EFFICACY OF ULTRAVIOLET RADIATION AS AN ALTERNATIVE TECHNOLOGY TO INACTIVATE MICROORGANISMS IN GRAPE JUICES AND WINES

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EFFICACY OF ULTRAVIOLET RADIATION AS AN ALTERNATIVE TECHNOLOGY TO INACTIVATE MICROORGANISMS IN GRAPE JUICES AND WINES

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There are two things to aim at in life; first to get what you want, and after that to enjoy it. Only the wisest of mankind has achieved the second.

Logan Pearsall Smith

DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that it has not previously, in its entirety or in part, been submitted at any other university for a degree.

Fredericks

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23/02/2012

Date

ABSTRACT

Sulphiting is considered as the most reliable and understood preservation technique in the wine industry. Since sulphur dioxide (SO_2) has been associated with possible health risks, legislation as well as consumers, are becoming more reluctant about the general use of SO_2 in wine production. In order to avoid economic losses due to spoilage, the wine industry is seeking feasible techniques to possibly reduce the levels of SO_2 in wine. The purpose of this study was, therefore to determine the efficacy of ultraviolet radiation (UV)-C (254 nm) as an alternative technology to inactivate microorganisms in white and red grape juices and wines.

Wine-associated microorganisms such as *Pediococcus acidilactici*, *Oenococcus oeni* 48, *Acetobacter aceti* DSM 3509^T, *Lactobacillus plantarum* 130, *Saccharomyces cerevisiae* VIN13 and *Brettanomyces bruxellensis* ISA 1649 were included in this study. A cocktail of these microorganisms were inoculated into 20 litre (l) Shiraz juice (21 °Brix, pH 3.73 and 4.51 g.l^{-1}) and 20 l Chenin blanc juice (22.5 °Brix, pH 3.50 and 5.0 g.l^{-1}) to obtain initial counts of ±1 x 10^6 cfu.ml^{-1}, respectively. Each microorganism was also inoculated into the following wines respectively: Chardonnay with ±0 mg.l^{-1} free SO_2, Chardonnay with ±20 mg.l^{-1} free SO_2, Pinotage with ±0 mg.l^{-1} free SO_2 and Pinotage with ±24 mg.l^{-1} free SO_2. A cocktail of the microorganisms were also inoculated into 20 l Pinotage wine with ±24 mg.l^{-1} free SO_2. The various 20 l batches were subjected to UV-C dosages ranging from 0, 459, 918, 1 377, 1 836, 2 295, 2 754 to 3 672 J.l^{-1}, at a constant flow speed of 4 000 l.h^{-1} using the novel pilot-scale UV-C reactor system of SurePure. The control samples of the wines containing SO_2 were kept at ambient temperature, while sampling had corresponded with the time that each UV-C dosage was completed. Microbiological analyses were performed on all samples. Three replicates of each experiment were performed and only the average microbial counts were used.

After 3 672 J.l^{-1}, an average $\log_{10}$ reduction of 5.24, 1.99, 5.52 was obtained in Shiraz juice for *L. plantarum* 130, *S. cerevisiae* VIN13 and *B. bruxellensis* ISA 1649, respectively. In Chenin blanc juice, an average $\log_{10}$ reduction of 5.38 and 3.57 was obtained for *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13, respectively.

Statistical analysis have demonstrated a significant difference ($p<0.05$) in microbial reduction of Chenin blanc and Shiraz juices. No significant difference ($p>0.05$) in microbial reduction was obtained in wines containing no and low levels of free SO_2. An average log_{10} reduction of 4.97 and 4.89 were respectively obtained in Chardonnay and Pinotage wine that contained low levels of free SO_2. A significant difference ($p<0.05$) in microbial reductions was also found in Chardonnay and Pinotage wine. In Pinotage wine, an average log_{10} reduction of 5.35 was obtained for the cocktail which was significantly lower ($p<0.05$) than the microbial reduction obtained for the individually inoculated microorganisms.

Significant microbial inactivation differences were obtained in grape juice and wine. The efficiency of UV-C radiation to inactivate microorganisms in grape juice and wine is rather challenging, due to variability in their physical properties such as colour and turbidity. The results obtained have also indicated no significant difference in the reduction of microorganisms in wines with no and low SO_2 levels, which verify significant germicidal activity of UV-C radiation in this study. Hence, UV-C radiation may represent an alternative technology to stabilise grape juice and wine microbiologically in conjunction with reduced SO_2 levels.

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DEDICATION

I dedicate this to my family, especially my parents **Pietie** and **Mary**, brothers **Garth** and **Enslin**. Thank you for granting me this opportunity and for always believing in me. Your support, encouragement and unconditional love throughout the years has made a world of difference...you guys are the best support system ever!

CONTENTS

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

Yeasts and bacteria play an imperative role in the production of wine by converting grape sugar to ethanol, CO_2 and other metabolites (Divol & Lonvaud-Funel, 2005; Goode, 2005; Volschenk *et al.*, 2006). However, certain species can cause spoilage once wine production suffers from a lack of efficient microbiological control systems (Sponholz, 1993; Jackson, 1994; Fugelsang, 1997). Since spoilage has a detrimental affect on the quality of the final product, it can lead towards great financial losses (Renouf & Lonvaud-Funel, 2007).

In attempt to limit the growth of spoilage microorganisms in must and wine, various disinfection regimes for winery contact surfaces together with numerous preservation techniques have been established over the years of which sulphiting is recognized as the most reliable technique to control microbial growth (Zoecklein *et al.*, 1995; Du Toit & Pretorius, 2000; Ribéreau-Gayon *et al.*, 2006; Fugelsang & Edwards, 2007; García-Ruiz *et al.*, 2008; Renouf *et al.*, 2008; Enrique *et al.*, 2009). Due to health concerns, mounting resistance dwell among consumers about the levels of sulphur dioxide (SO_2) that are currently used in wine production (Jackson, 1994). These concerns have become so far-fetched that the South African exporting market must oblige to the legislations of United States and European Union which requests that the presence of sulphites should be labelled on the bottle when above 10 $mg.l^{-1}$ (Salaha *et al.*, 2008). Other techniques such as filtration and fining are also effective in controlling microorganisms, but unfortunately these techniques are known to have a negative impact on the sensorial properties such as palate weight, mouthfeel and colour of the wine (Gergely *et al.*, 2003; Suárez *et al.*, 2007; Oelofse *et al.*, 2008). This causes a huge problem for the wine industry as the risk of wine spoilage will only increase if SO_2 is eliminated from wine production without the implementation of another feasible antimicrobial tool. In response to this, the wine industry is endeavoring to optimize current methods or to create new innovative

methods that have the potential of controlling microbial growth in grape juice and wine with no or reduced SO_2 levels.

A novel technology such as ultraviolet radiation (UV)-C (254 nm) is classified as a cold pasteurisation technique that inactivates a broad spectrum of spoilage microorganisms, without modifying the sensorial properties of the liquid matrix (Basaran *et al.*, 2004; López-Malo & Palou, 2005; Unluturk *et al.*, 2008). The principle of microbial inactivation caused by UV-C radiation is based on the absorbance of UV-C rays through the cell membrane, following dimerization of thymine and occasionally cytosince which hinder the cell from conducting normal transcription and replication (Sizer & Balasubramaniam, 1999; Thompson, 2003; Gabriel & Nakano, 2009).

To date, intensive research on the reduction of microorganisms in milk, juice and particularly water have been performed with UV-C radiation (Bintsis *et al.*, 2000; Hanes *et al.*, 2002; Ngadi *et al.*, 2003; Thompson, 2003; Matak *et al.*, 2005; Carrasco & Turner, 2006; Keyser *et al.*, 2008). Studies have indicated that the efficacy of UV-C radiation is in relation with the type of microorganism, concentration of the microorganism, design of the UV-C reactor, flow pattern of the liquid and physical appearance such as colour, turbidity, density, absorptive coefficient of the liquid food (Koutchma *et al*, 2004; Koutchma *et al.*, 2009). A juice such as, orange juice is more turbid than apple juice because of floating fibres and particles. Since some fibres and particles are impenetrable by UV-C light, it can reduce the efficacy of the technology by blocking the transmittance of UV-C light to microorganisms (Tran & Farid, 2004). In these situations, it is advised to optimize the technology by either using thin films of liquid or turbulent flow in the reactor to ensure that each part of the liquid comes equally exposed to the UV-C light (Koutchma *et al.*, 2009).

Since the use of UV-C radiation is a fairly new technology to microbiologically stabilise liquid foods, limited data currently exists in the literature on the effectiveness of UV-C radiation to inactivate microorganisms associated with grape juice and wine. Currently, only data on the efficacy of UV-C radiation to inactivate *Saccharomyces cerevisiae* in grape juice are available (Guerrero-Beltrán *et al.*, 2009). However, the

effect of UV-C radiation to inactivate other wine-related microorganisms in the grape juice and wine is still unknown.

The aim of this study was to evaluate the efficacy of UV-C radiation as an alternative technology to inactivate *Lactobacillus plantarum* 130, *Pediococcus acidilactici*, *Oenococcus oeni* 48, *Acetobacter aceti* DSM 3509^T, *Saccharomyces cerevisiae* VIN13 and *Brettanomyces bruxellensis* ISA 1649 in grape juices and wines, in order to reduce SO_2 levels in wines. Firstly, the efficacy of UV-C radiation to inactivate a mixture of these microorganisms in Shiraz and Chenin blanc juices was evaluated. Secondly, the efficacy of UV-C radiation to inactivate inoculated microorganisms in Chardonnay wine containing ± 0 and ± 20 mg.l^{-1} free molecular SO_2 was investigated. The efficacy of UV-C radiation to inactivate inoculated microorganisms in Pinotage wine containing ± 0 and ± 24 mg.l^{-1} free molecular SO_2 was also investigated. Lastly, the efficacy of UV-C radiation to inactivate a mixture of microorganisms in Pinotage wine containing ± 24 mg.l^{-1} free molecular SO_2 was evaluated.

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CHAPTER 2

LITERATURE REVIEW

Wine production is a very conservative process, with only a number of changes that have been implemented over the years (Plahuta & Raspor, 2007). Wine is created by complex biochemical and biological interactions between grapes and a wide range of microorganisms namely, yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB), fungi, mycoviruses and bacteriophages (Jolly *et al.*, 2006). Each one of these microorganisms plays a fundamental role in the production of a good quality wine. However, spoilage can occur if the growth of microorganisms are not properly regulated which rendering the product unpalatable with great economical losses (Pretorius & Bauer, 2002; Oelofse *et al.*, 2008).

The wine process, wine-associated microorganisms, wine preservation techniques along with the possible use of ultraviolet radiation (UV)-C in grape juice and wine as an alternative method to control microbial growth during wine production are discussed.

A. Wine process

White and red wine obtain their ultimate characteristics due to profound differences in the production of the respective wines, which are notably influenced by the selection and sequence of cellar methods applied by the winemaker (Fleet, 1998; Jackson, 1994). The diagram (Fig. 1) outlines the traditional process of wine production. The choices made by the winemaker are normally based upon the availability of equipment along with the desired quality of the final product (Ribéreau-Gayon *et al.*, 2006). As the grapes enter the winery it initially undergoes a process called destemming (Jackson, 1994). During this period the leaves and any extraneous material are removed, followed by the movement of grapes to the crusher. For red wine, the berries are ruptured and consequently, the juice, pulp, seeds and skins are collected into collecting containers, while only the juice and pulp is essential

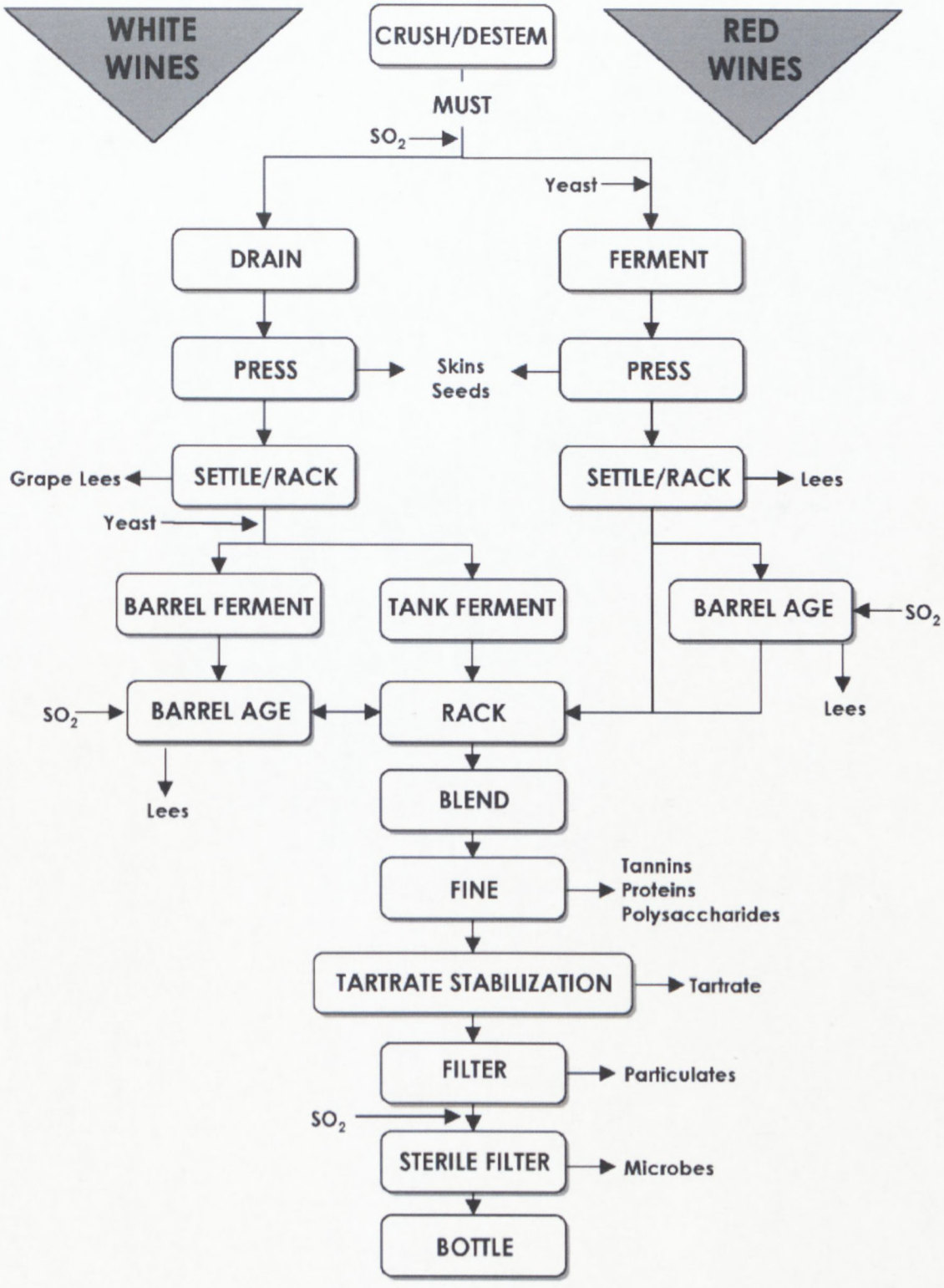

Figure 1 Schematic diagram on the production of white and red wine (adapted from Mills *et al.*, 2008).

for white wine (Fleet, 1998; Jackson, 1994). The grape juice released is highly susceptible to oxidative browning and microbial contamination (Dewey *et al.*, 2008; Salaha *et al.*, 2008). Microbial growth is especially enhanced by bruised/damaged grapes and harvesting in warm climatic conditions (Loureiro & Malfeito-Ferreira, 2003). Generally, winemakers endeavor to limit microbial growth early in wine production by intentional addition of sulphur dioxide (SO_2) at the moment of crushing (Bakker *et al.*, 1998; Gómez-Plaza *et al.*, 2002).

Red wine maceration follows crushing in view of extracting flavour compounds from the seeds, pulps, skins and also to favour the synthesis of essential compounds (Jackson, 1994; Gómez-Plaza *et al.*, 2002) that are required for its appealing appearance. The duration of maceration is controlled by parameters such as grape variety and the desired wine style (Gómez-Plaza *et al.*, 2002; Ribéreau-Gayon *et al.*, 2006). For instance, prolonged skin contact time for red wine is equivalent to a greater concentration of anthocyanins, ionized anthocyanin and polymeric compounds (Parish *et al.*, 2000; Gómez-Plaza *et al.*, 2002). Unlike red wine, maceration in white wine production is terminated quickly after its onset to avoid defects such as bad odours of spoiled grapes; astringent as well as bitter phenolic compounds are transmitted from skins, seeds and stems to the juice (Jackson, 1994; Ribéreau-Gayon *et al.*, 2006). In addition to the duration of maceration, the amount of phenolic compounds is also affected by temperature, addition of enzymes, SO_2 concentration and pressing (García-Ruiz *et al.*, 2008).

Often natural alcoholic fermentation (ALF) may start early, due to the presence of indigenous strains on the surfaces of grapes and poorly sanitized equipment (Loureiro & Malfeito-Ferreira, 2003). According to Jolly *et al.* (2006) variables, like the vineyard altitude, climatological conditions (temperature, rainfall, humidity), grape variety, viticultural practices, developmental stage of grapes, physical and health condition of grapes and winery waste disposal are all factors that influence the type of indigenous yeasts present and their population sizes on grapes (Jolly *et al.*, 2006). A successive growth pattern of indigenous yeast occurs during natural fermentation, which consists mainly of the genera *Kloeckera*, *Hanseniaspora* and *Candida* in the early stages of fermentation (Mateo *et al.*, 1991). When the ethanol concentration is

in the range of 3-4%, the latter is replaced by *Metschnikowia* and *Pichia* (Pretorius & Bauer, 2002), and lastly dominated by several alcohol-tolerant strains of *Saccharomyces cerevisiae* (Querol & Ramón, 1996), which then performs ALF (Divol & Lonvaud-Funel, 2005). Although wine produced via natural fermentation end up having a fuller and rounder palate structure (Pretorius & Bauer, 2002), it is often considered as a source of microbial spoilage (Jolly *et al.*, 2006). Therefore, in controlled ALF, the prevalence of these indigenous yeasts is traditionally suppressed by the addition of SO_2 (Henick-Kling *et al.*, 1998). Despite sulphiting, commercial yeast starter cultures establish themselves quickly and; consequently have an advantage above other indigenous yeasts and bacteria by the rapid production of alcohol (Mills *et al.*, 2002). Yeasts play a predominant role in the enrichment of wine aroma during inoculated ALF (Andorrà *et al.*, 2008). Naturally, *S. cerevisiae* can tolerate high doses of SO_2 and ethanol levels, thus it represents the most preferred commercial starter culture. The metabolic activity of selected *S. cerevisiae* strains generate higher alcohols, fatty acids, aldehydes and esters, which contribute towards the complexity of aroma and flavour compounds in wine (Malherbe *et al.*, 2003). Therefore, by knowing the characteristics of the yeast strain, it provides a platform of predicting the sensorial profile of the final product (Ribéreau-Gayon *et al.*, 2006).

