The production of reduced-alcohol wines using Gluzyme Mono® 10.000 BG-treated grape juice

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Submitted for publication: June 2009
Accepted for publication: August 2009
Key words: Glucose oxidase, gluconic acid, reduced- or low alcohol wine

High alcohol wines have become a major challenge in the international wine trade. Several physical processes are used to produce wines with reduced-alcohol content, all of which involve the selective extraction of ethanol based on volatility or diffusion. In this study, the possibility of Gluzyme Mono® 10.000 BG (Gluzyme) (Novozymes, South Africa) to reduce the glucose content of synthetic grape juice before fermentation was investigated in order to produce wine with reduced-alcohol content. Gluzyme is a glucose oxidase preparation from *Aspergillus oryzae*, currently used in the baking industry. Glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide (H_2O_2) in the presence of molecular oxygen. Gluzyme was initially used in synthetic grape juice, where different enzyme concentrations and factors influencing its efficiency were investigated under winemaking conditions. The results showed up to 0.5% v/v less alcohol at an enzyme concentration of 20 kU compared to the control samples. This reduction in alcohol was increased to 1 and 1.3% v/v alcohol at pH 3.5 and pH 5.5 respectively in aerated (8 mg/L O_2) synthetic grape juice using 30 kU enzyme. Secondly, Gluzyme was used to treat Pinotage grape must before fermentation. Gluzyme-treated wines at 30 kU enzyme concentration after fermentation contained 0.68% v/v less alcohol than the control wines. A decrease in acetic acid concentration of the treated compared to control wines was also observed.

In the production of fermented beverages such as wine, yeast facilitates the biochemical conversion of sugars to ethanol and carbon dioxide and produces a number of sensorially important metabolites such as higher alcohols, organic acids and esters that will consequently influence product quality (Romano *et al*., 1998; Lambrechts & Pretorius, 2000).

Saccharomyces cerevisiae is widely used as a wine yeast starter culture. Yeast species and strains vary in their abilities to utilise carbohydrates in the formation of alcohol and other by-products as well as in their ability to grow in various concentrations of alcohol (Zoecklein *et al*., 1995). Most strains of *S. cerevisiae* are inhibited as alcohol levels reach 14 to 15% v/v (Zoecklein *et al*., 1995). However, several strains are more alcohol tolerant. The quantity of alcohol and CO_2 formed as well as the nature and concentration of by-products vary with yeast strain, temperature of fermentation and the extent of aeration. Ethanol inhibits the growth of lactic acid bacteria, which then inhibits malolactic fermentation (Jackson, 1994). As a result of a growing demand worldwide for wines containing lower levels of alcohol, there is a continuous quest for new techniques that can be used to produce reduced-alcohol wines. However, in the New World, winemaking practices favour the production of wines with high flavour intensity, prepared from fully matured grapes. In most cases, the juice obtained from such grapes contains very high sugar concentrations, resulting in wines with high levels of alcohol. Furthermore, the high alcohol concentration may affect the quality of wines by altering the volatility of aroma compounds (Athes *et al*., 2004).

Several physical processes are available for the production of reduced-alcohol wines. However, these processes involve selective extraction of ethanol based on volatility or diffusion (Pickering, 2000). Despite their efficacy, these processes are expensive and difficult to perform, and can also affect the flavour balance through the loss of aroma compounds (Heux *et al*., 2006). One biological alternative would be to use yeast strains that produce low ethanol yields, a method that promises to be faster and less expensive (Heux *et al*., 2006). Unfortunately, such yeast is not yet available.

Several attempts have also been made through genetic engineering to reduce the ethanol yield of *S. cerevisiae* by diverting sugar metabolism into by-products other than ethanol, for instance yeast strains producing more glycerol and less ethanol (Remize *et al*., 1999; Nevoigt *et al*., 2002). Another strategy has been to express lactate dehydrogenase in yeast, resulting in the simultaneous conversion of pyruvate into ethanol and lactate, thereby reducing the ethanol yield (Dequin *et al*., 1999). In addition, other approaches have been based on the removal of fermentable sugar from grape must, which has been achieved by using glucose oxidase before fermentation to catalyse the oxidation of glucose to gluconolactone in the presence of molecular oxygen (Pickering *et al*., 1998, 1999a,b,c). A yeast strain able to produce glucose oxidase during fermentation and its potential to produce low-alcohol wine has been developed and produced lower ethanol levels (Malherbe *et al*., 2003).

