumerate *S. cerevisiae* VIN13. The incubation period of *A. aceti* DSM 3509 and *S. cerevisiae* VIN13 were 4–5 days at 30 C. Three replicates of each experiment were performed. The number of colonies counted were averaged, converted into logarithmic units and expressed as cfu ml⁻¹.

2.9. Statistical analysis

A repeated measures analysis of variance was performed, using general linear models. Probability levels of 5% or less was considered significant. The values obtained from the ANOVA Greenhouse-Geisser WS test were used.

3. Results

3.1. Grape juice

The efficacy of UV-C radiation to inactivate co-inoculated *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13 in unclarified Chenin blanc juice was determined. The average initial counts for *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13 was 1.35 × 10⁵ and 1.53 × 10⁶ cfu ml⁻¹ respectively (Fig. 1). At the lowest UV-C dosage of 459 J l⁻¹, a 0.47 and 1.07 log₁₀ reduction were obtained for *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13, respectively. After the 918 J l⁻¹ UV-C exposure, a 1.24 and 4.02 log₁₀ reduction were obtained for the same yeasts followed by a 1.83 and 5.38 log₁₀ reduction after a higher dosage of 1377 J l⁻¹. The UV-C dosage of 1377 J l⁻¹ was sufficient to completely inactivate *S. cerevisiae* VIN13.

Unclarified Shiraz juice was inoculated with *L. plantarum* 130, *S. cerevisiae* VIN13 and *B. bruxellensis* ISA 1649 to initial average
counts that ranged between 3.03 \times 10^4 and 7 \times 10^6 cfu ml^{-1} (Fig. 2). The Shiraz juice was exposed to the same UV-C dosages that were used to treat the Chenin blanc juice. A dosage of 918 J l^{-1} resulted in a log_{10} reduction of 1.86, 2.01 and 0.45 for L. plantarum 130, S. cerevisiae VIN13 and B. bruxellensis ISA 1649, respectively. However, a greater reduction of 3.38, 3.14 and 0.84 were obtained for the same microorganisms after the 1377 J l^{-1} UV-C exposure. The highest applied UV-C dosage of 3672 J l^{-1} reduced B. bruxellensis ISA 1649 and L. plantarum 130 to 1.99 and 1.99 log_{10}, respectively, while only S. cerevisiae VIN13 had been reduced to 1 log_{10}. A significant difference (p < 0.05) was found between the inactivation of B. bruxellensis ISA 1649 and S. cerevisiae VIN13 in Shiraz juice when compared to inactivation in Chenin blanc juice.

3.2. Wine

Chardonnay wine containing 0 mg l^{-1} and 20 mg l^{-1} free SO_2 was inoculated with L. plantarum 130, O. oeni 48, A. aceti DSM 3509^T, B. bruxellensis ISA 1649 and S. cerevisiae VIN13, respectively, to evaluate the feasibility of UV-C radiation to effect a significant reduction was due to UV-C radiation and not SO_2. Since SO_2 is generally applied in wine as an antioxidant and antimicrobial agent, further discussions will therefore only concentrate on the results obtained for the Chardonnay wine containing 20 mg l^{-1} free SO_2 that was subjected to UV-C dosages of 0, 459, 918, 1377, 1836, 2295, 2754 and 3672 J l^{-1}.