Although blending, chemical neutralization and precipitation are all recognizable techniques of deacidifying high acid wines (Volschenk *et al.*, 2006) a biological technique, malolactic fermentation (MLF), can also be applied in especially red wines and some white wines. In addition to deacidification, it creates a more stable wine in terms of microbial activity (Hernández-Orte *et al.*, 2009). This secondary fermentation process entails the transformation of dicarboxylic L-malic acid (malate) to monocarboxylic L-lactic acid (lactate) and carbon dioxide (CO_2) (Versari *et al.*, 1999; Liu, 2002; Beneduce *et al.*, 2004). Some species of *Pediococcus*, *Lactobacillus* and *Oenococcus* is ideal for conducting MLF due to its ability to tolerate low pH values, high SO_2 and ethanol concentrations (Liu, 2002; Bauer & Dicks, 2004; Saayman & Viljoen-Bloom, 2006; García-Ruiz *et al.*, 2008). According to some authors, MLF is not so easily conducted in wines with high pH (pH > 3.5) levels that are more likely encountered in warmer viticultural regions (Jackson, 1994; Fleet,

1998; Christaki & Tzia, 2002; Bauer & Dicks, 2004; Ribéreau-Gayon, *et al.*, 2006). This is, however, conflicting with what is happening in the industry. For instance, MLF plays an integral part in the quality of the South African wines irrespective of their pH values that are commonly above 3.5. In comparison with high pH (pH > 3.5) wine, the low pH wine (pH < 3.5) is a more hostile matrix for microorganisms to grow, hence, the possibility of wine spoilage is rather limited (Volschenk *et al.*, 2006).

Subsequently to MLF, red wines can be aged for weeks to even years (Jackson, 1994). A huge contribution is made by phenolic compounds towards the colour, flavour, bitterness and astringency of wine (Silva *et al.*, 2005), and aging conditions is one of the key factors in the development of this compounds (Valentão *et al.*, 2007). At the same time, it allows the precipitation of suspended material together with the release of yeasty odours and excess carbon dioxide that have originated through fermentation (Jackson, 1994). Aging normally occurs in wooden barrels, nevertheless other material like stainless steel, epoxy-lined carbon steel, fiberglass and cement containers can also be used alternatively (Pérez-Prieto *et al.*, 2002; De Beer *et al.*, 2008). Oak barrels have the advantage of enabling spontaneous clarification and formation of complex aroma. Since barrel aging is costly and time consuming, micro-oxygenation is a more rapid substitute for the acceleration of colour stability and polymerization of phenolics (Grainger & Tattersall, 2005; Hernández-Orte *et al.*, 2009). This technique is based on the deliberate addition of microscopic oxygen (O_2) concentrations to wine over a period of time (Parish *et al.*, 2000; Kelly & Wollan, 2003), therefore wines might be susceptible to wine spoilage microorganisms like AAB and *Brettanomyces*.

For wine, racking means the removal of sediment (lees) produced during fermentation and storage from wine (Pérez-Serradilla & Luque de Castro, 2008). Oxygen is naturally introduced to wine via racking; hence the conditions for AAB growth are more favourable (Christaki & Tzia, 2002; Bartowsky & Henschke, 2008). Undoubtedly, the O_2 concentration should be meticulously regulated and controlled in conjunction with the free SO_2 concentration in order to avoid any sort of spoilage defects. Colour, flavour and physical stability are obtained by fining treatments such as bentonite (Main & Morris, 1991), synthetic polymers (Gómez-Plaza *et al.*, 2002)

and proteins (gelatin, albumen) (Threlfall *et al.*, 1999). Finally, filtration is performed with the intention of removing colloids (Anon, 1998) and the remaining yeast and bacteria cells from the wine (Ubeda & Briones, 1999; Gergely *et al.*, 2003).

B. Microorganisms associated with winemaking

Since microorganisms are naturally present on the grape surfaces, it contaminates the juice and ultimately wine upon the rupture of the berries, followed by the winery and its equipment that it comes into contact with (Heard & Fleet, 1986). External factors like the type of rainfall, temperature and grape variety influence the diversity of microflora on grapes (Querol & Ramón, 1996). For example, the pathogen *Botrytis cinerea* (Dewey *et al.*, 2008) can cause several types of rot, known as noble, grey and sour rot as a result of alternating wet and dry periods (Loureiro & Malfeito-Ferreira, 2003, Klaasen *et al.*, 2006). These defects generally result in the growth of a wide variety of microorganisms than what is normally encountered on healthy grapes (Coetzee *et al.*, 2008).

Wine-associated microorganisms are represented by yeasts, LAB and AAB which are involved in the complex biochemical process of converting grape must to wine (Goode, 2005; Andorrà *et al.*, 2008). Since *S. cerevisiae* performs ALF it can be regarded as the most important microorganism that metabolise grape sugar to CO_2, ethanol and other metabolites (Divol & Lonvaud-Funel, 2005). In addition to this, deacidification, flavour modification and microbial stability of grape wines are established by LAB during MLF (Du Plessis *et al.*, 2004). Unlike yeasts and LAB, AAB are primarily considered as spoilage microorganisms.

Lactic acid bacteria

This group of bacteria can be divided into two families and three genera (Fugelsang, 1997). The rod shaped Gram-positive *Lactobacillus* genus is a representative of the Lactobacillaceae family whereas the Streptococcaceae family is represented by the genera *Pediococcus*, *Leuconostoc* and *Oenococcus*. The former is Gram-positive and its isolates appear coccoid- or coccobacilloid in shape. The bacteria are classified according to the end products that result through their metabolism

(Ribéreau-Gayon *et al.*, 2006). Glucose is degraded to pyruvic acid throughout homofermentation via the Embden-Meyerhoff Parmas (EMP) pathway, with lactic acid as the primarily end-product produced. In comparison with homofermentation, more than one end-product - lactic acid, ethanol and CO_2 result from heterofermenters (Zoecklein *et al.*, 1995). The number of ATP molecules generated per glucose molecule also serves as a manner of distinction between the two forms of fermentations. *Lactobacillus* are both homofermentative and heterofermentative, *Oenococcus* are heterofermentative while the species of the genus *Pediococcus* are also homofermenters (Ribéreau-Gayon *et al.*, 2006).

Lactic acid bacteria have fastidious nutrient requirements and, therefore, metabolize a range of vitamins, minerals, nucleotides and amino acids in wine (Terrade & De Orduña, 2009). Among the other factors that influence the growth of LAB, the pH of a matrix plays a distinctive role on which species will be present on a given time (Ruiz *et al.*, 2010). High pH wines (pH > 3.5) stimulates the growth of *Lactobacillus* and *Pediococcus*, whilst the presence of *Oenococcus* are more indicative of low pH wines (pH < 3.5) (Mills *et al.*, 2008). Since these bacteria are well adapted to acid and ethanol environments (Mills *et al.*, 2008), they are likely encountered in all stages of wine production (García-Ruiz *et al.*, 2008; Moreno-Arribas & Polo, 2008). Species of lactic acid bacteria *Lactobacillus plantarum, Pediococcus damnosus, Pediococcus pentosaceus* and *Oenococcus oeni* were previously isolated during ALF (Ribéreau-Gayon *et al.*, 2006). From the aforementioned species, the more resistant *O. oeni* remain active on the completion of ALF (Du Plessis *et al.*, 2004). However, certain *Pediococcus* and *Lactobacillus* species have evolved higher tolerances towards the harsh conditions of wine and are, therefore, still capable of survival after MLF (Bauer & Dicks, 2004; Groenewald *et al.*, 2006; Andorrà *et al.*, 2008; García-Ruiz *et al.*, 2008).

As a consequence of MLF performed by LAB, favourable transformations in the sensorial, chemical and microbiological profile of wine occur (Beneduce *et al.*, 2004). This process entails the decarboxylation of bicarboxylic L-malic acid into monocarboxylic L-lactic acid. Despite this, microbial stabilization is still required once

MLF is completed in order to avoid the emergence of unwanted LAB growth, which can result into spoilage (Millet & Lonvaud-Funel, 2000).

Mousey and mannitol taints, bitterness, off-flavours and buttery flavours are all indicators of spoilage caused by LAB (Fugelsang, 1997; Fleet, 1998). The genus of *Pediococcus* are more likely to secrete exopolysaccharides (Delaherche *et al.*, 2004; Walling *et al.*, 2005; Martínez-Viedma *et al.*, 2008), that result in a more viscous, ropy and oily product, rendering the wine unpalatable (Renouf *et al.*, 2006). Furthermore, LAB species can also produce biogenic amines (Arena & De Nadra, 2001). Although biogenic amines are not necessarily perceived as a spoilage defect, it is, however, of toxicological interest on the health of susceptible consumers (Moreno & Azpilicueta, 2004; Moreno-Arribas & Polo, 2008; Rosi *et al.*, 2009).

Acetic acid bacteria

The Acetobacteriaceae family can be categorized into three genera namely *Acetobacter*, *Gluconobacter* and *Gluconacetobacter* (König & Berkelmann-Löhnertz, 2009). All three genera have previously been detected on grapes (König & Berkelmann-Löhnertz, 2009), in musts and wine (Ribéreau-Gayon *et al.*, 2006; Bartowsky & Henschke, 2008). Species of *Gluconobacter* are more dominant on the grapes as they prefer sugar as a carbon source, whilst *Acetobacter* and *Gluconacetobacter* are more common in alcohol-rich environments such as wine. Due to the alcohol sensitivity of *Gluconobacter,* it is hardly perceived as a spoilage microorganism in wine (Du Toit & Lambrechts, 2002). The aerobic, Gram-negative AAB are rod-like or ellipsoidal in shape with dimensions of 0.6-0.8 µm by 1-4 µm (Fugelsang, 1997). Some of them have the ability to move due to locomotive organs, which can either surround the cell or are located at the ends (Ribéreau-Gayon *et al.*, 2006).

Acetic acid bacteria are introduced to wine via either one of the following sources: wooden barrels, damaged grapes or grapes that are infected with *Botrytis cinerea* (Fleet, 1993). In the presence of small amounts of O_2, this group of bacteria has the ability to oxidise ethanol into acetic acid, which causes a significant increase in the volatile acidity of wine (Du Toit & Lambrechts, 2002; Bartowsky *et al.*, 2003).

Since this phenomenon has a deteriorating effect on the quality of wine, AAB are regarded as spoilage microoganisms of wine. The oxidation of glycerol to dihydroxy acetone had been linked to the metabolism of AAB (Fugelsang, 1997). It also has the ability to form extracellular cellulose that result into a ropy wine (Fleet, 1998).

The occurrence of AAB are in correlation with the availability of oxygen, therefore, their growth are more common during barrel aging, where approximately 30 $mg.l^{-1}$ of oxygen permeates through wood into wine per year. In addition, O_2 is also naturally incorporated in wine via pumping; transferring and racking that can stimulate AAB and cause a drastic increase in their cell number (Du Toit *et al.*, 2006b; Mills *et al.*, 2008). After the introduction of 7.5 $mg.l^{-1}$ of O_2, AAB have increased by 30-40 fold in numbers. Du Toit & Pretorius (2002) have reported that the population of *A. aceti* and *A. pasteurianus* have increased within a few days from 10^4 to 10^8 $cells.ml^{-1}$ in fully aerated (100%) wine. Even though both species still persisted in wine saturated with 70% dissolved oxygen, their final population sizes were slightly reduced to 10^6-10^7 $cells.ml^{-1}$.

Micro-oxygenation is a relatively new method and, therefore, the affect of this technique on the growth of AAB is still unclear. However, it was demonstrated in a study of Du Toit *et al.* (2006a) that the counts of AAB have increased significantly in micro-oxygenation treated wine. Futhermore, Bartowsky and co-workers have indicated in their study that the bottle position of wine is also essential in the prevention of AAB spoilage. This was discovered after different levels of spoilage caused by *A. pasteurianus* was detected in bottled Shiraz wine that was stored in a vertical position (Bartowsky *et al.*, 2003). A growth ring is normally visible in vertical positioned bottles at the junction between the surface of the wine and headspace that is created by bad bottle closures (Bartowsky & Henschke, 2008), which induce the entrance of oxygen (Bartowsky *et al.*, 2003). Nevertheless, AAB can also survive under anaerobic to semi-anaerobic conditions from the partial ethanol oxidation to acetaldehyde (Fugelsang, 1997; Bartowsky *et al.*, 2003). Under these circumstances of limited O_2, AAB regress into a viable but non-culturable state (VBNC), and only recovers from this state as soon as O_2 becomes available (Millet & Lonvaud-Funel, 2000).

Yeasts

Wine yeasts can be categorized into two groups, i.e. the *Saccharomyces* group and non-*Saccharomyces* group of which include *Debaryomyces*, *Brettanomyces*, *Hanseniaspora*, *Citeromyces*, *Issatchenkia*, *Kluyveromyces* and many more (Jolly *et al.*, 2006). The *Saccharomyces* group is mainly represented by *S. cerevisiae* (Henick-Kling *et al.*, 1998) that is acknowledged as the most important yeast in wine production due to its ability of converting grape sugar to ethanol and CO_2 (König & Berkelmann-Löhnertz, 2009).

The following genera are more likely to be isolated from juice in the early stages of wine production: *Hanseniaspora* (anamorph *Kloeckera*), *Pichia*, *Metschikowia*, *Candida*, *Kluyveromyces*, *Rhodotorula*, *Cryptococcus*, *Debaryomyces* and *Trichosporan* (Loureiro & Malfeito-Ferreira, 2003). The persistence of these microorganisms in wine is dependant on their O_2 requirements and sensitivities towards temperature, ethanol, carbon dioxide and SO_2 (Mills *et al.*, 2002). *Saccharomyces* strains become apparent once the juice is released from grapes (Fernández-González *et al.*, 2001). As mentioned before, non-*Saccharomyces* yeasts (*Hanseniaspora*, *Metschnikowia*, *Candida*, *Pichia*, and *Kluyveromyces*) dominate the early stages of spontaneous fermentation, followed by different strains of *S. cerevisiae* (Fernández-González *et al.*, 2001). Although other yeasts are also of importance in wine production, only *Saccharomyces* and *Brettanomyces* will be discussed.

Saccharomyces spp.

Indigenous *Saccharomyces* species are scarce on healthy grapes and are present in much higher numbers in fermenting must and on winery equipment where sugar are freely available (Fugelsang, 1997; Mills *et al.*, 2008). The morphological characteristics of the genus are spherical to ellipsoidal in shape with approximate dimensions of 8 x 7 µm (Fugelsang, 1997). The primarily role of commercial selected *S. cerevisiae* strains is to perform ALF in wine, a process where grape sugar is converted into ethanol, CO_2 and other additional products (Cappello *et al.*, 2004). Metabolites such as higher alcohols, fatty acids and esters carbonyls and thiols are

also produced from grape juice components through the metabolism of *S. cerevisiae* (Mauriello *et al.*, 2009). These compounds contribute towards the wine flavour of the final product. Compared to other yeasts, *S. cerevisiae* is more resistant to high levels of alcohol and SO_2 (Divol & Lonvaud-Funel, 2005) and is, therefore, the most appropriate and preferred yeast to conduct ALF (Mateo *et al.*, 1991; Andorrá *et al.*, 2008). Even though *S. cerevisiae* is essential for ALF in wine production, it is only considered as a spoilage microorganism when it re-ferments finished or bottled wines (Divol & Lonvaud-Funel, 2005; Martorell *et al.*, 2005; Nisiotou & Gibson, 2005).

Brettanomyces spp.

The genus *Brettanomyces* and its teleomorph *Dekkera* was first encountered in the beer industry (Delaherche *et al.*, 2004). These spoilage species can tolerate harsh conditions such as lack of nutrients and high ethanol concentrations in a matrix (Renouf *et al.*, 2008). Even though *Brettanomyces* are virtually present in all the stages of wine production, it only dominates the late stages of wine production (Fugelsang, 1997; Suárez *et al.*, 2007; Renouf *et al.*, 2008). Once introduced to the winery, the microorganisms accumulate in pumps, drains, isolated pockets of juice and wine, wooden barrels, transfer lines and valves (Fugelsang, 1997; Nisiotou & Gibson, 2005). Normally, it accumulates away in hard to clean places like wood, rough surfaces of equipment and cracks in the floor. These environments serve as a shield for the yeasts against disinfection techniques. Thus, in most cases *Brettanomyces* is generally introduced to wine via contamination (Fugelsang, 1997; Delaherche *et al.*, 2004; Suárez *et al.*, 2007).

Wooden barrels are regarded as the most classical habitat of microorganisms among all the other winery equipment (Ciani & Ferraro, 1997). According to Suárez *et al.* (2007), *Brettanomyces* have been previously discovered 8 mm down within the wood of barrel staves. It is believed that the porous nature of wood provides a shelter for microorganisms against sterilization techniques, thus it creates an environment in which microorganisms can survive (Rayne & Eggers, 2008). As microorganisms develop in old barrels, they are becoming more likely to contaminate wine (Oelofse *et al.*, 2008). New barrels on the contrary do not contain any wine associated yeasts

and bacteria, but they can be introduced to the barrel once the barrel is filled with contaminated wine (Bartowsky & Henschke, 2008). Approximately 30 $mg.l^{-1}$ of O_2 permeates through oak staves per year during barrel aging (Bartowsky *et al.*, 2003), which can stimulate the growth of aerobic spoilage microorganisms particularly *Brettanomyces* (Du Toit *et al.*, 2006b). Compared to old barrels, new barrels permit a higher influx of O_2 (Suárez *et al.*, 2007) and, therefore, it is assumed to favour the development of these aerobic microorganisms even more. Apart from wooden barrels, *Brettanomyces* have also been isolated using selective media from wine stored in concrete or steel vats (Agnolucci *et al.*, 2009).

The production of volatile phenols such as 4-vinylphenol, 4-vinylguaiacol, 4-ethylphenol, 4-ethylguaiacol, vinyl catechol and ethyl catechol is characteristic of *Brettanomyces* growth in wine (Pollnitz *et al.*, 2000; Dias *et al.*, 2003; Romano *et al.*, 2009). The origin of these molecules is based upon the enzyme-linked transformation of hydroxycinnamic acid (ferulic, p-coumaric or caffeic acid) into hydroxystyrenes (vinylphenols) (Rodrigues *et al.*, 2001; Pérez-Prieto *et al.*, 2002; Couto *et al.*, 2005; Suárez *et al.*, 2007). The first reaction is catalysed by hydroxycinnamate decarboxylase, followed by the reduction of hydroxysterenes to ethyl derivatives by vinylphenol reductase. The presence of volatile phenols (4-ethylphenol and 4-ethylguaiacol) in wine is reminiscent of "horse sweat", "wet wool", "medicinal", "smoky", "spicy", "leather" and "animal" (Delaherche *et al.*, 2004; Couto *et al.*, 2005; Hayashi *et al.*, 2007; Renouf & Lonvaud-Funel, 2007).

Species of *Brettanomyces* are well acclimatized to survive unfavourable conditions of wine more, especially red wine, where it cause detrimental affects on the visual and organoleptic properties of the wine (Oelofse *et al.*, 2009). This includes the production of volatile acidity, volatile phenols, off-flavours (tetrahydropyridines) and loss of colour together with the fruity sensorial qualities of the wine (Ciani & Ferraro, 1997; Delaherche *et al.*, 2004; Renouf & Lonvaud-Funel, 2007; Suárez *et al.*, 2007; Renouf *et al.*, 2008, Oelofse *et al.*, 2009). Apart from all this, some species have been linked to film formation which can significantly contribute towards an increase in the cloudiness in wine (Hayashi *et al.*, 2007).