Gluzyme Mono® 10.000 BG (Novozymes), hereinafter referred to as Gluzyme, is a glucose oxidase preparation from *Aspergillus*

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Acknowledgements: We would like to thank the Institute for Wine Biotechnology and Stellenbosch University for funding this project and our sincere appreciation to J. van Aswagen of Novozyme, South Africa for his valuable discussions on Gluzyme.

niger, produced by a genetically modified *Aspergillus oryzae* microorganism. Gluzyme catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide. This enzyme is relatively inexpensive compared to pure glucose oxidase. Its main application is to strengthen gluten as well as to facilitate the baking process. The present study aimed at investigating the effect of Gluzyme on the glucose content of synthetic grape juice under winemaking conditions in order to reduce the alcohol content of the resultant wine after fermentation. Different factors influencing Gluzyme's efficiency such as aeration, pH and temperature in synthetic grape juice were also assessed. Finally, Gluzyme was used to treat grape juice before fermentation in order to confirm results obtained in synthetic grape juice.

MATERIALS AND METHODS

Gluzyme treatment in a synthetic grape juice: laboratory-scale fermentation

Media preparation

Synthetic grape juice was prepared as described by Bely *et al*. (1990). Initially, 40 g/L sugar (glucose: fructose ratio of 1:1) was used. In subsequent trials using synthetic grape juice, the sugar concentration was increased to 200 g/L (glucose: fructose ratio of 1:1). The acid concentrations were adjusted as follows: 0.5 g/L citric acid, 1.0 g/L malic acid and 2.0 g/L tartaric acid. The pH of the synthetic grape juice was adjusted to pH 3.3 using sodium hydroxide (10 M NaOH). All the trials performed using synthetic grape juice were carried out in 500 mL Erlenmeyer flasks with each containing 200 mL of synthetic grape juice in triplicate. The synthetic grape juice was sterilised by filtration through 0.22 µm pores and all equipments were sterilised before use. The Erlenmeyer flasks were sealed with fermentation airlocks.

Gluzyme treatments

Gluzyme (Novozymes, (Pty) Ltd, Benmore, South Africa) was used to treat synthetic grape juice before fermentation. Its concentration is 10,000 glucose oxidase units per gram (GODU/g). Gluzyme recommended dosage range for the baking industry is 0.25–5 g per 100 kilogram (kg) of flour, which corresponds to 25–500 GODU per kg flour. Gluzyme optimum dosage varies depending on the flour quality, formulation and processing and should therefore be determined through baking trials.

According to the manufacturer, like all other glucose oxidases, a higher dosage of Gluzyme may cause off-flavours during a long fermentation. The active components of Gluzyme are readily soluble in water at all concentrations found under normal usage. However, water solutions will become turbid because the enzyme is encapsulated with wheat flour.

The effect of Gluzyme dosage on glucose content was investigated initially in a synthetic grape juice at four different enzyme concentrations: 0, 5, 10 and 20 kilo units (kU) conducted at pH 3.3 using 40 g/L sugar (glucose: fructose ratio of 1:1).

The synthetic grape juice was treated with Gluzyme before fermentation. The enzyme reaction was performed for a period of nine to 24 hours with the first sampling one hour after enzyme addition and thereafter every three hours for nine hours and after 24 hour. In order to stop the enzymatic reaction, 200 µL of 4 M sulphuric acid was added to the sample (1.8 mL). At the end of the enzyme treatment, all samples were inoculated with *S. cerevisiae* VIN13 (Anchor Yeast Biotechnologies (Pty) Ltd, South Africa) ($1x10^6$ cells/mL) from pre-grown cultures at 30°C for 72 hours in a Yeast Peptone Dextrose (YPD, Merck, Johannesburg, South Africa) broth. The yeast ($1x10^6$ cells/mL) was spinned down at 5 000 rpm for five minutes and re-suspended in 20 mL sterile distilled water before inoculation. Fermentation was allowed to proceed to dryness. The fermentation process was monitored by recording weight loss on a daily basis. Ethanol concentration at the end of fermentation was determined using HPLC (see below).

Glucose and gluconic acid determination

D-Glucose and D-gluconic acid concentrations were measured enzymatically using enzymatic kits (R-Biopharm AG, D-64293 Darmstadt, Germany) with a 1 mL total assay volume in disposable cuvettes using spectrophotometer measurements at 340 nm.

Ethanol determination

Ethanol concentration was determined by HPLC (Castellari *et al*., 2000). The standard solutions were prepared as follows: 1, 4, 8 and 12% v/v ethanol, using 96% ethanol (Merck) and diluted five times with MilliQ water (Millipore, Microsep, Sandton, South Africa). Undiluted samples were filtered through a 0.22 µm sterile syringe filter and diluted five times with MilliQ water for analysis on the HPLC to ensure that ethanol content was within the calibration range. The solvent, standard solutions and all samples were also filtered through a 0.22 µm filter paper before running them on the HPLC. A Waters 717 auto sampler and refractive index detector was used with Agilent 1100 binary pump and Millennium software. Separation was achieved on a Biorad, Aminex HPX-87H ion exclusion column 300x7.8 mm at an injection volume of 5 µL. An isocratic flow rate of 5 mM H_2SO_4 was used at 0.6 mL/min at 45°C.