The Chardonnay wine containing 20 mg l^{-1} free SO_2 that received no UV-C treatment was used as the control. The initial average counts for L. plantarum 130, O. oeni 48, P. acidilactici, A. aceti DSM 3509^T, B. bruxellensis ISA 1649 and S. cerevisiae VIN13 were 7.6, 10^5; 4 \times 10^5; 2.44 \times 10^5; 1.17 \times 10^5; 4.32 \times 10^5; and 5.37 \times 10^5, respectively (Fig. 3). It is, however, clear that the various microbial counts were relatively constant for approximately 30 min whereafter a 0.13, 0.62, 0.66, 1.00, 1.63 and 2.21 log_{10} reduction had followed for B. bruxellensis ISA 1649, A. aceti DSM 3509^T, S. cerevisiae VIN13, P. acidilactici, O. oeni 48 and L. plantarum 130, respectively. The UV-C treated Chardonnay wine with 20 mg l^{-1} free SO_2 resulted in a 3.66, 4.37, 4.67, 2.00, 3.73 and 3.57 log_{10} reduction for O. oeni 48, L. plantarum 130, P. acidilactici, A. aceti DSM 3509^T, S. cerevisiae VIN13 and B. bruxellensis ISA 1649, respectively, after a UV-C dosage of only 459 J l^{-1} (Fig. 4). Similarly, a 3.65, 4.90, 5.00, 2.53, 5.39 and 4.76 log_{10} reduction was obtained after a higher dosage of 918 J l^{-1}.

Microbial reduction was caused by UV-C radiation or the SO_2 present in wine.

Statistical analysis were done on the results obtained for the inoculated Chardonnay wine containing 0 mg l^{-1} and 20 mg l^{-1} free SO_2, respectively. According to repeated measures of ANOVA, no significant differences (p > 0.05) in microbial reduction were observed in Chardonnay wine containing 0 mg l^{-1} and 20 mg l^{-1} free SO_2. Only the results of P. acidilactici in Chardonnay wine containing 0 mg l^{-1} free SO_2 was significantly different (p < 0.05) from that of P. acidilactici in Chardonnay wine containing 20 mg l^{-1} free SO_2. The contrast values for the reduction at each dosage have clearly indicated a significant difference (p < 0.05) within dosage intervals of 459 to 918 J l^{-1} and 2754 to 3672 J l^{-1}. Contrary, no significant difference (p > 0.05) was observed in the reduction of L. plantarum 130, O. oeni 48, A. aceti DSM 3509^T, B. bruxellensis ISA 1649 and S. cerevisiae VIN13 in Chardonnay wine containing 0 mg l^{-1} and 20 mg l^{-1} free SO_2. Statistical analysis demonstrated that microbial reduction was due to UV-C radiation and not SO_2. Since SO_2 is generally applied in wine as an antioxidant and antimicrobial agent, further discussions will therefore only concentrate on the results obtained for the Chardonnay wine containing 20 mg l^{-1} free SO_2 that was subjected to UV-C dosages of 0, 459, 918, 1377, 1836, 2295, 2754 and 3672 J l^{-1}.
In order to investigate the efficacy of UV-C radiation with respect to different wines, similar experiments were done using Pinotage wine in addition to Chardonnay wine. Statistical analysis also showed no significant difference ($p > 0.05$) between the microbial reduction in Pinotage wine containing 0 mg l $^{-1}$ and 24 mg l $^{-1}$ free SO$_2$ after each UV-C dosage. Since there was no significant difference only the results of Pinotage wine containing 24 mg l $^{-1}$ free SO$_2$ will be further discussed in this study for its practical significance.

**A. aceti** DSM 3509$^{T}$, **B. bruxellensis** ISA 1649, **L. plantarum** 130, **O. oeni** 48, P. acidilactici and **S. cerevisiae** VIN13 were individually inoculated into Pinotage wine containing 24 mg l $^{-1}$ free SO$_2$ that was not exposed to UV-C radiation. The microbial reductions for the respective microorganisms were obtained as $1.26$ and $1.07$ log$_{10}$ after 459 J l $^{-1}$ dosage for **B. bruxellensis** ISA 1649, **L. plantarum** DSM 3509$^{T}$, respectively. The results of the control confirm that microbial inactivation found in Pinotage wine may be attributed to UV-C radiation.