There is some controversy about the effect of the volatile phenols in wine, a certain part of the consumers appreciate the aromatic notes of spices, leather or smoke in red wine while others believe that it deteriorate the sensory profile of wine (Loureiro & Malfeito-Ferreira, 2003). Concentrations of volatile phenols below the established limit of 620 µg.l-1 are still acceptable, whilst higher levels are seen as spoilage (Loureiro & Malfeito-Ferreira, 2003). However, the sensory threshold of volatiles is not definite as the degree of olfactory detection levels vary between human individuals. The intensity of the compounds is also influenced by the style and structure of wine that can either enhance or reduce the aromatic notes in wine (Couto *et al.*, 2005; Rayne & Eggers, 2008).

C. Preservation techniques

Wine preservation should be considered with a holistic approach, as microorganisms are present everywhere on winery surfaces, walls, floors and equipment which can contaminate wine. Hygienic and sanitation regimes are normally implemented as early as from the vineyard to the winery to control the growth of undesirable microorganisms. Yet, wine can still become unpalatable due to negligence or a lack of control over the growth of microorganisms. Since it is unethical to sell spoiled wine, defective wines are rejected by the board of spirits and as a consequence the winery stares great economical losses in the eye. For this purpose, numerous microbiological preservation techniques have surfaced over the years to control microorganisms in wine (Jackson, 1994; Fugelsang, 1997; Delfini & Formica, 2001; Goode, 2005; Fugelsang & Edwards, 2007). Some techniques are more preferred than others in view of their mode of action, efficacy and practical limitations. This section is classified into three categories: (1) chemical preservation, (2) biological preservation and (3) physical preservation.

Chemical Preservation

Sulphur dioxide

Traditionally, microbiological stability of grape juice and wine are obtained by the antimicrobial property of SO_2 (Bakker *et al.*, 1998; Renouf *et al.*, 2008). For this

reason, extensive research regarding the use of SO_2 as a chemical preservative in oenology currently exists. Numerous studies have indicated that only the molecular form of SO_2 possess antimicrobial activity towards microorganisms (Barbe *et al.*, 2000; Frivik & Ebeler, 2003). Molecular SO_2 penetrates the cells via active transport or simple diffusion after which, it is modified to HSO_3^- due to the intracellular pH of the cell (Stratford *et al.*, 1987; Romano & Suzzi, 1993; Bauer & Dicks, 2004; Ribéreau-Gayon *et al.*, 2006). The duration of absorption into the cell is directly proportional to the metabolic activity of the microorganisms at that given moment, for instance AAB is much more susceptible to SO_2 at its optimum growth temperature of 30°C (Du Toit & Lambrechts, 2002). Once the sulphite reaches the inside of the cell, it interacts with vitamins, coenzymes and cofactors, thereby inhibiting the proliferation of microorganisms (Bauer & Dicks, 2004; Ribéreau-Gayon *et al.*, 2006).

It is, however, unclear to which concentration is the most reliable to control microbial growth as inconsistent results in terms of microbial inactivation was previously reported. For instance, the viability of *A. pasteurianus* was reduced and inhibited at a molecular SO_2 concentration of 0.25 $mg.l^{-1}$ (Du Toit *et al.*, 2005) while a concentration of 60 $mg.l^{-1}$ had hardly any affect on AAB (Andorrá *et al.*, 2008). It is clear that the effectiveness of the undissociated molecular property of SO_2 are in relation with the composition of the matrix, such as the pH, concentration of ethanol, phenolic compounds and O_2 (Goode, 2005; García-Ruiz *et al.*, 2008; Oelofse *et al.*, 2008; Renouf *et al.*, 2008). Higher molecular SO_2 concentrations are encountered in low pH wines in comparison with high pH wines (Barbe *et al.*, 2000). A concentration of 30 $mg.l^{-1}$ free SO_2 is roughly equivalent to 0.4 $mg.l^{-1}$ molecular SO_2 at pH 3.4 and 0.8 $mg.l^{-1}$ at pH 3.7 (Oelofse *et al.*, 2008).

Compounds such as acetaldehyde (ethanal), sugars, ketonic acids and dicarbonyl groups inherent in wine (Stratford *et al.*, 1987; Romano & Suzzi, 1993; Barbe *et al.*, 2000), have a high affinity for SO_2 and forms complexes of bound SO_2 that has absolutely no antimicrobial activity (Bauer & Dicks, 2004). *Brettanomyces* were hardly affected by this property of SO_2 (Du Toit *et al.*, 2006b). Even though the above-mentioned compounds are present in healthy wine, excessive amounts are found in grape juice that was infected by *B. cinerea* and AAB (Du Toit *et al.*, 2005).

Hypothetically, acetaldehyde present in the range of 30 and 130 $mg.l^{-1}$ is almost equivalent to 44 and 190 $mg.l^{-1}$ of bound SO_2 in wine (Ribéreau-Gayon *et al.*, 2006). Sometimes microorganisms can also enter a viable but non-culturable state which is caused by the pressure of free SO_2; the regression of microorganisms from this state dependant on the alcohol concentration and osmotic pressure of the matrix (Divol & Lonvaud-Funel, 2005). In all these situations, the use of SO_2 concentrations should be cautiously adjusted to ensure a high quality end-product with microbial stability, in order to avoid any types of wine spoilage.

Since possible health risks and organoleptic changes had been associated with sulphites (Ruiz-Capillas & Jiménez-Colmenero, 2009; Du Toit & Pretorius, 2000), the use of SO_2 is currently being reviewed (Salaha *et al.*, 2008). As a concern, the Food and Drug Administration (FDA) have set the acceptable daily intake (ADI) of SO_2 as 0.70 $mg.kg^{-1}$ body weight (Ruiz-Capillas & Jiménez-Colmenero, 2009). Furthermore, the legislation of European Union and United States requests that the sulphite concentration of the final product should be labelled when it exceeds 10 $mg.l^{-1}$ (García-Ruiz *et al.*, 2008). Without a valid alternative method to replace SO_2, the risk of wine spoilage will only increase. Therefore, as an incentive, the wine industry is faced with the challenge of finding an alternative technology to control microbial growth in grape juice and wine, that will comply to the world wide trend to produce wines with reduced SO_2 levels.

Dimethyl dicarbonate

Dimethyl dicarbonate (DMDC), commercially known as Velcorin®, does also display inhibitory activity towards microorganisms (García-Ruiz *et al.*, 2008). Inhibition follows when DMDC targets glycolytic enzymes notably alcohol-dehydrogenase and glyceraldehydes-3-phosphate by methoxycarbonylation of the nucleophilc residues (Costa *et al.*, 2008). As differential sensitivity towards DMDC is found among microbiota, different concentrations are required to inactivate microorganisms (Renouf *et al.*, 2008). In a temperature range of 20 - 40°C, a 100–fold increase in effectiveness against *S. cerevisiae* and *L. plantarum* are obtained (Basaran-Akgul *et al.*, 2009). In the study of Renouf *et al.* (2008) a concentration of 600 $mg.l^{-1}$ was

sufficient in the inactivation of all species *Pichia anomala*, *Rhodotorula mucilaginosa*, *S. cerevisiae*, *O. oeni* and *Brettanomyces* tested. Although the authorized level of 200 $mg.l^{-1}$ DMDC (Enrique *et al.*, 2008) is sufficient to control contaminative yeast, the activity of LAB and AAB are hardly affected (Costa *et al.*, 2008). DMDC should be added more than once to protect aged and bottled wine, since DMDC corrode with time which renders it inhibitory affect futile.

Fining agents

Substances like bentonite, activated carbon, gelatine, and potassium caseinate are categorized as fining agents for wine (Jackson, 1994; Zoecklein *et al.*, 1995). Fining has been well documented as a technique to limit microbial instabilities in wine. According to Oelofse *et al.* (2008), fining proteins can decrease *Brettanomyces* populations by 40 to 2 000-fold. For example, liquid gelatine used at a dosage of 0.6 $ml.l^{-1}$ has decrease the initial population of *Brettanomyces* in red wine from 10^4 $cfu.ml^{-1}$ to 170 $cfu.ml^{-1}$ (Suárez *et al.*, 2007). Even though fining agents has inhibitory effects on microorganisms, it influences the sensorial property of wine negatively by reducing the volatile and aromatic content (López *et al.*, 2001; Armada & Falqué, 2007). Due to the deteriorating effect of fining agents on the sensorial property of wine, the wine industry does not consider fining as a feasible technique to microbiologically stabilize grape and wine.

Weak acids

Weak acids, such as fumaric, benzoic and sorbic acids have been recognized for years as antimicrobial agents in wine (Techakriengkrai & Surakarnkul, 2007; Han *et al.*, 2008). Although there are some uncertainties about their mode of action, it is believed that the weak acids are in their undissociated form at low pH values which allow them to permeate through the cell membrane (Papadimitriou *et al.*, 2007). Once it reaches the inside of the cell, the high pH of the cell induces dissociation where the protons and anions are automatically released, rendering the cytoplasm of the cell acidic. Sorbic acid, especially the undissociated form of sorbic acid induces inhibition by targeting its cell membrane function (Du Toit & Pretorius, 2000). Its

efficacy in the matrix is directly linked to control parameters such as pH, ethanol levels, SO_2 concentrations and initial counts of spoilage yeasts. The metabolic activity of the yeast is also of importance in the prediction of spoilage occurrence (Quintas *et al.*, 2005). Even though weak acids are more effective in low pH matrixes, some yeasts have revolutionized tolerance mechanisms to counteract its mode of action (Loureiro, 2000; Papadimitriou *et al.*, 2007; Suárez *et al.*, 2007). It also seems as if sorbic acid has differential activity towards yeasts, after a 156 and 168 $mg.l^{-1}$ were sufficient to cause inhibition of *Kloeckera apiculata* and *Pichia anomala, respectively,* while a concentration ≥672 $mg.l^{-1}$ are recommended for both *Schizosaccharomyces pombe* and *Zygosaccharomyces* (Fugelsang & Edwards, 2007).

Biological preservation

Natural products

A vanillin concentration in the range of 30 - 100 $mg.l^{-1}$ is also bioactive against spoilage yeasts but the specified concentration range can alter the aroma of wine, hence it may affect the acceptability of the final product (Renouf *et al.*, 2008). However, the oxidative form of vanillin known as vanillic acid is more applicable in wine production (Mourtzinos *et al.*, 2009). It is also suggested that natural occurring polyphenols such as gallic acid, ferulic acid and (+)-catechin in must and wine can be used in oenology to control the growth of LAB (García-Ruiz *et al.*, 2008).

Zymocins

The killer toxins produced by yeasts also dictate the extent of microbial growth in wine (Jolly *et al.*, 2006). The proteinaceous killer toxin produced by *Kluyveromyces phaffii* DBVPG 6076 has displayed inhibitory affects against *Hanseniaspora uvarum* (Ciani & Fatichenti, 2001). Its optimum fungicidal activity was encountered in a pH range of 3 to 5 and temperatures below 40°C of which corresponds to wine production conditions. Thus, this toxin can be regarded as a suitable biopreservative agent in wine production. Five years ago two toxins produced by *Pichia anomala* and *Kluyveromyces wickerhamii* have displayed inhibitory affects against *Brettanomyces*

in Sangiovese wine for a period of ten days (Comitini *et al.*, 2004), which have indicated that it can be of use during wine aging and storage.

Bacteriolytic enzymes

A non-toxic preservative in the food industry, lysozyme (1,4-ß-*N*-acetylmuramidase), renders specifically Gram-positive bacteria inactive by cleaving the ß-1,4 glycosidic bonds in the peptidoglycan layer of microbial cells (Du Toit & Pretorius, 2000). It is known that the cell wall of Gram-positive bacteria consists of a thick peptidoglycan layer while the thin peptidoglycan layer of Gram-negative bacteria is surrounded with an outer membrane (Prescott *et al.*, 2005). Since the outer membrane provide a protective layer for Gram-negative bacteria, the antimicrobial properties of lysozyme is mainly limited to Gram-positive bacteria such as LAB. Lysozyme is therefore ideal for inhibiting MLF which are conducted by Gram-positive LAB. According to Delfini & Formica (2001), lysozyme has variable activity within bacterial strains after it was noticed that *Leuconostoc oenos* L230 was resistant to 400 $mg.l^{-1}$ of lysozyme while strain L239 was sensitive to only 50 $mg.l^{-1}$ of lysozyme. It seems also as if the activity of lysozyme are influenced by polyphenolic content, since its more effective in white than red wines (Fugelsang & Edwards, 2007). Little application of lysozyme has been made thus far in wine production, due to high cost (Bauer & Dicks, 2004).

Peptides

The antimicrobial activities of synthetic peptides on wine spoilage microorganisms have been investigated *in vitro* for three consecutive years by a group of Spanish researchers. Firstly, the antimicrobial action of synthetic peptides towards wine spoilage yeasts such as *Cryptococcus albidus*, *Dekkera bruxellensis*, *Pichia membranifaciens*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bisporus* and *Zygosaccharomyces bailii* were investigated (Enrique *et al.*, 2007). It was found that each peptide had distinct fungicidal activities towards specific microorganisms. The degree of microbial inactivation in laboratory growth medium was also not in relation to those found in the wine matrix, which indicates that, the efficacy of the peptides is dependant on the composition of the food matrix. For example, *Z. bailii*, *Z. bisporus*

and *S. cerevisiae* were completely inactivated in GPY medium when exposed to concentration of 25 - 100 µM PAF 26L and $LfcinB_{17-31}$ while no affect was found in wine at the same concentration range (Enrique *et al.*, 2007). This is attributed to wine salts that interfere with the electrostatic interaction of the cationic antimicrobial peptide and negatively charged surfaces of the microorganisms (Enrique *et al.*, 2008). The fungicidal activity of peptides towards *Z. bailii* was significantly enhanced by the removal of these salts. In a most recent study, the selectivity of the synthetic peptide $LfcinB_{17-31}$ was demonstrated on fermenting must (Enrique *et al.*, 2009). The peptide was only active towards *O. oeni*, *Lactobacillus brevis* and *Pediococcus damnosus* whereas the growth kinetics and ALF of *S. cerevisiae* T73 was hardly affected. The synthesis of $LFcinB_{17-31}$ might be a limiting factor to its application in wine production; nevertheless lactoferrin hydrolysates can be used as alternatives (Enrique *et al.*, 2009).

Bacteriocins

A diverse group of LAB species generate bacteriocins that inhibit the growth of other microorganisms (Knoll *et al.*, 2008). The polypeptide bacteriocin, nisin (pM 5000; 34 amino acids) mainly targets Gram-positive bacteria by permeating the cytoplasmic membrane through transient pore formation, thereby ensuring cell death (Konings *et al.*, 2000; Solomakos *et al.*, 2008). A MIC_{50} value for *O. oeni* and other LAB was respectively, 0.024 $\mu g.ml^{-1}$ and 12.5 $\mu g.ml^{-1}$, after the addition of nisin (Rojo-Bezares *et al.*, 2007). Nisin is already commercially available for the inhibition of spoilage bacteria, even though its use in oenology has not yet been approved by law (García-Ruiz *et al.*, 2008). In a study of De Nadra *et al.* (1998), LAB isolated from wine was sensitive to pediocin N5p produced by *Pediococcus pentosaceus*. Since the absorptivity and stability of the compound was not affected by either ethanol (5 - 14%) or SO_2 (20 - 200 $mg.l^{-1}$) concentrations correlating to vinification conditions, it seems to be a relevant additive in the industry.

Development of yeast strains with antimicrobial activity

The price of purified peptides and bacteriocins are very costly (Oelofse *et al.*, 2008), nevertheless this obstacle can be circumvented by incorporating these antimicrobial activities into target microorganisms through genetic modification (Pretorius & Bauer, 2002). A study pertaining to the construction of a bactericidal strain of *S. cerevisiae* that produces pediocin PA-1 have been investigated by Schoeman *et al.* (1999), who have discovered that bactericidal yeast strains can be developed to inhibit the growth of spoilage bacteria. In 2003, Malherbe *et al.* have expressed a gene of *Aspergillus niger* that encodes glucose oxidase in *S. cerevisiae*. The inactivation of LAB and AAB was mainly attributed to the final product of glucose oxidase enzymatic reaction, hydrogen peroxide (H_2O_2). The efficacy of this technique is based on the sensitivity of nucleic acids and proteins to H_2O_2. (Malherbe *et al.*, 2003). Additionally, a broad spectrum of microorganisms can also be targeted by expressing both K1 and K2 killer toxins in wine yeasts (Querol & Ramón, 1996).

Physical preservation

Filtration

As more cells are encountered in unfiltered wine (Nisiotou & Gibson, 2005), filtration are performed prior to bottling to sterilize wine (Gergely *et al.*, 2003). Yet, yeast strains can still persist in bottled wine and conduct spoilage as a result of inaccurate filtration (Loureiro & Querol, 1999). Sometimes after a prolonged storage in wine, microorganisms exist in a viable but nonculturable state (VBNC) (Divol & Lonvaud-Funel, 2005) and tend to pass through the filter membrane (Millet & Lonvaud-Funel, 2000). But as soon as they recover their normal metabolic activity, they are retained by the membrane. Species of *Brettanomyces*, can pass through 0.45 µm pore size membrane (Suárez *et al.*, 2007), even though its average size is (5-8) x (3-4) µm (Oelofse *et al.*, 2008). However, smaller pore size membranes and fining agents can deteriorate the colloidal structure of wine, and thereby diminish its color intensity. Some winemakers are sceptical about filtration since they tend to believe that it strips wine from its fruity character, reduces body and viscosity and influences the mouth feel of wine negatively (Couto *et al.*, 2005).

Additional approaches

Applied pressure of 400 - 500 MPa for 5 - 15 min at 5 - 20°C has achieved huge reductions in the cell population of AAB and LAB (Suárez *et al.*, 2007). This process has the advantage of not modifying the physicochemical properties, enzymatic activity as well the sensorial properties of wine. Even though the efficacy of heat treatment is dependant on the ethanol content and degree of phenolic compounds (Bartowsky & Henschke, 2008), thermal inactivation at 35°C has successfully inactivated *Dekkera/Brettanomyces* (Couto *et al.*, 2005). The growth of aerobic microorganisms during barrel-aging can also be inhibited via the removal of air by using nitrogen (N_2), regularly wetting the bung and maintaining completely full barrels (Christaki & Tzia, 2002).

Pulsed electrical fields

Pulsed electrical fields (PEF) technology comprise one of the most recent emerging technologies in the food industry that has the ability of controlling microorganisms in wine production, thereby preventing wine of becoming spoiled (Puértolas *et al.*, 2009). After intensive research, it was established that an optimum treatment of 186 $kJ.kg^{-1}$ at 29 $KV.cm^{-1}$ was sufficient to limit growth in must and wine. Irrespective of the applied treatment, LAB (*Lactobacillus hilgardii* and *L. plantarum*) were generally more resistant than *B. anomala* and *B. bruxellensis* (Puértolas *et al.*, 2009). This is mainly attributed to the much larger size of yeasts as lower field strengths is required to electroporate their cell membranes. Recently, a study was performed on ways to optimize the inactivation efficacy of PEF on a mixture of spoilage microorganisms in grape juice (Marsellés-Fontanet *et al.*, 2009). The predictions by response surface methodology (RSM) models did successfully comply with the results of factorially designed experiments, meaning that RSM could be used to optimize efficacy of PEF in fruit juices.

Ultraviolet (UV-C) radiation

UV-C radiation is acknowledged as an efficient inactivation method for a wide range of bacteria, viruses and parasites (Carrasco & Turner, 2006), in liquid food products

such as water, milk and fruit juices (Keyser *et al.*, 2008). UV is located in the electromagnetic spectrum, with wavelengths ranging from 100 to 400 nm (Shama, 2007). This range is formally categorized in three sections: UV-A (320 – 400 nm), UV-B (290 – 320 nm) and UV-C (200 – 290 nm) (Madronich *et al.*, 1998). The germicidal properties of UV light are correlated to the short energetic UV wavelengths of UV-C (254 nm) (Bintsis *et al.*, 2000; Thompson, 2003; Dumètre *et al.*, 2008).

Upon UV treatment, germicidal UV light is absorbed through the outer membrane of microorganisms, followed by the dimerization of thymine and occasionally cytosine, thus normal transcription and replication of DNA are hindered (Sizer & Balasubramaniam, 1999; Thompson, 2003; Unluturk *et al.*, 2008). Consequently, microorganisms lack the ability to reproduce and die off eventually (Gabriel & Nakano, 2009). UV-C inactivation can be identified by the presence of photoproducts such as cyclobutyl-type dimers (pyrimidine dimers), pyrimidine adducts and DNA-protein cross-links (Bintsis *et al.*, 2000). As the thickness of cell walls and ability of microorganisms to repair DNA differ, the resistance to UV radiation will also vary significantly (Tran & Farid, 2004; Shama, 2007). The UV resistance pattern of microorganisms is as follow: Gram-negatives < Gram-positives < yeast < bacterial spores < molds < viruses (López-Malo & Palou, 2005). Yeasts and molds contain less thymine in their DNA in comparison with bacteria; therefore it is has an advantage of being more resistant to UV radiation (Tran & Farid, 2004). But yeast (5-8 x 3-7 µm) cells is a lot bigger than bacteria (0.5-1.2 x 0.7-8 µm) in size (Holt, 2000; Hammes & Vogel, 1995; Simpson & Taguchi, 1995; Fugelsang, 1997; Boulton *et al.*, 1999; Millet & Lonvaud-Funel, 2000), thus it is assumed that the UV rays will easily penetrate the greater surface area of the yeasts, rendering them more sensitive. UV-induced lesions can be repaired by repair mechanisms that have evolved in some microorganisms (Goosen & Moolenaar, 2008). This process arise by either two major pathways i) photolyase in the presence of light (photoreactivation) and ii) dark repair (nucleotide excision repair (NER), postreplication recombinational and error-prone repair) (Trombert *et al.*, 2007).

To date, intensive research on the efficacy of UV light for the reduction of microorganisms in milk, water and juice have been performed with either continuous

flow reactors or bench top collimated beam apparatus (Sommer *et al.*, 1996; Unluturk *et al.*, 2008). For example, applied UV dose of ≥ 6500 μ W s.cm^{-2} have obtained an approximate reduction of 6 $\log_{10}$ *Esherichia coli* in apple cider (Basaran *et al.*, 2004). Hanes *et al.* (2002) have reported that experimentally contaminated apple cider exposed to 14.32 mJ of UV irradiation.cm^{-2} for ≤1.9 seconds, achieved a 5 $\log_{10}$ reduction of viable *Cryptosporidium parvum* oocysts. Results have indicated that the inactivation of *E. coli* strain (ATCC 8739), *E. coli* O157:H7 and *Salmonella typhimurium* depends on the depth of the liquid food medium, UV light intensity and exposure time in a collimated beam apparatus (Ngadi *et al.*, 2003; Unluturk *et al.*, 2008). In a continuous flow reactor, a reduction of 5 $\log_{10}$ *Listeria monocytogenes* in milk were achieved with a UV dose of 15.8 ± 1.6 mJ.cm^{-2} (Matak *et al.*, 2005). Nevertheless, the application of UV-C radiation on the microbial stabilization of wine is not well-documented.

Initially, UV-C radiation was mainly used to sterilize surfaces (Koutchma *et al.*, 2009). Its application in liquids was previously limited due to the poor transmittance of UV-C light in liquids (Keyser *et al.*, 2008). According to Sizer & Balasubramaniam (1999), the penetration of UV light in juice is only 1 mm. A number of parameters such as the size of the microbial population and intrinsic parameters such as UV-C absorptivity, soluble solids and suspended matter of the flow medium effect the efficacy of UV-C light (Thompson, 2003; Basaran *et al.*, 2004; Keyser *et al.*, 2008). It is also found that colour and turbidity of the liquid play an imperative role in the inactivation efficacy of UV-C radiation (Koutchma *et al.*, 2004; López-Malo & Palou, 2005). Due to absorption, UV-C transmission is reduced in products that are darker in colour. At 253.7 nm, the absorption coefficients of white and red wine are 10 and 30 cm^{-1}, respectively (López-Malo & Palou, 2005). Better microbial inactivation can therefore be expected in light coloured white wines compare to red wines. Nevertheless, the lack of UV-C penetration in liquids can be optimized by modifying critical control parameters such as flow pattern of the medium in the UV reactor, reactor design and UV light distribution (Koutchma *et al.*, 2004).

UV-C radiation is a safe and effective method in reducing microorganisms to acceptable levels (Thompson, 2003), and in addition to that, it is considered as one of

the simplest and environmental friendliest techniques (Bintsis *et al.*, 2000). It is postulated that this technology will be effective in ensuring microbiological safe wine without affecting its chemical and sensorial properties, since there was no reports on the modification of organoleptic (colour, flavour, odour or pH) properties of juice (Basaran *et al.*, 2004). In response to the demands of consumers and legislation to produce wines with reduced SO_2 levels, UV-C radiation can be applied as an alternative method, to produce a microbiological safe and healthy product with no or low amounts of chemical preservatives. In light of these facts, UV-C radiation can be regarded as an applicable technology in controlling spoilage microorganisms in grape juice and wine.

D. Conclusion

Sulphur dioxide is generally used as an antimicrobial agent in grape juice and wine to ensure microbial stability. However, there are mounting concerns about sulphite usage, as it has been associated with possible health risks. Due to these concerns the use of SO_2 in the production of wine is currently being reviewed. Other techniques, such as filtration and fining are also efficient in controlling microbial growth, but unfortunately these techniques have detrimental affects on the sensorial properties of the wine.

Not only is a feasible preservation strategy in demand to microbiologically stabilize wine, but also one that will not deteriorate the other qualities of the wine. Meanwhile, is it senseless to discontinue the use of SO_2 without a valid technique to control the growth of microorganisms. As this will only increase the risk of wine spoilage. Hence, the wine industry is seeking alternative strategies to either substitute or enhance the affect of lower SO_2 levels without modifying the chemical and sensorial properties of the wine.

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CHAPTER 3

EFFICACY OF ULTRAVIOLET RADIATION AS AN ALTERNATIVE TECHNOLOGY TO INACTIVATE MICROORGANISMS IN GRAPE JUICES AND WINES

Abstract

Since the use of SO_2 is under review, the wine industry is seeking a feasible technique to fulfill the role of the chemical preservatives used in grape juice and wine. The aim of this study is, therefore to investigate the efficacy of ultraviolet radiation (UV)-C (254 nm) as an alternative technology to inactivate microorganisms in white and red grape juices and wines.

Lactic acid bacteria, acetic acid bacteria and yeasts were respectively inoculated into 20 litre (l) Chardonnay wine containing ± 0 and ± 20 $mg.l^{-1}$ free SO_2. The same experimental procedure was followed in 20 l batches of Pinotage wine containing ± 0 and ± 24 $mg.l^{-1}$ free SO_2. The microorganisms were also co-inoculated in 20 l batches of Pinotage wine containing ± 24 $mg.l^{-1}$ free SO_2, Chenin blanc and Shiraz juices, respectively. The various 20 l triplicate batches of each experiment were subjected to UV-C dosages ranging from 459 to 3 672 $J.l^{-1}$, at a constant flow speed of 4 000 $l.h^{-1}$.

After 3 672 $J.l^{-1}$, an average $\log_{10}$ reduction of 4.97 and 4.89 were obtained in Chardonnay and Pinotage wine with SO_2, respectively. The microbial reductions obtained in Chardonnay were significantly different ($p<0.05$) from Pinotage. UV-C radiation of co-inoculated Pinotage wine resulted in an average $\log_{10}$ reduction of 5.35 which have demonstrated a significant difference ($p<0.05$) from individual inoculated microorganisms in Pinotage wine. An average $\log_{10}$ reduction of 4.48 and 4.25 was obtained in Chenin blanc and Shiraz juice, respectively. The microbial reductions obtained in Chenin blanc juice was also significantly different ($p<0.05$) from Shiraz juice.

Significant microbial inactivation differences were obtained in grape juice and wine. In view of the results, the ability of UV-C radiation to inactivate microorganisms in grape juice and wine is rather challenging due to variability in physical properties such as colour, absorptivity and turbidity. The results obtained have also indicated no significant difference in the reduction of microorganisms in wines containing no and low SO_2 levels, which verify the good germicidal activity of UV-C radiation. Hence, UV-C radiation may represent an alternative technology to stabilise grape juice and wine microbiologically in conjunction with reduced SO_2 levels.

Introduction

Although microorganisms play an imperative role in wine production (Liu, 2002; Cappello *et al.*, 2004; Jolly *et al.*, 2006; Saayman & Bloom, 2006; Mauriello *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). These spoilage defects are usually recognized by haze formation, increase in acetic acid or volatile acidity, ethanol concentration, volatile phenols, volatile sulphur and viscosity of wine (Jackson, 1994; Fleet, 1998; Fugelsang, 1997; Bauer & Dicks, 2004; Delaherche *et al.*, 2004; Divol & Lonvaud-Funel, 2005; Du Toit *et al.*, 2006; Renouf *et al.*, 2006; Fugelsang & Edwards, 2007, Bartowsky & 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 SO_2 has always been an effective means of stabilising grape juice and wine microbiologically (Ribéreau-Gayon *et al.*, 2006a). But ever since SO_2 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 obliged to obey stricter regulations that demand the use of reduced SO_2 levels (Enrique *et al.*, 2008; García-Ruiz *et al.*, 2008). Other techniques such as filtration and fining are also efficient in controlling microbial growth, but unfortunately these techniques have detrimental affects on the sensorial properties of the wine

(López *et al.*, 2001; Gergely *et al.*, 2003; Armada & Falqué, 2007; Suárez *et al.*, 2007). Hence, the wine industry is seeking alternative strategies to either substitute or enhance the effect of reduced SO_2 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 & 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 & Farid, 2004; Gabriel & 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 & Balasubramaniam, 1999; López-Malo & Palou, 2005; Guerrero-Beltrán & 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 pasteurization technology has been studied in a range of products including apple cider (Hanes *et al.*, 2002; Koutchma *et al.*, 2004), orange juice (Tran & Farid, 2004), apple juice (Guerrero-Beltrán & Barbosa-Cánovas, 2005), strawberry nectar (Keyser *et al.*, 2008) and mango nectar (Guerrero-Beltrán & Barbosa-Cánovas, 2006).

Apparently, UV-C sensitivity also differs among microorganisms, species, strains and growth stage of the culture (Guerrero-Beltrán & Barbosa-Cánovas, 2005; López-Malo & Palou, 2005; Koutchma *et al.*, 2009). Guerrero-Beltrán *et al.*, 2009 have recently evaluated the effect of UV-C radiation on *Saccharomyces cerevisiae* in grape juice that resulted in a 0.53 $\log_{10}$ reduction; yet, 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.

Materials and methods

Cultivation of cultures

Pure cultures used were obtained from the Institute for Wine Biotechnology, Stellenbosch University, South Africa. Different culture media and incubation times were used to obtain optimum growth of LAB, AAB and yeasts. For the sub-culture, a colony of an overnight pure culture was suspended in liquid media in order for the cultures to acclimatise to the growth conditions (Table 1). Subsequently, an aliquot of the sub-culture was transferred to a bigger volume of media in a 1% (v/v) ratio to start with a final concentration of 6 x 10^6 colony-forming units per millilitre (cfu.ml^{-1}). This culture was then used as the inoculum.

Lactobacillus plantarum 130 and *Pediococcus acidilactici,* were cultivated in 50 g.l^{-1} MRS (Biolab, Merck, South Africa) broth, whereas *Oenococcus oeni* 48 was grown in MRS broth supplemented with 10% (v/v) tomato juice (MRST) at a final pH range of 4.80 - 5.20. The inoculums of *L. plantarum* 130 and *P. acidilactici* were ready after an incubation period of 2 - 3 days, when the desired final concentration of approximately 6 x 10^6 cfu.ml^{-1} was obtained. Since the growth of *O. oeni* 48 is slightly slower than the other LAB, the inoculum was only ready after 6 days.

The aerobe, *Acetobacter aceti* DSM 3509^T was cultivated for 6 days in yeast peptone mannitol (YPM) broth 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. For optimum growth, aerobic microorganisms were incubated in a shaker-incubator (Memmert, Lasec) at 250 rev.min^{-1}. Aerobic yeasts, *Brettanomyces bruxellensis* ISA 1649 and *Saccharomyces cerevisiae* VIN13 were cultivated in a rich medium, yeast peptone dextrose (YPD) 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. *Saccharomyces cerevisiae* VIN13 was incubated in a rotary shaker with the speed set at 250 rev.min^{-1} whereafter the inoculum was ready after 3

Table 1 The culture media, incubation temperature and time that was used to cultivate the different microorganisms

Microorganisms	Sub-culture	Culture
Lactobacillus plantarum 130	MRS; pH 6.5; 30°C; 36h	MRS; 30°C; 24h
Pediococcus acidilactici	MRS; pH 6.5; 30°C; 36h	MRS; 30°C; 24h
Oenococcus oeni 48	MRST; pH 4.8–5.2; 30°C; 72h	MRST; pH 4.8-5.2; 30°C; 72h
Acetobacter aceti DSM 3509^T	YPM; pH 5.5; 30°C; 72h	YPM; pH 5.5; 30°C; 72h
Saccharomyces cerevisiae VIN13	YPD; pH 6.5; 30°C; 24h	YPD; 30°C; 36h
Brettanomyces bruxellensis ISA 1649	YPD; pH 6.5; 30°C; 72h	YPD; 30°C; 96h

days. Similarly, *B. bruxellensis* ISA 1649 was also incubated at 250 rev.min^{-1}. The inoculum of *B. bruxellensis* ISA 1649 has required an incubation period of 7 days in order to obtain the desired final concentration of 6 x 10^6 cfu.ml^{-1}. Water additions were made by Milli-Q water with a resistivity of 18MΩ. All the media were sterilized in an autoclave at 121°C for 15 min.

Stock cultures

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

White and red grape juice

Unclarified grape juice of Chenin blanc and Shiraz varieties were used in this study. The juices were obtained from cellars in the Western Cape. The Chenin blanc juice consisted of 22.5 °Brix, pH 3.5 and 5.0 g.l^{-1} titritable 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.

White and red wine

Chardonnay 2008 and Pinotage 2009 were also supplied by a cellar situated in the heart of the Cape Winelands region. Both wines were analysed by a certified laboratory, VinLAB which is based in Stellenbosch, South Africa. The chemical composition of Chardonnay 2008 was as follow: 12.43% (v/v) alcohol, pH 3.45, 6.67 g.l^{-1} TA, 20 mg.l^{-1} free molecular SO_2 and 50 mg.l^{-1} total SO_2. 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 SO_2 and 34 mg.l^{-1} total SO_2.

Pilot-scale UV-C reactor system

A company SurePure, based in Milnerton, South Africa, has designed and manufactured a novel pilot-scale UV-C reactor system. The UV-C reactor consists of

a stainless steel inlet and outlet chamber with a stainless steel corrugated spiral tube situated between the chambers. A cylindrical low pressure mercury lamp that emits power at 100 watts (W) is placed in a quartz sleeve. The product flows in the space between the corrugated spiral tube and the quartz sleeve. As a result of the tangential inlet of the reactor, the product increase in velocity and turbulence in the inlet chamber. This causes maximum exposure of the product to UV-C radiation. Apart from the reactor design and UV-C light distribution, the flow pattern of the product in the UV-C reactor is also considered as a critical control parameter that affects the performance efficacy of the UV-C radiation (Koutchma *et al.*, 2004). Therefore, the product was pumped from the inlet chamber into the UV-C reactor at a constant flow rate of 4 000 $l.h^{-1}$ in order to achieve a Reynolds value (Re) of 7 500 which indicates turbulent flow. Not only does the turbulent flow improve the efficiency of UV-C radiation, but also prevents the microorganisms forming clumps (Keyser *et al.*, 2008).

Only 18 s is required for the 20 litre (l) product to pass through the system once at a flow rate of 4 000 $l.h^{-1}$, thus one pass of the product through the system is equivalent to a UV-C dose of 22.95 $J.l^{-1}$. 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.

UV-C dosage measurement

The design of the pilot-scale UV-C reactor in this study is identical to the one that was used by Keyser *et al.* (2008), and therefore the same theoretical calculations were applicable. Initially, UV-C radiation was primarily used to disinfect surfaces and therefore is expressed by watts per square centimetre ($W.cm^{-2}$) or joules per square centimetre ($J.cm^{-2}$), whereas radiant exposure (dosage) is expressed as watts per second per square centimetre ($W.s.cm^{-2}$) or joules per square centimetre ($J.cm^{-2}$). The exposure time (T) and irradiance (I) are characterised as critical control parameters of UV-C dosage. Since the UV-C energy penetrates into liquid flow medium instead of area, it is 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. A relationship between UV-C dosage per volume ($J.l^{-1}$) and area ($J.cm^{-2}$) as

well as contact time was meticulously calculated (Table 2) for 20 l product circulating at a constant flow rate of 4 000 $l.h^{-1}$.

UV-C dosage per area

The quartz sleeve used was 0.860 m in length with an outer surface area (A_s) of 661.93 cm^2. The annulus is defined as the area between the quartz sleeve and rigid spiral tubing, of which the volume was determined as 0.675 l or 0.00068 m^3. Seeing that the effective area (A_s) of UV-C was determined at a distance of 5 mm, the lamp had also been placed 5 mm away from the outer surface of the sleeve. The UV-C reactor system was also designed in such a way that an energy transmission rate (total UV-C output) of 25.5 W UV-C was delivered from the lamp to the constant surface of the quartz sleeve (A_s = 661.93 cm^2). The following calculations are based on the effective A_s of the quartz sleeve alone, irrespective of the volume of the annulus and the type of product in the annulus.