After a UV-C dosage of 459 J l $^{-1}$ a 0.03, 0.63, 0.96, 0.26, 0.19 and 0.46 log$_{10}$ reduction (Fig. 6) was obtained in Pinotage wine for **L. plantarum** 130, **P. acidilactici**, **O. oeni** 48, **A. aceti** DSM 3509$^{T}$, **B. bruxellensis** ISA 1649 and **S. cerevisiae** VIN13, respectively. The microbial log$_{10}$ reductions had slightly increased to $0.34, 1.56, 2.61, 0.82, 0.69$ and $0.89$ following a dosage of 918 J l $^{-1}$. Exposure to a higher UV-C dosage of 1377 J l $^{-1}$ had resulted in a 1.62, 3.20, 4.10, 1.66, 1.66 and 1.75 log$_{10}$ reduction for the same microorganisms. In view of the results, it is clear that the microbial counts decreased with an increase of UV-C dosage.

The initial microbial load in a matrix is also a function of the effectiveness of UV-C radiation as an inactivation technology. Therefore, the efficacy of UV-C radiation to inactivate a cocktail of microorganisms comprising of **L. plantarum** 130, **B. bruxellensis** ISA 1649 and **S. cerevisiae** VIN13 in Pinotage wine containing 24 mg l $^{-1}$ free SO$_2$ was also investigated. Each of the microorganisms was inoculated to a concentration of $5 \times 10^5, 2.53 \times 10^5$ and $4.11 \times 10^6$ cfu ml $^{-1}$, respectively. The Pinotage wine containing 24 mg l $^{-1}$ free SO$_2$ that received no UV-C radiation was used as the control (Fig. 7). For the control, the microbial counts of **L. plantarum** 130, **B. bruxellensis** ISA 1649 and **S. cerevisiae** VIN13 were relatively constant for 48 min after which a 0.08, 0.84 and 0.15 log$_{10}$ reduction occurred for the respective microorganisms.

After 459 J l $^{-1}$, a 0.41, 0.26 and 0.26 log$_{10}$ reduction (Fig. 8) were obtained for **L. plantarum** 130, **B. bruxellensis** ISA 1649 and **S. cerevisiae** VIN13, respectively. A UV-C dosage of 918 J l $^{-1}$ resulted in a 1.79, 1.18 and 1.28 log$_{10}$ reduction followed by a reduction of 4.02, 2.74 and 2.17 for the same microorganisms after 1377 J l $^{-1}$.  

**Fig. 4.** The average log$_{10}$ microbial counts of **Brettanomyces bruxellensis** ISA 1649, **Lactobacillus plantarum** 130, **Pediococcus acidilactici**, **Saccharomyces cerevisiae** VIN13, **Oenococcus oeni** 48 and **Acetobacter aceti** DSM 3509$^{T}$ that were individually inoculated in Chardonnay wine containing 20 mg l $^{-1}$ free SO$_2$ after exposure to various UV dosages (J l $^{-1}$) at a constant flow rate of 4000 l h $^{-1}$.

**Fig. 5.** The average log$_{10}$ microbial counts of the controls for **Brettanomyces bruxellensis** ISA 1649, **Lactobacillus plantarum** 130, **Pediococcus acidilactici**, **Saccharomyces cerevisiae** VIN13, **Oenococcus oeni** 48 and **Acetobacter aceti** DSM 3509$^{T}$ that were individually inoculated in Pinotage wine containing 24 mg l $^{-1}$ free SO$_2$.