The intensity (I) of the UV-C reactor is calculated accordingly:

Intensity (I) = Total UV-C output per unit (W)/Area (cm^2)
= 25.5 W/661.93 cm^2
= 0.039 $W.cm^{-2}$
= 38.5 $mW.cm^{-2}$

The product retention time (T) in the reactor is directly proportional to the volume of the reactor, and is therefore calculated as follow:

Retention time (T) = Volume of the reactor (l)/Flow rate ($l.h^{-1}$)
= 0.675 l/4 000 $l.h^{-1}$
= 0.675 l/1.111 $l.s^{-1}$
= 0.608 s

Table 2 The comparison between UV-C dosage as $J.l^{-1}$ and $mJ.cm^{-2}$, and contact time for a 20 l of sample volume in a pilot-scale UV-C system using only one UV-C lamp (Adapted from Keyser *et al.*, 2008)

Passes	Dose per volume ($J.l^{-1}$)	Dose per area ($mJ.cm^{-2}$)	Contact time (min) for 20 litre of grape juice or wine
0	0	0	0
20	459	467.96	6
40	918	935.93	12
60	1 377	1 403.89	18
80	1 836	1 871.86	24
100	2 295	2 339.82	30
120	2 754	2 807.79	36
160	3 672	3 743.72	48

Hence, the retention time (T) of the product at a flow rate (Fr) of 4000 $l.h^{-1}$ was calculated as 0.608 s per reactor. The UV dosage (D) per surface area using only one reactor with continuous flow can be determined as follows:

Dosage = Intensity (I) x Time (T)
= 38.50 $mW.cm^{-2}$ x 0.608 s
= 23.408 $mW.s.cm^{-2}$
= 23.408 $mJ.cm^{-2}$

UV-C dosage per volume

Based on the calculations in the previous section of UV-C dosage per area, the product retention time (T) per reactor that is operating at a flow rate (Fr) of 4 000 $l.h^{-1}$, is 0.608 s. Therefore, the UV-C dosage per litre of liquid treated for only one reactor with continuous flow can be calculated as follows:

Dosage = Total UV-C output per unit (W)/Flow rate ($l.s^{-1}$)
= 25.50 W/1.11 $l.s^{-1}$
= 22.95 $W.s.l^{-1}$
= 22.95 $J.l^{-1}$

Operation of the pilot-scale UV-C reactor system

Initially, the holding tank of the UV-C system was filled with 20 l of treating volume. The product was circulated at a constant flow rate of 4 000 $l.h^{-l}$. The temperature was continuously regulated by a thermometer that is installed adjacent to the lamps. UV-C radiation can be classified as an adiabatic process, since the temperature had remained constant subsequent to each UV-C treatment. 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.

Cleaning of the UV-C reactor system

A standard 'Cleaning In Place' (CIP) process was implemented prior to and following each UV treatment to ensure that the UV-C reactor system is microbiological safe. The pilot-scale unit was firstly rinsed with warm tap water (50°C) for 10 min, followed by the circulation of a 1.0% alkaline solution for 30 min at 75°C. Subsequently, the unit was rinsed once more with warm tap water at 50°C for approximately 5 min. Perasan solution (Divosan System, JohnsonDiversey, South Africa) at a concentration of 0.5% were circulated for 10 min, whereafter the system was rinsed with cold tap water. The hygiene of the system was determined by microbiological analysis of the cold water.

UV-C treatment of white and red grape juice

Brettanomyces bruxellensis ISA 1649 and *S. cerevisiae* VIN13 were co-inoculated into 20 l Chenin blanc juice with an initial microbial count of 1 x 10^6 cfu.ml^{-1}, respectively. Twenty litre of Shiraz juice was also inoculated with *B. bruxellensis* ISA 1649, *S. cerevisiae* VIN13 and *L. plantarum* 130 to also obtain initial microbial counts of 1 x 10^6 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, 1 377, 1 836, 2 295, 2 754 and 3 672 (J.l^{-1}), after which microbiological analysis were performed.

UV-C treatment of white and red wine

Duplicate 50 ml samples of Chardonnay 2008 and Pinotage 2009 were taken prior to inoculation and UV-C treatment. The wines were inoculated with *L. plantarum* 130, *P. acidilactici*, *O. oeni* 48, *B. bruxellensis* ISA 1649, *S. cerevisiae* VIN13 and *A. aceti* DSM 3509^T, respectively, to obtain an initial count of 1 x 10^6 cfu.ml^{-1}. Triplicate 50 ml samples were taken after inoculation of Chardonnay and Pinotage that contained ± 20 mg.l^{-1} free and ± 24 mg.l^{-1} free SO_2, respectively. These samples were not exposed to the UV-C light and therefore, were labelled as the control. Consequently, the wines were subjected to UV-C dosages of 459, 918, 1 377, 1 836, 2 295, 2 754 and 3 672 J.l^{-1}. After each dosage (J.l^{-1}), duplicate 50 ml samples were taken. The control samples were kept at ambient temperature, while sampling had corresponded

with the time that each UV-C dosage was completed. Microbiological analysis was performed on all the samples.

Additionally, Pinotage containing ± 24 $mg.l^{-1}$ free SO_2 were inoculated to an initial count of 1 x 10^6 $cfu.ml^{-1}$ with a cocktail comprising of *L. plantarum* 130, *S. cerevisiae* VIN13 and *B. bruxellensis* ISA 1649, respectively. Duplicate 50 ml samples were taken before inoculation and after inoculation, and subsequently to each UV-C dosage. The control was obtained in the same manner as mentioned above. Microbiological analysis was performed on all the samples.

Duplicate 50 ml samples were taken after the removal of SO_2 and were labelled as before inoculation. Free SO_2 was removed from the wine through meticulous addition of 40 volume hydrogen peroxide (Zoecklein *et al.*, 1995). The same experimental procedure was followed as for wine that contained SO_2.

The enumeration of microorganisms from grape juices and wines

One milliliter (ml) of the grape juice and wine samples was transferred aseptically to 9 ml quarter strength Ringer's solution (Merck) and vortexed thoroughly. Serial tenfold dilution range were prepared (10^{-1} to 10^{-7}) and 1 ml of each dilution was pour plated with selective media (Table 3) to determine the viability of the microorganisms after UV-C treatment.

Lactobacillus plantarum 130 and *P. acidilactici* were enumerated with MRS agar supplemented with 50 $mg.l^{-1}$ Delvocid (DSM Food Specialties, 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. 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. Since a CO_2 enriched atmosphere stimulate the growth of *O. oeni* 48 (Fleet, 1993), the plates were incubated for 7 days under anaerobic conditions (Anaerojar, Quantum Biotechnologies). Wallenstein (WL) nutrient (Quantum Biotechnologies) agar supplemented with 30 $mg.l^{-1}$ kanamycin sulphate, 50 $mg.l^{-1}$ cycloheximide

Table 3 The selective media that were used for the enumeration of microorganisms in grape juice and wine

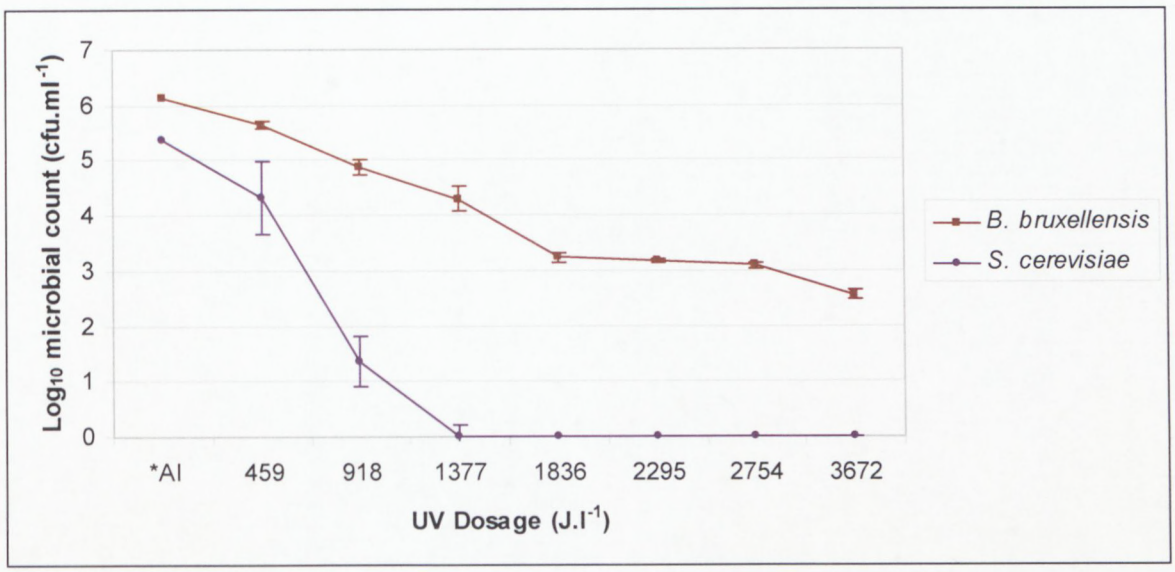
Culture media	*L. plantarum* 130 *P. acidilactici*	*O. oeni* 48	*S. cerevisiae* VIN13	*B. bruxellensis* ISA 1649	*A. aceti* DSM 3509^T
Medium					
Wallenstein Nutrient (WLN)				+	
De Mann Rogosa Sharpe (MRS)	+	+			
Yeast peptone dextrose (YPD)			+		
Yeast peptone mannitol (YPM)					+
Antibiotics					
Chloramphenicol				+	+
Cycloheximide				+	
Kanamycin sulphate	+	+		+	
Delvocid	+	+			+
Additional constituents					
Sodium metabisulfite			+		
Ethanol			+		
Tomato juice		+			

(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, respectively (Du Plessis *et al.*, 2002; Fugelsang & Edwards, 2007; Oelofse *et al.*, 2009). Growth of *B. bruxellensis* ISA 1649 was detected after an incubation period of 7 days. Delvocid (50 mg.l^{-1}) and chloramphenicol (50 mg.l^{-1}) were added to YPM agar for the enumeration of *A. aceti* DSM 3509^{T}. The YPD agar supplemented with sodium metabisulfite (Sigma-Aldrich) at a final concentration of 0.02% and 12% (v/v) absolute ethanol (Merck, Saarchem) was used to enumerate *S. cerevisiae* VIN 13. The incubation period of *A. aceti* DSM 3509^{T} and *S. cerevisiae* VIN13 were 4 - 5 days at 30°C. The number of colonies counted was expressed as cfu.ml^{-1} and converted into logarithmic units. Three replicates of each experiment were performed and only the average microbial counts were used.

Results and discussion

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. Each inoculum had a microbial count of approximately 5 – 6 $\log_{10}$ of which three replicates were performed. Chenin blanc juice was subjected to UV-C dosages that ranged from 459, 918, 1 377, 1 836, 2 295, 2 754 to 3 672 J.l^{-1}, at a constant flow rate of 4 000 l.hr^{-1}. At the lowest UV-C dosage of 459 J.l^{-1}, a 0.47 and 1.07 $\log_{10}$ reduction were obtained for *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13, respectively. After the 918 J.l^{-1} UV-C exposure of co-inoculated Chenin blanc juice, a 1.24 and 4.02 $\log_{10}$ reduction (Fig. 1) were obtained for the same yeasts followed by a 1.83 and 5.38 $\log_{10}$ reduction after a higher dosage of 1 377 J.l^{-1}. The UV-C dosage of 1 377 J.l^{-1} was sufficient to completely eliminate *S. cerevisiae* VIN13 from the matrix. It is, therefore, clear that *S. cerevisiae* VIN13 was more sensitive to UV-C radiation than *B. bruxellensis* ISA 1649. According to the literature, yeasts is expected to be more resistant to UV-C radiation since less tyrosine or cytosine pyrimidines are encountered in their genetic DNA material, which is the primar site



*AI : After inoculation

Figure 1 The log_{10} microbial counts of co-inoculated *Brettanomyces bruxellensis* ISA 1649 and *Saccharomyces cerevisiae* VIN13 in unclarified Chenin blanc juice after exposure to various UV-C dosages.

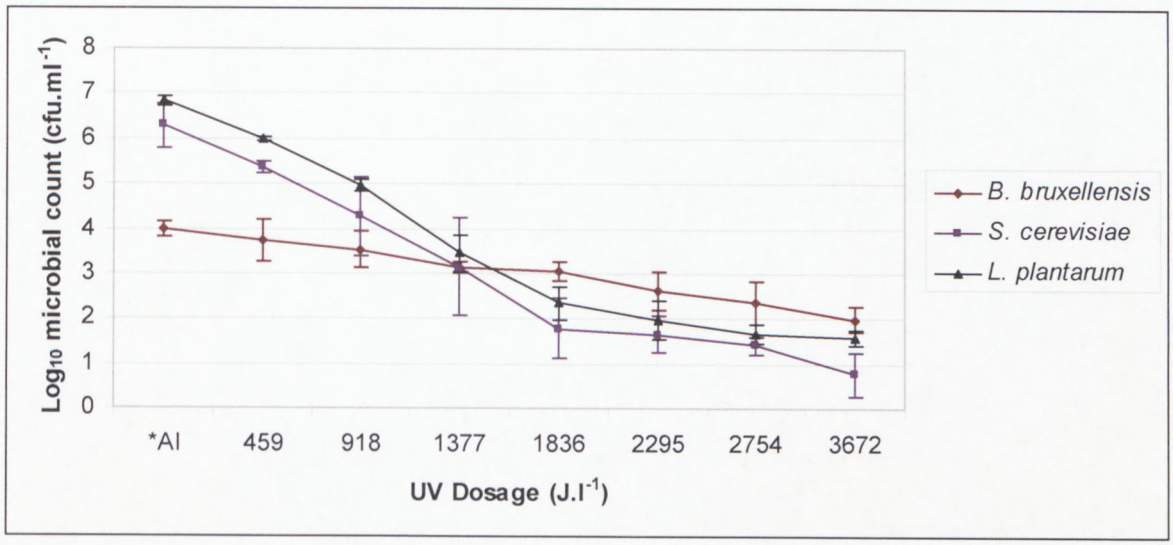
where UV-C radiation targets and causes microbial inactivation (Thompson, 2003). However, it is assumed that the size of yeasts cells may have played a role in the activation of *S. cerevisiae* VIN13 (Boulton *et al.*, 1999). Apparently, microorganisms larger than 1 micron are more sensitive to UV-C radiation because of their cytoplasm that easily absorbs UV-C light (Koutchma *et al.*, 2009). Hence, UV-C rays may penetrate the greater surface area of *S. cerevisiae* VIN13 cells more easily and render them more sensitive to UV-C radiation based on their size.

Cold pasteurisation of liquid foods with UV-C radiation is a fairly new technology in the food and beverage industry. Consequently, no reports on UV-C effectiveness against *B. bruxellensis* exist in the literature whereas limited data is available on the effectiveness of UV-C radiation against *S. cerevisiae*. It has been reported by Koutchma *et al.* (2009) that *S. cerevisiae* was effectively inactivated by pulsed UV light. A research group have performed two studies on the inactivation of *S. cerevisiae* in apple juice and mango nectar with a laminar flow UV-C system consisting of two low pressure mercury lamps (25W) (Guerrero-Beltrán & Barbosa-Cánovas, 2005; Guerrero-Beltrán & Barbosa-Cánovas, 2006). An UV-C dose of 450 $kJ.m^{-2}$ had resulted in a 1.34 log reduction for *S. cerevisiae* in apple juice at 0.548 $L.min^{-1}$, whereas a 2.71 log reduction had followed in mango nectar at a flow rate of 0.451 $L.min^{-1}$. It is rather difficult to compare the reductions of *S. cerevisiae* found in grape juice with apple juice and mango nectar, since their physical appearance such as colour, viscosity and turbidity vary which may perhaps have different affects on the inactivation efficacy of UV-C radiation (Tran & Farid, 2004; Keyser *et al.*, 2008; Koutchma *et al.*, 2009). Apart from the flow mediums, the UV-C reactor design in this study is not identical to the one that was used by Guerrero-Beltrán & Barbosa-Cánovas (2005; 2006); and may, therefore, also explain the different reductions obtained for *S. cerevisiae* (Ngadi *et al.*, 2003; Koutchma *et al.*, 2004).

In red wine production, the pulps, seeds and skins remain into contact with the grape juice as it is essential in the extraction of flavour and colour compounds (Jackson, 1994). In comparison with Chenin blanc juice, the unclarified Shiraz juice appeared dark red and more turbid with floating grape skins and solid particles. The

Shiraz juice were inoculated with *L. plantarum* 130, *S. cerevisiae* VIN13 and *B. bruxellensis* ISA 1649 in order to start with initial counts ranging from 4 to 7 $\log_{10}$. The Shiraz juice was exposed to exactly 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 (Fig. 2) 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 1 377 $J.l^{-1}$ UV-C exposure. It is clear from the results obtained that microbial reduction increases with exposure to higher dosages. The highest applied UV-C dosage of 3 672 $J.l^{-1}$ reduced *B. bruxellensis* ISA 1649 and *L. plantarum* 130 to 1.99 and 1.59 $\log_{10}$, respectively, while only *S. cerevisiae* VIN13 had sufficiently been reduced to 1 $\log_{10}$.

Significant differences between the inactivation of the inoculated microorganisms in Chenin blanc and Shiraz juice were evaluated using repeated measures analysis of variance (ANOVA). A significant difference ($p<0.05$) was found between the inactivation of *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13 in Shiraz juice with regards to inactivation in Chenin blanc juice. According to López-Malo & Palou (2005), a turbid matrix causes shadowing and scattering of the UV-C rays and thus reduces the efficacy of the technology. A study reported by Koutchma *et al.* (2009) have illustrated that the inactivation rate of *Escherichia coli* K12 was negatively reduced after the turbidity of the apple juice had been increased from 1 400 to 2 400 NTU. Regrettably, the turbidity of Chenin blanc and Shiraz juices could not be determined in this study; however, Shiraz juice visibly appeared more turbid with fibres and small amounts of grape skins. In addition to turbidity, Guerrero-Beltrán *et al.*, 2009 have indicated that the colour of the liquid may also be an obstacle that may negatively influence the penetration ability of UV-C light in the liquid. This was observed after a 0.53 log reduction of *S. cerevisiae* was obtained in deep violet grape juice (*Vitis vinifera*). The reduction of *S. cerevisiae* found in grape juice (*Vitis vinifera*) is very low compare to previous log reductions of 2.42 and 1.34 ± 0.354 found in grapefruit juice and apple juice, respectively (Guerrero-Beltrán & Barbosa-Cánovas, 2005; Guerrero-Beltrán *et al.*, 2009). It was implied that the dark violet colour of the grape juice (*Vitis vinifera*) had obstructed UV-C transmittance to *S.*



*AI : After inoculation

Figure 2 The log_{10} microbial counts of *Brettanomyces bruxellensis* ISA 1649, *Saccharomyces cerevisiae* VIN13 and *Lactobacillus plantarum* 130 in unclarified Shiraz juice after exposure to various UV-C dosages.

cerevisiae in juice. Therefore, it is may be assumed that the penetration intensity of UV-C radiation to *S. cerevisiae* VIN13 and *B. bruxellensis* ISA 1649 in this study was limited in Shiraz juice because of its higher level of turbidity and dark red colour, which may have blocked UV-C light from reaching the microorganisms in the juice. 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 a previous study had reported that the pH and °Brix levels had no effect on the inactivation rate of *E. coli* in model apple juice (Koutchma *et al.*, 2009). *Lactobacillus plantarum* 130 was also included as additional microorganisms in the experiment that may have reduce the efficacy of UV-C radiation. Thus, a higher dosage is possibly required to reduce the microorganisms to zero in Shiraz juice in order to optimize the effectiveness of the technology as a microbiological control system.

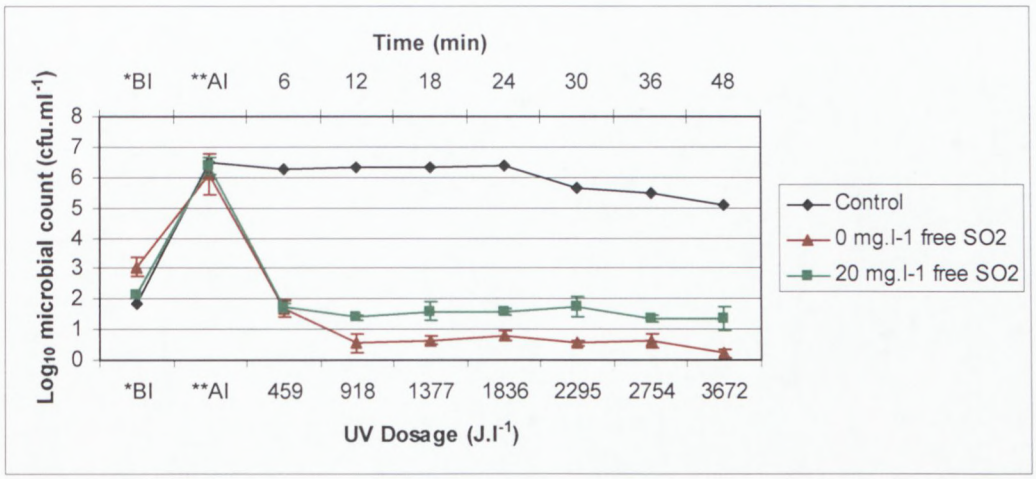
In the wine industry, SO_2 is generally added to the grape juice after crushing to selectively reduce the number of unwanted wild yeasts and bacteria that have originated from the grape surfaces (Henick-Kling *et al.*, 1998). A novel technology such as pulsed electric field (PEF) technology also holds promise to control spoilage microorganisms but pilot plant studies are lacking to illustrate the feasibility of PEF technology in wineries (Puértolas *et al.*, 2009). In view of the results obtained, UV-C radiation may be applied as an alternative technology to stabilise grape juice from microbial infected grapes with the use of reduced SO_2 levels as such matrixes are rich in SO_2 binding compounds such as acetaldehyde, anthocyanins, sugars, keto-acids (gluconic and pyruvic acids) and dihydroxyacetone that commonly necessitates elevated SO_2 concentrations to compensate for the loss of the antimicrobial activity (Barbe *et al.*, 2000; Ribéreau-Gayon *et al.*, 2006a). UV-C radiation may also be applied to the juice after pressing or before primary fermentation to inactivate the wild yeast and bacteria cells, allowing a more homogenous fermentation that will be facilitated by inoculated starter cultures.

Wine

The inactivation effect of UV-C radiation on *L. plantarum* 130, *O. oeni* 48, *P. acidilactici*, *A. aceti* DSM 3509^T, *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13

were investigated in Chardonnay wine. The microorganisms were inoculated to an initial concentration of ± 1 x 10^6 cfu.ml^{-1} in Chardonnay wine containing ± 0 mg.l^{-1} and ± 20 mg.l^{-1} free SO_2, respectively, in order to discriminate between microbial reduction caused by UV-C radiation and SO_2. The Chardonnay wine that received no UV containing ± 20 mg.l^{-1} free SO_2 was used as the control. It is, however, clear that the various microbial counts were relatively constant for approximately 30 minutes 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 results of the control had confirmed that microbial reduction was mainly due to UV-C radiation and not because of the SO_2 present in Chardonnay wine. Repeated measures of ANOVA was used to test for significant differences between the microbial reduction in Chardonnay wine containing ± 0 mg.l^{-1} and ± 20 mg.l^{-1} free SO_2. 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 (Fig. 3). 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 2 754 to 3 672 J.l^{-1}. 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 (Figs. 4 to 8) in Chardonnay wine containing ± 0 mg.l^{-1} and ± 20 mg.l^{-1} free SO_2 which means that the inactivation was mainly due to UV-C radiation. Although a significant difference ($p<0.05$) between the reduction of *P. acidilactici* was found, the discussion will only concentrate on the results obtained for the Chardonnay wine containing ± 20 mg.l^{-1} free SO_2, since SO_2 is generally applied to wine as an antioxidant and antimicrobial agent.

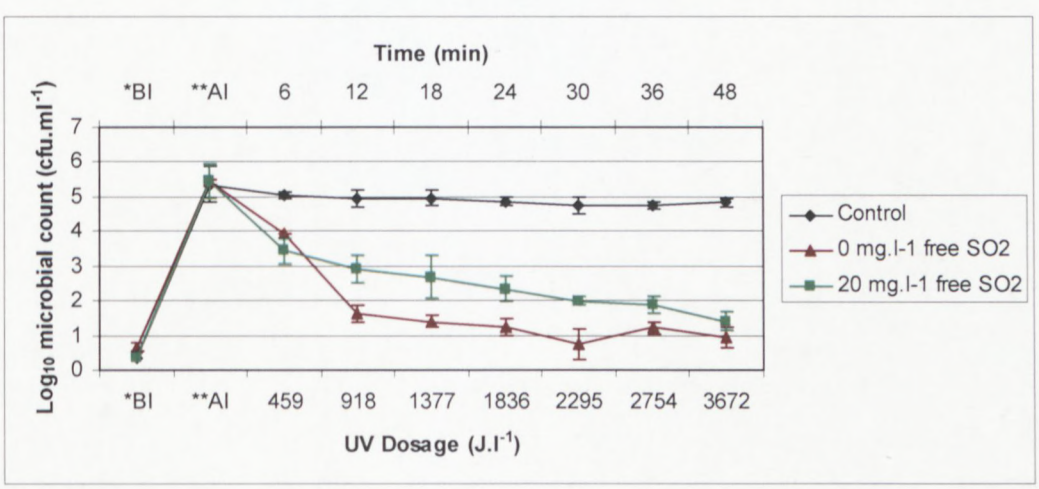
The UV-C treated Chardonnay wine with ± 20 mg.l^{-1} free SO_2 resulted in a 3.66, 4.37, 4.67, 2.00, 3.73 and 3.57 log_{10} reduction for *O. oeni* 48, *L. plantarum* 130, *P. acidilactici*, *A. aceti* DSM 3509^T, *S. cerevisiae* VIN13 and *B. bruxellensis* ISA 1649, respectively, after a UV dosage of only 459 J.l^{-1} (Fig. 9). 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}.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

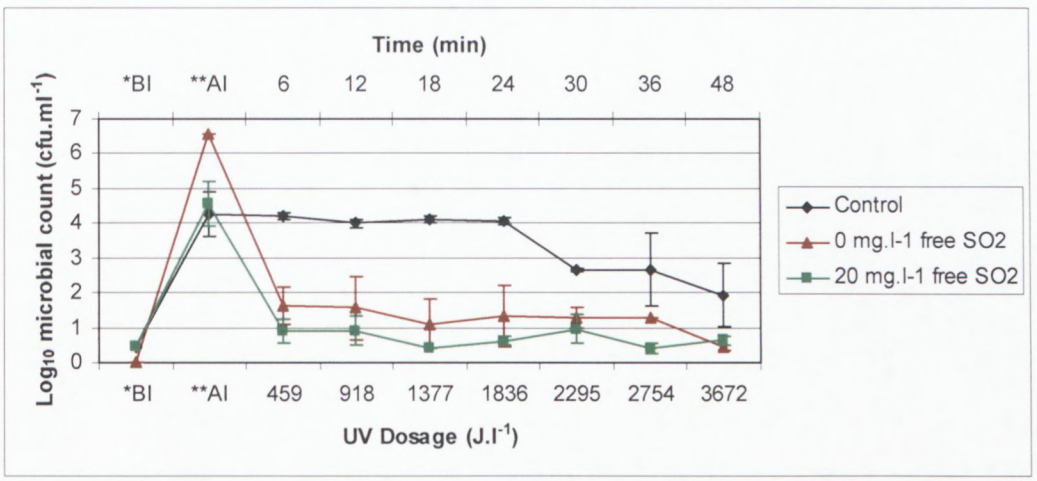
Figure 3 The log_{10} microbial counts of *Pediococcus acidilactici* in Chardonnay wine 2008 at each $J.l^{-1}$. The Chardonnay wine with ± 20 $mg.l^{-1}$ free SO_2 that was not exposed to UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

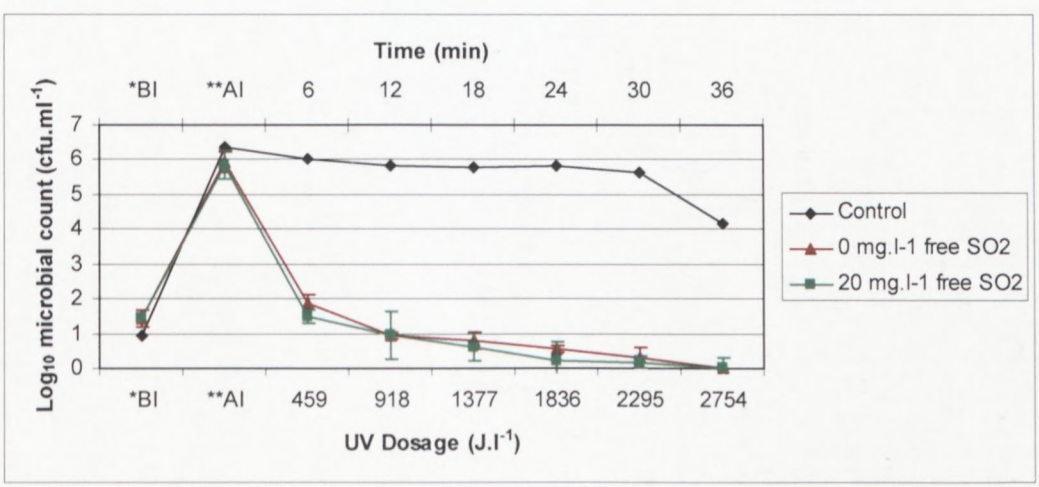
Figure 4 The log_{10} microbial counts of *Acetobacter aceti* DSM 3509^T in Chardonnay wine 2008 at each $J.l^{-1}$. The Chardonnay wine with ± 20 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

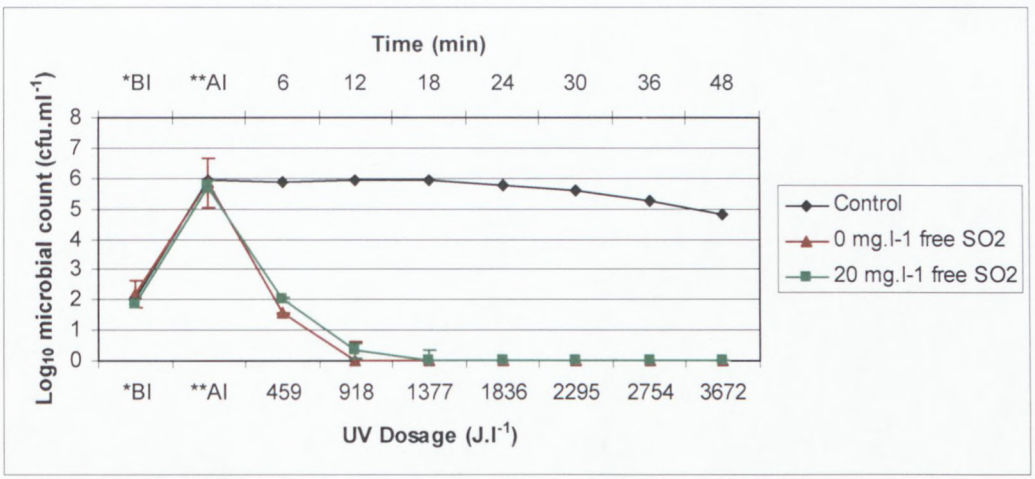
Figure 5 The log_{10} microbial counts of *Oenococcus oeni* 48 in Chardonnay wine 2008 at each $J.l^{-1}$. The Chardonnay wine with ± 20 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

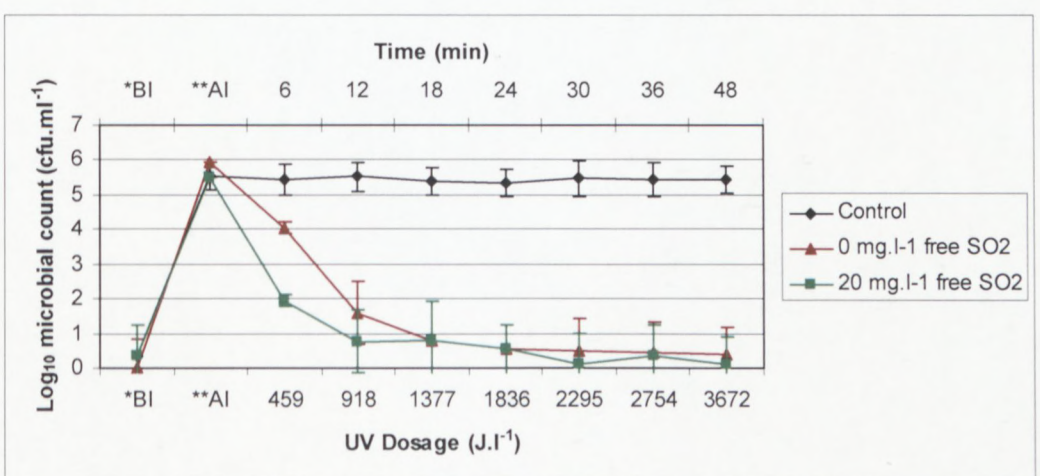
Figure 6 The log_{10} microbial counts of *Lactobacillus plantarum* 130 in Chardonnay wine 2008 at each $J.l^{-1}$. The Chardonnay wine with ± 20 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

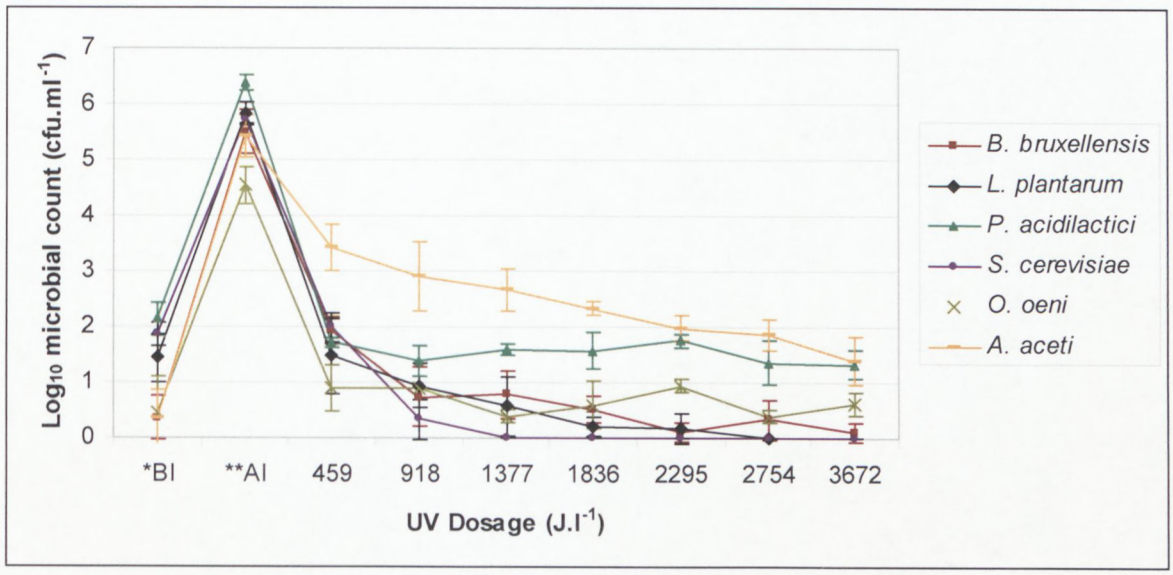
Figure 7 The $\log_{10}$ microbial counts of *Saccharomyces cerevisiae* VIN13 in Chardonnay wine 2008 at each $J.l^{-1}$. The Chardonnay wine with ± 20 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

Figure 8 The $\log_{10}$ microbial counts of *Brettanomyces bruxellensis* ISA 1649 in Chardonnay wine 2008 at each $J.l^{-1}$. The Chardonnay wine with ± 20 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation (No UV-C radiation)

**AI : After inoculation

Figure 9 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 at a constant flow rate of 4 000 l.hr^{-1}.