**Fig. 6.** The average log$_{10}$ microbial counts of **Brettanomyces bruxellensis** ISA 1649, **Lactobacillus plantarum** 130, **Pediococcus acidilactici**, **Saccharomyces cerevisiae** VIN13, **Oenococcus oeni** 48 and **Acetobacter aceti** DSM 3509$^{T}$ that were individually inoculated in Pinotage wine containing 24 mg l $^{-1}$ free SO$_2$ after exposure to various UV dosages (J l $^{-1}$) at a constant flow rate of 4000 l h $^{-1}$.
In this study, the gemicidal effect of UV-C radiation on different microorganisms that are typically present in grape juice and wine were evaluated. Currently, no studies pertaining to the inactivation of wine-related microorganisms has been reported. However, the use of UV-C radiation against \textit{S. cerevisiae} in other juices such as apple juice, grape juice (\textit{Vitis vinifera}) and grapefruit juice have been investigated (Guerrero-Beltrán and Barbosa-Cánovas, 2005, 2006; Guerrero-Beltrán et al., 2009). In this study \textit{S. cerevisiae} VIN38 was inactivated in Chenin blanc juice and showed a 5.38 log_{10} reduction after a UV-C dosage of 1377 J l^{-1}. \textit{S. cerevisiae} are known to express UV-C resistance proteins that are involved in DNA repair systems (Qiu et al., 1998). It is believed that this mechanism of \textit{S. cerevisiae} may contribute significantly to their resistance to UV-C radiation. Lu et al. (2010) showed that only a 1±2 log_{10} reduction was obtained in beer after a UV-C dosage of 6.9 mJ cm^{-2}. Contradictory, \textit{S. cerevisiae} was more sensitive to UV-C radiation in this study when compared to the findings of Lu et al. (2010). The UV-C system used in their study was based on laminar flow and may have played a role in the low log_{10} reduction of this yeast.

According to Koutchma et al. (2004), the average flowing particle travels 1.1±1.2 times faster than the volume average particle in turbulent flow pattern at high flow rates compare to laminar flow where the fastest flowing particle travels twice as fast as the average particle. The particles are therefore longer exposed to the UV-C light in a turbulent flow reactor which is proportional to better inactivation (Koutchma et al., 2009). Furthermore, a turbulent flow profile also facilitates better mixing which ensures that each part of the liquid are equally exposed to the UV-C light (Kevery et al., 2008). The novel turbulent flow reactor of SurePure may have been the cause of better microbial reduction in this study.

The microbial reductions obtained for \textit{S. cerevisiae} VIN13 and \textit{B. bruxellensis} ISA 1649 in Chenin blanc juice (Fig. 1) was significantly different from those obtained in Shiraz juice (Fig. 2). Even though Chenin blanc and Shiraz juice differ in Brix (soluble solids content) and pH levels, it may be insignificant to the difference found in microbial reductions since the inactivation rate of \textit{Escherichia coli} in model apple juice was independent of pH and Brix (Koutchma et al., 2009). In comparison with Chenin blanc juice, Shiraz juice appeared red in colour and more turbid although both juices were unclarified. According to López-Malo and Palou (2005), a turbid matrix causes shadowing and scattering of the UV-C rays and thus reduces the efficacy of the technology. This finding correlates to a study reported by Koutchma et al. (2004) that have indicated reduced inactivation rates of \textit{E. coli} K12 in apple juice with increased levels of turbidity.

Although slight microbial reductions were observed for the wine controls after ±30 min (Figs. 3, 5 and 7), this has demonstrated that microbial reductions may be primarily attributed to UV-C radiation and not because of the antimicrobial effect of the SO₂ present. As observed, it seems as if the UV-C resistance pattern in Chardonnay wine (Fig. 4) was as follows: \textit{A. aceti} DSM 3509¹ < \textit{P. acidilactici} < \textit{O. oeni} 48 < \textit{B. bruxellensis} ISA 1649 < \textit{L. plantarum} 130 < \textit{S. cerevisiae} VIN13. This is in contradiction with previous finding which claims that Gram-negative bacteria are more sensitive to UV-C radiation than Gram-positive bacteria and yeasts (Thompson, 2003). \textit{A. aceti} DSM 3509¹ is Gram-negative whereas \textit{O. oeni} 48, \textit{P. acidilactici} and \textit{L. plantarum} 130 are classified as Gram-positive bacteria. In comparison to bacteria, yeasts do contain less thymine or cytosine pyrimidines on their DNA strands. UV-C radiation primarily targets these pyrimidines, whereafter dimers are formed rendering the microorganisms incapable of reproducing (Bintsis et al., 2000; Thompson, 2003; Tran and Farid, 2004). It is, therefore, expected that yeasts should be more resistant to UV-C radiation than bacteria.