Based on the data in Table 4, it is clear that the reduction of microorganisms increases as the UV-C dosages increases. As observed, it seems as if the UV-C resistance pattern in Chardonnay wine was as follow: *A. aceti* DSM 3509^T < *P. acidilactici* < *O. oeni* 48 < *B. bruxellensis* ISA 1649 < *L. plantarum* 130 < *S. cerevisiae* VIN13. This is in contradiction with the data reported in the literature which claims that Gram-negative bacteria are more sensitive to UV-C radiation than Gram-positive bacteria and yeasts (López-Malo & Palou, 2005). *Acetobacter aceti* DSM 3509^T is Gram-negative whereas *O. oeni* 48, *P. acidilactici* and *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 (Tran & Farid, 2004). It is, therefore, expected that yeasts should be more resistant to UV-C radiation than bacteria. Yet, the results have indicated that *P. acidilactici* and *O. oeni* 48 were slightly more resistant to UV-C radiation than *B. bruxellensis* ISA 1649. In addition to this, *S. cerevisiae* VIN13 was sufficiently reduce to zero $cfu.ml^{-1}$ after 1 377 $J.l^{-1}$. The higher sensitivity of *S. cerevisiae* VIN13 to UV-C radiation may be attributed to its larger size which is perhaps easily targeted by the UV-C rays. Although the principle of microbial inactivation caused by PEF treatment and UV-C radiation differ, the same phenomena were observed in a study of Puértolas *et al.* (2009) where yeasts such as *Brettanomyces anomala*, *B. bruxellensis* and *Saccharomyces bayanus* were more PEF sensitive than bacteria because of their larger sizes. In Chardonnay wine, *L. plantarum* 130 was the most sensitive bacteria to UV-C radiation. Similar to *S. cerevisiae* VIN13, *L. plantarum* 130 is substantially larger in size compare to the rest of the LAB (Hammes & Vogel, 1995; Simpson & Taguchi, 1995). Due to its larger size, the *L. plantarum* 130 cells may be easily targeted by the UV-C rays. Yeasts, LAB and AAB also vary in cell wall structure, thickness, and composition; and therefore may differ in terms of UV-C sensitivity (Koutchma *et al.*, 2009). Since *P. acidilactici* was moderately UV resistant, it is postulated that the bacteria have gained an advantage from its unique ability to secrete exopolysaccharides (Renouf *et al.*, 2006). The thicker cell wall may have

Table 4 The log_{10} microbial counts ($cfu.ml^{-1}$) of *Brettanomyces bruxellensis* ISA 1649, *Acetobacter aceti* DSM 3509^T, *Saccharomyces cerevisiae* VIN13, *Lactobacillus plantarum* 130, *Oenococcus oeni* 48 and *Pediococcus acidilactici* individually inoculated into 20 litre Chardonnay wine containing ± 20 $mg.l^{-1}$ free SO_2 at different UV dosages ($J.l^{-1}$)

UV Dosage ($J.l^{-1}$)	Log_{10} microbial counts ($cfu.ml^{-1}$) of the microorganisms					
	B. bruxellensis ISA 1649	*A. aceti* DSM 3509^T	*S. cerevisiae* VIN13	*L. plantarum* 130	*O. oeni* 48	*P. acidilactici*
*BI	0.36	0.35	1.87	1.43	0.45	2.12
**AI	5.50	5.42	5.72	5.84	4.55	6.38
459	1.93	3.42	1.99	1.47	0.89	1.71
918	0.74	2.89	0.33	0.94	0.90	1.38
1 377	0.79	2.66	0	0.58	0.39	1.59
1 836	0.52	2.32	0	0.20	0.60	1.56
2 295	0.10	1.97	0	0.16	0.95	1.74
2 754	0.33	1.86	0	0	0.39	1.36
3 672	0.10	1.39	0	0	0.63	1.32

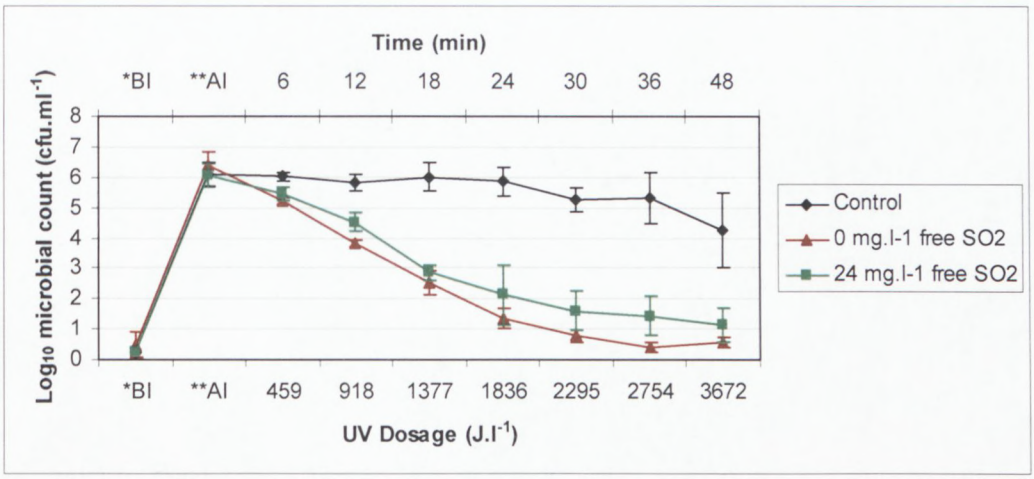
*BI : Before inoculation (No UV-C radiation)

**AI : After inoculation

protected the DNA of the *P. acidilactici*, thus the penetration ability of the UV-C rays may have been obstructed.

The efficacy of UV-C radiation vary with respect to the matrix been treated, and therefore the germicidal effect of UV-C radiation were investigated on *L. plantarum* 130, *O. oeni* 48, *P. acidilactici*, *A. aceti* DSM 3509^T, *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13, respectively, in Pinotage wine with ± 0 mg.l^{-1} free SO_2. The same experiment was also repeated in Pinotage wine with ± 24 mg.l^{-1} free SO_2 in order to determine whether microbial inactivation was due to the presence of SO_2 in wine or UV-C radiation. The microorganisms were individually inoculated into Pinotage wine to an initial concentration of ± 1 x 10^6 cfu.ml^{-1}. Stastical analysis was done by using the repeated measures ANOVA tests. The analysis have confirmed that no significant differences ($p>0.05$) were found between the microbial reduction in Pinotage wine with ± 0 mg.l^{-1} and ± 24 mg.l^{-1} free SO_2 after each UV-C dosage (Figs. 10 to 15). Therefore, it may be assumed that inactivation was mainly due to UV-C radiation. The Pinotage wine with ± 24 mg.l^{-1} free SO_2 that was not exposed to UV-C radiation was labelled as the control. After 48 minutes, a 0.63, 1.83, 0.29, 0.00, 0.06 and 0.14 $\log_{10}$ reduction had resulted for *L. plantarum* 130, *P. acidilactici*, *B. bruxellensis* ISA 1649, *S. cerevisiae* VIN13, *O. oeni* 48 and *A. aceti* DSM 3509^T, respectively. The controls have displayed that the inactivation found in Pinotage wine may not be attributed to the presence of SO_2 but as a result of UV-C radiation. Since there was no significant difference ($p>0.05$) between the results obtained in Pinotage wine with ± 0 mg.l^{-1} and ± 24 mg.l^{-1} free SO_2, 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.

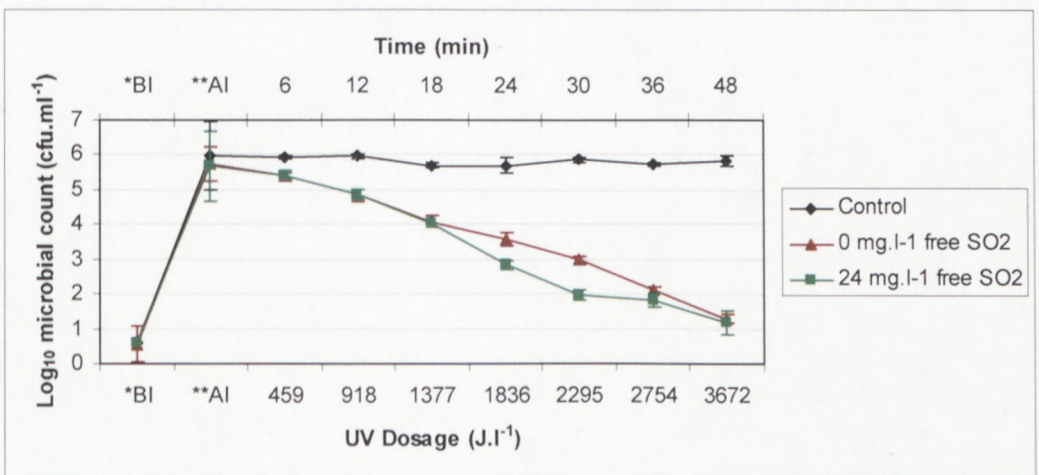
After a dosage of 459 J.l^{-1}, 0.03, 0.63, 0.96, 0.26, 0.19 and 0.46 $\log_{10}$ reduction (Fig. 16) were obtained in Pinotage wine for *L. plantarum* 130, *P. acidilactici*, *O. oeni* 48, *A. aceti* DSM 3509^T, *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13, respectively. The microbial $\log_{10}$ 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 1 377 J.l^{-1}, had resulted in a 1.62, 3.20, 4.10, 1.66, 1.66 and 1.75 $\log_{10}$



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

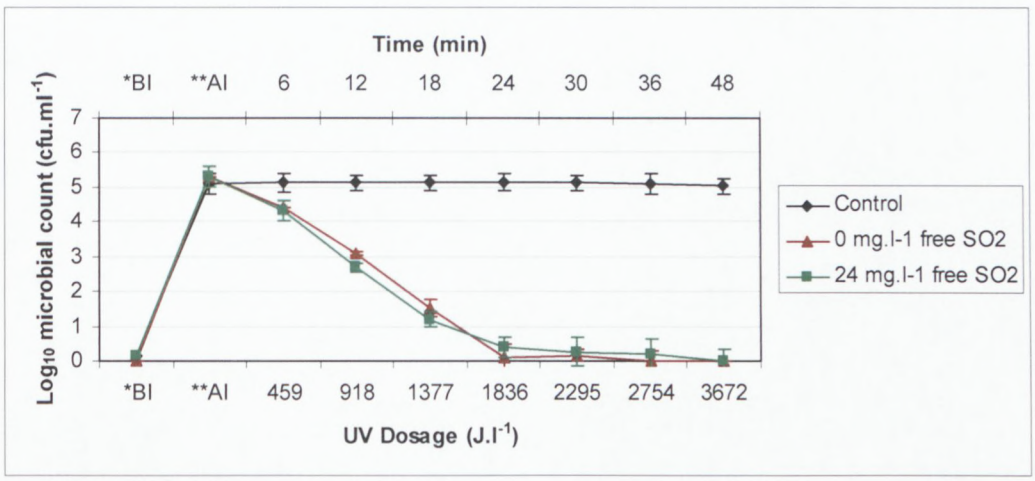
Figure 10 The $\log_{10}$ microbial counts of *Pediococcus acidilactici* in Pinotage wine 2009 at each $J.l^{-1}$. The Pinotage wine containing ± 24 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

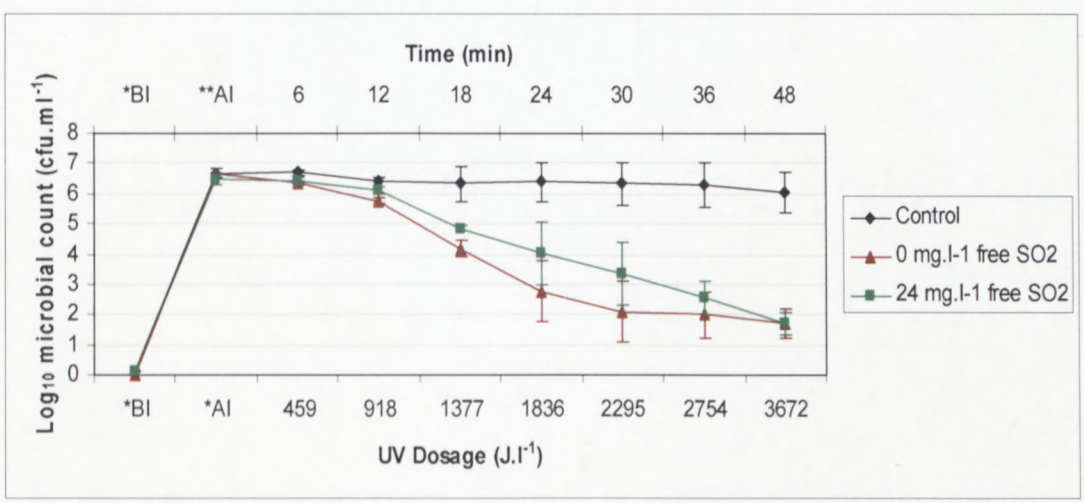
Figure 11 The $\log_{10}$ microbial counts of *Acetobacter aceti* DSM 3509^T in Pinotage wine 2009 at each $J.l^{-1}$. The Pinotage wine containing ± 24 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

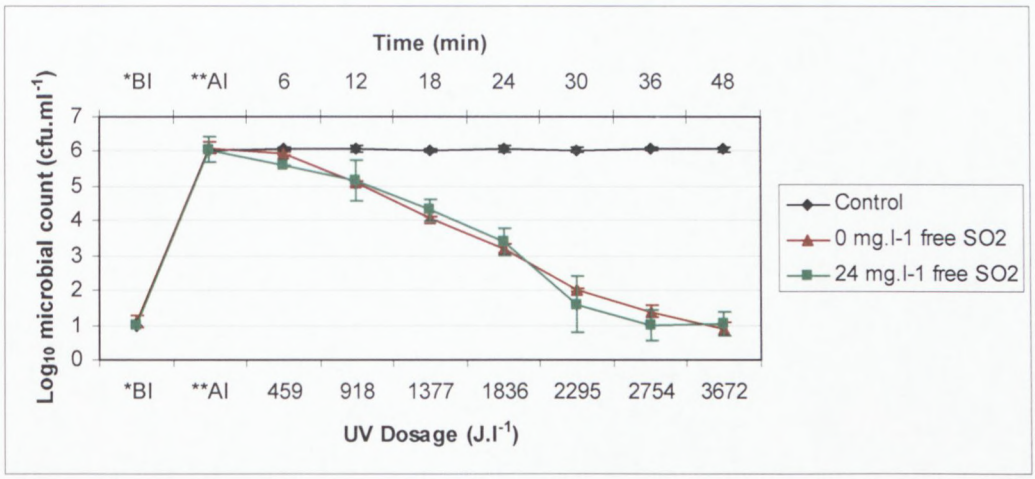
Figure 12 The $\log_{10}$ microbial counts of *Oenococcus oeni* 48 in Pinotage wine 2009 at each $J.l^{-1}$. The Pinotage wine containing ± 24 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

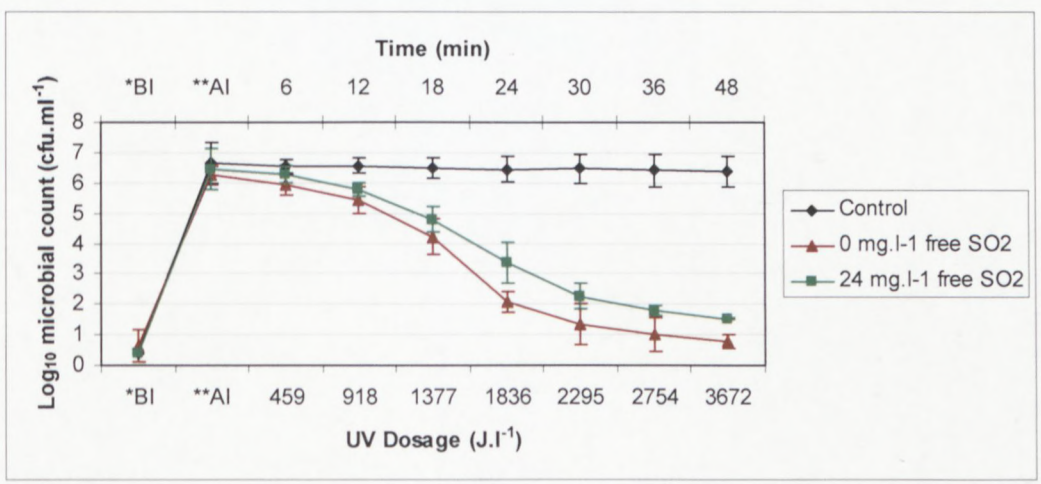
Figure 13 The $\log_{10}$ microbial counts of *Lactobacillus plantarum* 130 in Pinotage wine 2009 at each $J.l^{-1}$. The Pinotage wine containing ± 24 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

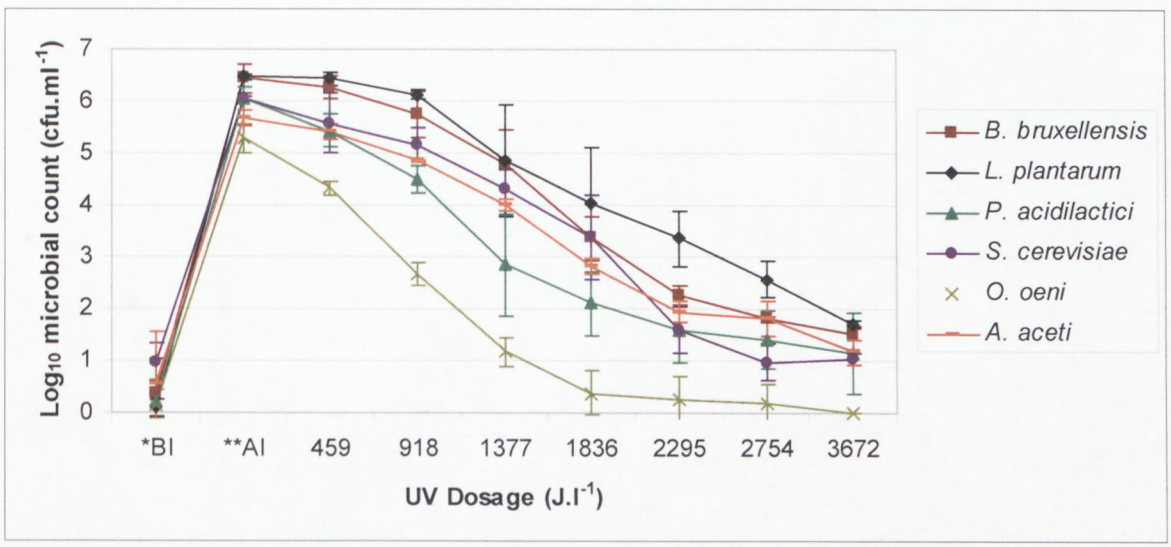
Figure 14 The $\log_{10}$ microbial counts of *Saccharomyces cerevisiae* VIN13 in Pinotage wine 2009 at each $J.l^{-1}$. The Pinotage wine containing ± 24 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation; after H_2O_2 addition (No UV-C radiation)

**AI : After inoculation

Figure 15 The $\log_{10}$ microbial counts of *Brettanomyces bruxellensis* ISA 1649 in Pinotage wine 2009 at each $J.l^{-1}$. The Pinotage wine containing ± 24 $mg.l^{-1}$ free SO_2 that received no UV-C radiation was used as the control.



*BI : Before inoculation (No UV-C radiation)

**AI : After inoculation

Figure 16 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 at a constant flow rate of 4 000 $l.hr^{-1}$.

reduction for the same microorganisms. From the results obtained in Pinotage wine with ± 24 mg.l^{-1} free SO_2 (Table 5), it is clear that the microbial counts decreased with an increase in UV-C dosage. It may, therefore, be assumed that the degree of microbial reduction is in correlation with the applied UV-C dosage.

Significant microbial inactivation differences ($p<0.05$) were obtained for Chardonnay and Pinotage wine. This is clear from the UV-C resistance patterns of the inoculated microorganisms in Chardonnay wine (Fig. 9) that were completely different from Pinotage wine (Fig. 16). The resistance pattern of the microorganisms to UV-C radiation was as follow: *L. plantarum* 130 < *B. bruxellensis* ISA 1649^{T} < *S. cerevisiae* VIN13 < *A. aceti* DSM 3509^{T} < *P. acidilactici* < *O. oeni* 48. The microbial reductions obtained for microorganisms in Pinotage wine were also much lower than those that were found in Chardonnay wine. The higher resistance of the microorganisms in Pinotage wine may be because of the differences found in the characteristics of Chardonnay wine and Pinotage wine, for example colour. In comparison with Chardonnay wine, Pinotage wine is darker in colour as a result of the higher phenol concentration present. The authors, López-Malo & Palou (2005), have reported that the absorption coefficient (α) at the germicidal wavelength (253.7 nm) of white and red wine is 10 and 30 cm^{-1}, respectively. Unfortunately, the actual absorption coefficients of the Chardonnay and Pinotage wine could not be tested in this study. The higher absorption coefficient of red wine may be attributed to the presence of anthocyanins (Zoecklein *et al.*, 1995). It is already known that a range of phenolic compounds in red wine absorb radiation in the UV region of the electromagnetic spectrum (Gómez-Cordovés, 2004; Ribéreau-Gayon *et al.*, 2006b). It is, therefore, postulated that the UV-C rays may have been absorbed by the Pinotage wine instead of being transmitted to the microorganisms. However, significant microbial reduction was still obtained meaning that UV-C light was only limited to a certain extent in Pinotage wine.

The initial microbial load in a matrix is also a function of the effectiveness of UV-C radiation as a pasteurisation technology. In view of this, 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 were investigated.

Table 5 The log_{10} microbial counts ($cfu.ml^{-1}$) of *Brettanomyces bruxellensis* ISA 1649, *Acetobacter aceti* DSM 3509^T, *Saccharomyces cerevisiae* VIN13, *Lactobacillus plantarum* 130, *Oenococcus oeni* 48 and *Pediococcus acidilactici* individually inoculated into 20 litre Pinotage wine containing ± 24 $mg.l^{-1}$ free SO_2 at different UV dosages ($J.l^{-1}$)

UV Dosage ($J.l^{-1}$)	Log_{10} microbial counts ($cfu.ml^{-1}$) of the microorganisms					
	B. bruxellensis ISA 1649	*A. aceti* DSM 3509^T	*S. cerevisiae* VIN13	*L. plantarum* 130	*O. oeni* 48	*P. acidilactici*
*BI	0.38	0.57	0.96	0.10	0.16	0.23
**AI	6.44	5.66	6.03	6.47	5.28	6.05
459	6.25	5.40	5.57	6.44	4.32	5.42
918	5.75	4.84	5.14	6.13	2.67	4.49
1 377	4.78	4.00	4.28	4.85	1.18	2.85
1 836	3.36	2.83	3.38	4.04	0.38	2.10
2 295	2.25	1.94	1.59	3.36	0.26	1.59
2 754	1.80	1.81	0.98	2.56	0.20	1.42
3 672	1.54	1.18	1.03	1.71	0	1.14

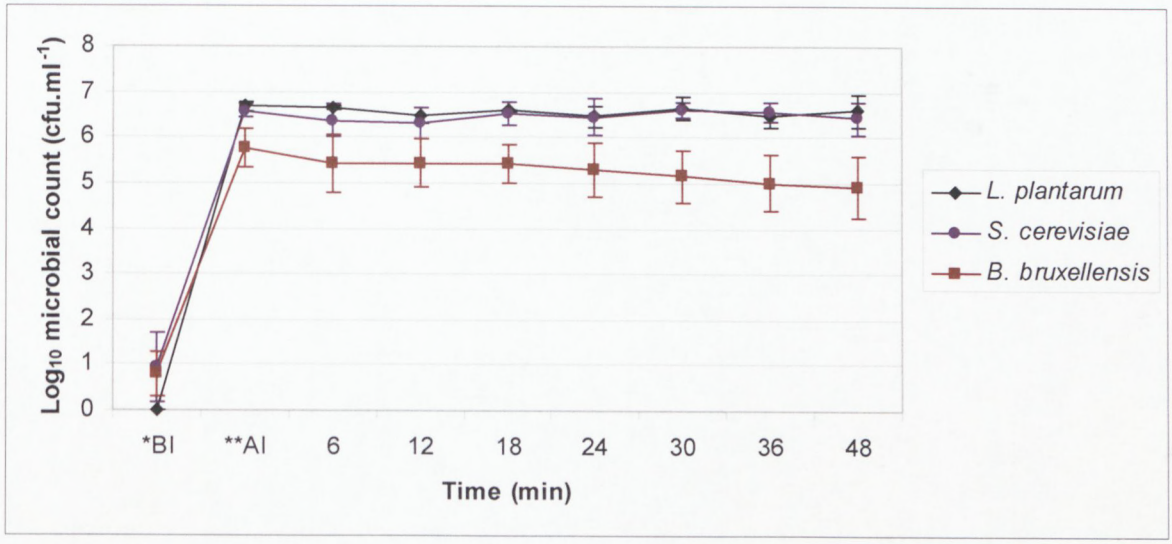
*BI : Before inoculation (No UV-C radiation)

**AI : After inoculation

Each of the microorganisms was inoculated to a concentration of 1 x 10^6 cfu.ml^{-1}. Since no significant difference were found between the inactivation of microorganisms in Pinotage wine containing ± 0 mg.l^{-1} and ± 24 mg.l^{-1} free SO_2, this experiment was only performed in Pinotage wine containing ± 24 mg.l^{-1} free SO_2. The Pinotage wine containing ± 24 mg.l^{-1} free SO_2 that received no UV-C radiation was used as the control. The microbial counts of *L. plantarum* 130, *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13 were relatively constant for ± 48 minutes whereafter a 0.08, 0.84 and 0.15 log_{10} reduction had followed for the respective microorganisms. Based on the results of the control (Fig. 17), it may be assumed that microbial reduction may be primarily attributed to UV-C radiation and not because of the antimicrobial effect of the SO_2 present in Pinotage wine.

After 459 J.l^{-1}, a 0.41, 0.26 and 0.26 log_{10} reduction (Fig. 18) were obtained for *L. plantarum* 130, *B. bruxellensis* ISA 1649 and *S. cerevisiae* VIN13, respectively. A dosage of 918 J.l^{-1} had 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 1 377 J.l^{-1}. 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 SO_2 (Fig. 16) were significantly higher ($p<0.05$) than the reductions that was found for the cocktail (Fig. 18). It is, however, possible that the high microbial concentrations of the cocktail might have influenced the absorbance coefficient of the Pinotage negatively, thereby reducing the UV-C efficacy in the matrix (Koutchma *et al.*, 2009). Therefore, the population size should also be considered as a function of UV-C efficacy.

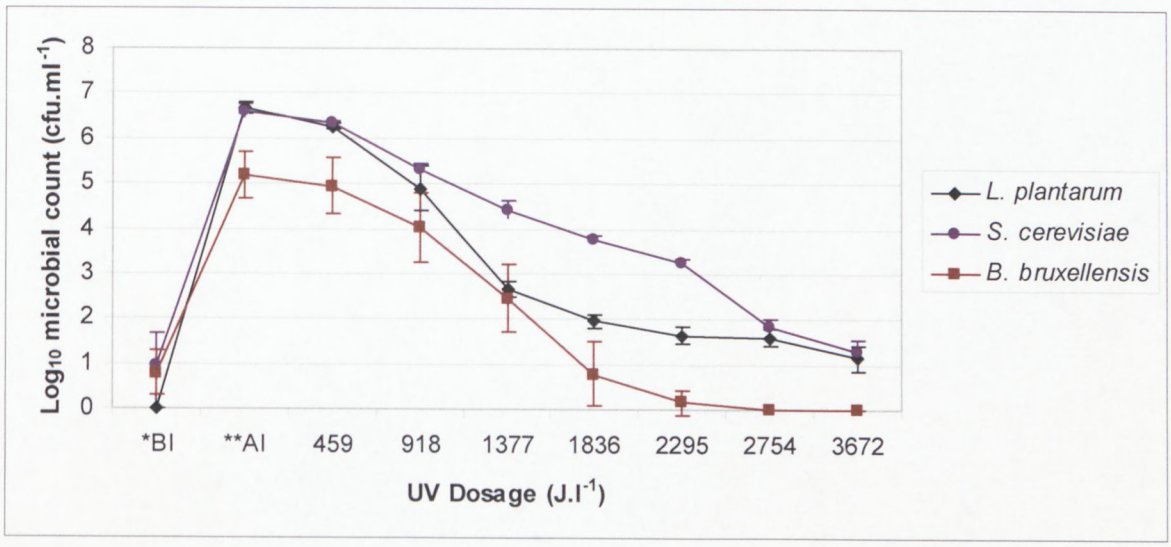
Currently, no studies have been reported pertaining to the inactivation of wine-specific microorganisms with UV-C radiation. Based on the results obtained, UV-C radiation holds promise as an alternative technology to limit microbiological induced defects in white and red wine. UV-C radiation may be applied before alcoholic fermentation and malolactic fermentation to facilitate the growth of inoculated starter cultures by eliminating indigenous microflora. Apart from sulphiting, the microbial load is effectively reduced in red wines and some white wines after MLF or just before bottling by filtration in order to prevent wine of becoming spoiled (Ubeda & Briones, 1999). However, microorganisms such as AAB, LAB and *Brettanomyces* can enter a



*BI : Before inoculation (No UV-C radiation)

**AI : After inoculation

Figure 17 The average $\log_{10}$ microbial counts of the control for *Lactobacillus plantarum* 130, *Saccharomyces cerevisiae* VIN13 and *Brettanomyces bruxellensis* ISA 1649 inoculated into Pinotage wine containing ± 24 $mg.l^{-1}$ free SO_2.



*BI : Before inoculation (No UV-C radiation)

**AI : After inoculation

Figure 18 The average log_{10} microbial counts of *Lactobacillus plantarum* 130, *Saccharomyces cerevisiae* VIN13 and *Brettanomyces bruxellensis* ISA 1649 inoculated into Pinotage containing ± 24 $mg.l^{-1}$ free SO_2 after exposure to various UV dosages at a constant flow rate of 4 000 $l.hr^{-1}$.

viable but non-culturable (VBNC) state due to the presence of SO_2 and limited availability of oxygen in wine (Millet & Lonvaud-Funel, 2000). Since the cells persist with a smaller size in the VBNC state than usual, the cells can pass through the membrane (0.45 µm), and eventually cause spoilage once favourable conditions arise. Smaller pore size membranes can effectively retain the microorganisms in a VBNC state, but it can have a detrimental affect on the aroma, flavour, colour (pigments and tannins) and palate weight of the wine (Jackson, 1994; Suárez *et al.*, 2007; Oelofse *et al.*, 2008). This novel technology may serve as a cost effective alternative to filtration to microbiologically stabilise wine after MLF without affecting the sensorial properties of the wine. UV-C radiation may also be applied before aging to inactivate existing microorganisms, thereby avoiding undesirable modifications of wine and microbial contamination of contact surfaces such as barrels and winery equipment. Finally, UV-C radiation may represent the ultimate preservation technique before bottling.

Conclusion

This is the first report on the use of UV-C radiation to inactivate microorganisms associated with wine production. The findings of this study have revealed that the microbial reduction is in correlation with the applied 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 3 672 $J.l^{-1}$. The applied UV-C dosage ($J.l^{-1}$) depends largely on the physical composition of the liquid such as turbidity (suspended and soluble solids), colour and absorbance.

Significant differences ($p<0.05$) in microbial reductions were obtained in Chenin blanc and Shiraz juice. This may be due to the darker colour and higher level of turbidity of Shiraz juice which might have lowered the transmittance of UV-C rays to the microorganisms because of absorbance. The suspended and soluble solids in Shiraz juice were probably impenetratable to light and therefore may have provided effective shields for microorganisms against UV-C rays, allowing them to survive UV-C radiation.

Statistical analysis has also demonstrated no significant difference ($p<0.05$) in microbial reductions obtained in wine containing no and low levels of SO_2. The controls have also verified that microbial reductions obtained in this study were mainly due to the germicidal activity of UV-C radiation and not because of the antimicrobial effect of SO_2 present in wine. Significant microbial reduction differences ($p<0.05$) were also found in Chardonnay and Pinotage wine. It is clear that better microbial reductions were obtained in white wine compare to red wine which indicates that the clear Chardonnay wine was more UV-C transparent than Pinotage wine. Since Pinotage was dark red in colour, the transmittance of the UV-C rays in the wine might have been limited because of absorbance. Colour compounds such as anthocyanins and phenols present in Pinotage wine might have absorbed the UV rays which most probably had interfered with the transmittance of UV-C rays to the microorganisms, allowing them to survive UV-C radiation. This finding show similar trend to the Beer-Lambert-Bouger's law, which claims that light intensity is indirectly proportional to absorbance of a matrix.

A significant difference ($p<0.05$) in microbial reductions were also obtained in Pinotage with individual and cocktail inoculated microorganisms. It is postulated that the high microbial concentrations of the cocktail might have influence the absorbance coefficient of Pinotage negatively, thereby reducing the transmittance of UV-C rays. These results are inaccordance with previous findings that have indicated that the initial population size is also a function of UV-C efficacy.

The morphology of each microorganism was different, and, therefore different inactivation profiles were obtained for each microorganism. UV-C resistance of each microorganism is based on the amount of thymine and cytosine pyrimidines in the DNA material, which is the primary site where UV-C light form dimers and eventually prevents the cell of performing normal replication. Since yeasts contain less of these essential pyrimidines in their genetic material, it is expected to be more UV-C resistant than bacteria. Yet, *S. cerevisiae* VIN13 were less UV-C resistant than bacteria in Chardonnay wine. This may be attributed to the larger size of yeast cells which may be easily penetratable to UV-C rays, rendering them less UV-C resistant.

UV-C radiation has offered a wide spectrum of effectiveness against wine-associated microorganisms such as *Brettanomyces*, *Saccharomyces*, *Acetobacter*, *Lactobacillus*, *Pediococcus* and *Oenococcus*; therefore UV-C radiation may hold promise as an alternative technology to inactivate spoilage microorganisms at different stages of vinification in conjunction with reduced SO_2 levels. These results clearly indicate that UV-C radiation of grape juice and wine is rather challenging due to variable UV-C resistance of microorganisms and physical properties of the liquids such as colour and turbidity that influence UV-C efficacy. A standard procedure of UV-C radiation for controlling spoilage microorganisms in different varieties of grape juices and wine styles will not be feasible. The effective UV-C dosage to reduce microorganisms to acceptable levels in grape juices and wines should therefore be adjusted according to the type of liquid (colour, turbidity and absorbance), microorganism (bacteria, yeasts and moulds) and initial concentration of microorganisms.

Preliminary tests were done by SurePure to determine the effect of UV-C radiation on the chemical structure of wine. No modifications on the chemical structure such as pH, TA and alcohol content of wine were detected, after 918 and 1 377 $J.l^{-1}$. The brown pigment concentrations, which generally indicate oxidation in wine have also remained constant; thus the UV-C dosages tested had no effect on the chemical composition and colour of wine. Although, 3 672 $J.l^{-1}$ resulted in the best microbial inactivation in this study, no tests was done to verify its effect on the chemical and sensorial structure of wine.

Further studies pertaining to the long and short term effect of UV-C radiation on the sensorial and chemical profile of wine are imperative. More specifically, the effect of UV-C radiation on the pH, TA, volatile acidity, phenolic compounds, protein stability and oxidation stability in wines should be analysed. The ability of UV-C radiation to cause pinking in white wine should also be confirmed. Kinetic inactivation models for target spoilage microorganisms such as LAB, AAB, yeasts and moulds should be calculated and confirmed.

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CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

UV-C (254 nm) radiation is an adiabatic process that has a germicidal effect on a variety of wine-associated microorganisms such as *Lactobacillus*, *Pediococcus*, *Oenococcus*, *Acetobacter*, *Brettanomyces* and *Saccharomyces* in white and red South African grape juices and wines. The germicidal efficacy of UV-C radiation is in correlation with the applied UV-C dosage ($J.l^{-1}$). As a result, the best microbial inactivation was obtained after 3 672 $J.l^{-1}$ in this study. However, the UV-C transmittance, insoluble solids (turbidity) and viscosity of the liquid food is a function of the applied UV-C dosage.

A significant difference ($p<0.05$) in microbial reduction was obtained in Chenin blanc and Shiraz juice. Unlike Chenin blanc juice, Shiraz juice appeared visibly more turbid due to the presence of soluble and suspended solids. Solids generally have a high light absorbance and are unable to transmit UV-C light. These solids may have provided effective shields for the microorganisms against the UV-C rays in Shiraz juice, allowing them to survive UV-C radiation. Shiraz juice is also dark red in colour, which may have limited the transmittance of UV-C rays to the microorganisms in the juice because of absorbance.

Statisical analysis has also demonstrated a significant difference ($p<0.05$) in the efficacy of UV-C radiation to inactivate the microorganisms in Chardonnay and Pinotage wine. In general, red wine has a higher absorption coefficient than white wine at 254 nm due to the presence of colour compounds such as anthocyanins and phenols. Lower microbial reductions were obtained in Pinotage wine in comparison with Chardonnay wine. The lower microbial reductions obtained in Pinotage wine may therefore be attributed to its darker colour that resulted in a high absorption coefficient, which may have reduced the percentage UV-C transmittance in the wine. Since Chardonnay is a clear white wine, it has a lower absorption coefficient meaning that it is more UV-C transparent and thus better microbial reductions resulted in Chardonnay wine compare to Pinotage wine. The results obtained show similar

trends to the Beer-Lambert-Bouger's law, which claims that light intensity is indirectly proportional to absorbance of the liquid.

The microbial reductions of the cocktail were significantly different ($p<0.05$) from the individually inoculated microorganisms in Pinotage wine. The high microbial concentrations of the cocktail may have affected the absorption coefficient of the Pinotage wine negatively, and thereby reducing the efficacy of UV-C radiation. In light of this, it may also be assumed that the initial microbial population play a significant role on UV-C efficacy.

No significant difference ($p>0.05$) in microbial reductions were obtained in wine containing no and low levels of sulphur dioxide (SO_2). The controls of the wines have also verified that the antimicrobial effect of SO_2 was only visible after ±30 and ±48 minutes for Chardonnay and Pinotage wine, respectively. This finding clearly indicates that the microbial reductions obtained may be attributed to UV-C radiation and not as a result of the SO_2 present in wine.

Since the microorganisms have unique morphology features, a significant difference ($p<0.05$) in UV-C resistance of the microorganisms were observed. The amount of thymine and cytosine pyrimidines in the DNA material play an important role in the UV-C resistance of the microorganisms, as UV-C radiation especially targets these pyrimidines to form dimers, rendering the cell incapable of performing normal transcription and replication. The yeast, *Saccharomyces cerevisiae,* was less resistant to UV-C radiation than bacteria in the Chardonnay wine. Based on the literature, yeasts are expected to be more UV-C resistant than bacteria as it contains less of the essential pyrimidines such as thymine and cytosine in their DNA material which are required for inactivation. However, it is postulated that the UV-C rays may have easily penetrated the yeasts cells due to their larger size.

According these results, it is clear that the use of UV-C radiation to inactivate microorganisms in grape juices and wines are rather challenging due to variability in liquid properties such as colour and turbidity that may influence UV-C efficacy. The effective UV-C dosage to reduce microorganisms to acceptable levels in grape juices and wines should therefore be adjusted according to the type of liquid (colour, turbidity and absorbance), microorganism (bacteria, yeasts and moulds) and

concentration of microorganisms. Regardless, UV-C radiation has represented significant germicidal effect against wine-associated microorganisms in white and red grape juices and wines. Therefore, UV-C radiation may hold potential of stabilising grape juice and wine microbiologically in conjunction with reduced SO_2 levels.

Recommendations

Further research is required on the effect of UV-C radiation on other problematic spoilage microorganisms such as *Botrytis, Cryptococcus*, *Candida*, *Pichia*, *Metschnikowia* and *Zygosaccharomyces*. Kinetic inactivation models for different target microorganisms such as yeasts, acetic acid bacteria, lactic acid bacteria and moulds should be calculated and established. The benefits of using this technology in the wine industry should also be quantified by studies pertaining to the effect of UV-C radiation on the chemical structure of wine. These studies should specifically focus on the effect of UV-C radiation on the pH, titratable acidity and volatile acidity of grape juice and wine. The effect of UV-C radiation on protein stability and oxidative stability of grape juice and wines should also be evaluated. The long and short term effect of UV-C radiation on the sensorial quality such as colour and polyphenol levels of wines should also be investigated.