A significant difference ($p < 0.05$) in microbial reduction was obtained when comparing Chardonnay and Pinotage wine. In Pinotage wine (Fig. 6), the resistance pattern of the microorganisms to UV-C radiation was as follows: \textit{L. plantarum} 130 < \textit{B. bruxellensis} ISA 1649¹ < \textit{S. cerevisiae} VIN13 < \textit{A. aceti} DSM 3509¹ < \textit{P. acidilactici} < \textit{O. oeni} 48. In addition to this, the degree of microbial reductions obtained in Pinotage wine (Fig. 6) was also much lower than those found in Chardonnay wine (Fig. 4). This finding correlates to the Beer × Lambert-Bouger’s law stating that light intensity is indirectly proportional to absorbance of a matrix (Koutchma et al., 2009). The same was also observed for the red and white grape juices, meaning that the efficacy of UV-C radiation is mean dependent. This may be attributed to the colour of the product that can affect the absorbance of the liquid. The authors, Guerrero-Beltrán and Barbosa-Cánovas (2004), have reported that the absorption coefficient ($a$) at the gemicidal wavelength (253.7 nm) of white and red wine is 10 and 30 cm$^{-1}$, respectively. The higher absorption coefficient of red
wine may be due to presence of anthocyanins (Zoecklein et al., 1995). A range of phenolic compounds in red wine are capable of absorbing radiation in the UV region of the electromagnetic spectrum (Gómez-Cordovés, 2004; Ribéreau-Gayon et al., 2006b). It may therefore be assumed that the UV-C light was absorbed by the phenolic compounds present in Pinotage wine instead of being transmitted to the microorganisms. Yet, significant microbial reduction was obtained in Pinotage wine meaning that the penetration ability of UV-C light in the wine was only limited to a certain extent.

In this study, the efficacy of UV-C radiation was also affected by the initial microbial concentration in Pinotage wine. Based on the results, the reduction profiles for the individually inoculated L. plantarum 130, B. bruxellensis ISA 1649 and S. cerevisiae VIN13 in Pinotage containing 24 mg l⁻¹ free SO₂ (Fig. 6) were significantly higher (p < 0.05) than the reductions observed for the cocktail (Fig. 8). The lower microbial reductions found for the cocktail could be related to the high initial microbial load in Pinotage wine that may have influence the absorbance of the wine (López-Malo and Palou, 2005; Koutchma et al., 2009). Moreover, high microbial concentrations could also facilitate clump formation where the outer layered cells may protect the more deep-seated ones from the UV-C light and in doing so decrease UV-C efficacy (Keyser et al., 2008).

Importantly, this study has revealed that microbial reduction is in correlation with the UV-C dosage (J l⁻¹): higher microbial reductions were obtained with exposure to higher UV-C dosages (J l⁻¹). As a result, the highest microbial reduction were obtained after 3672 J l⁻¹. Also, the applied UV-C dosage (J l⁻¹) depends largely on the physical appearance of the liquid such as turbidity (suspended and soluble solids), colour and initial microbial load (López-Malo and Palou, 2005; Koutchma et al., 2009). This might be reason for better microbial reduction observed in white wine comparing to red wine.

In conclusion, UV-C radiation has offered a wide spectrum of effective inactivation of wine-associated microorganisms such as Brettanomyces, Saccharomyces, Acetobacter, Lactobacillus, Pediococcus and Oenococcus; therefore may hold promise as an alternative technology to inactivate spoilage microorganisms at different stages of vinification in conjunction with reduced SO₂ levels. From a practical point of view, further studies pertaining to the long and short term effect of UV-C radiation on the sensorial and chemical properties of wine are imperative.