Factors influencing Gluzyme efficiency

Factors such as aeration, pH and temperature were investigated using Gluzyme-treated synthetic grape juice containing 200 g/L sugar (glucose: fructose ratio of 1:1). Each factor was investigated as a separate experiment according to the methods as described below. The amount of enzyme used in all the experiments was 30 kU. The enzyme treatment was performed for nine hours. At the end of the nine-hour period, all samples were inoculated with VIN13 ($1x\ 10^6$ cells/ mL). Fermentation was monitored by recording the weight loss on a daily basis. All the experiments were carried out at a laboratory temperature of 20 to 22 °C, except for the temperature experiment which was conducted at 15 and 25°C. The completion of alcoholic fermentation was confirmed by an estimation of the sugar content using Clinitest tablets (Bayer Corporation, New York, USA 10591-5097). Ethanol concentration was determined by HPLC as described in the above mentioned method for ethanol analysis.

Aeration

Air was introduced into the synthetic grape juice by pouring the synthetic grape juice from one container to another to obtain three different dissolved oxygen levels, which were measured using oxygen meter (Oxi 330 and Oxi 330i, Merck). The control synthetic grape juice was sparged with nitrogen gas (N_2) (Afrox, Epping, Cape Town, South Africa) to lower the oxygen concentration to <1 mg/L O_2. In the second treatment, the oxygen level was 2 mg/L. In the third treatment, 4 mg/L oxygen was achieved. At each level of dissolved oxygen, a control (without enzyme) and Gluzyme-treated samples were included. Once the different oxygen levels achieved, the synthetic grape juice was transferred into 500-mL

Erlenmeyer flasks that had been sparged with N_2. The oxygen levels used are normally found in winemaking conditions in the grape must (Du Toit, 2006).

Further trials with aeration included increased levels of dissolved oxygen up to 8 mg/L. The pH of the synthetic grape juice was adjusted to pH 3.5 and pH 5.5.

pH

The pH of the synthetic grape juice was adjusted to three different pH levels using 10 M NaOH: pH 3, pH 3.5 and pH 4. At each pH level, control without enzyme and enzyme treatment were included. Further pH experiments were carried out under the same conditions, which included pH 3.5 and pH 5.5 with aeration; with the latter pH regarded as the optimum pH for the enzyme activity (Pickering *et al*., 1998).

Temperature

Two different temperatures 15 and 25°C, were evaluated for their effect on Gluzyme efficiency in synthetic grape juice adjusted to pH 3.3. The synthetic grape juice was left overnight to achieve the desired temperature. The synthetic grape juice was inoculated with the enzyme the following day.

Statistical analysis

The ANOVA method was used for the statistical analysis of the data to determine if there were differences between control and treated samples using Statistica version 8, Statsoft. One-way ANOVA or two-way ANOVA, depending on the parameters of interaction, was used. Statistical analysis was performed for all treatments carried out in synthetic grape juice, but not in Gluzyme-treated wines due to limited replications.

Small-scale wine vinification of Gluzyme Mono® 10.000 BG-treated grape juice

Preparation of must

Pinotage grapes from the Stellenbosch area, South Africa, were used from the 2007 harvesting season. The grapes were crushed and destemmed, and the must was divided equally into 10-L buckets. The skins were separated, mixed and divided equally in buckets to ensure that every treatment received equal amounts of juice and skins. The analysis of the must pH, sugar concentration (°Brix), titratable acidity (TA) and SO_2 was performed by using a Metrohm Titrino apparatus (702 SM Titrino, Swiss lab) equipped with a 722 stirrer (Swiss lab). No acid adjustment or SO_2 additions were made to the must before Gluzyme treatment. Full analysis of the juice was performed using the Grape Scan FT120 instrument (Foss Electric, Denmark). All samples were degassed by filtration prior to the analysis by using a filtration unit (type 79500, Foss Electric, Denmark) with filter paper circles graded at 20–25 µm with a diameter of 185 mm.

Treatment of grape juice with Gluzyme

The effect of Gluzyme on the glucose conversion efficiency in Pinotage grape juice was investigated using 3 g/L (30 kU) enzyme. Mean composition parameters of the juice analysed were as follows: sugar concentration (°Brix), pH, acidity as well as FSO_2 and TSO_2. The juice was treated with Gluzyme prior to fermentation. These treatments were performed with two different batches of Pinotage grapes (A and B). The enzymatic reaction was performed for six hours in wine A and the sugar concentration was analysed after the enzyme treatment for both control (without enzyme) and treated samples. The treatments were carried out in triplicate. In wine B, Pinotage grape juice was treated with the enzyme prior to fermentation for 48 hours. In this case treatments were carried out in duplicate.

Wine fermentation

At the end of the Gluzyme treatments, all samples were inoculated with VIN13 strain at 0.3 g/L. All fermentations were conducted at 25°C. Pumping-over was performed two to four times a day.

The sugar content of the fermenting must was monitored by using the Grape Scan FT120. The must was fermented dry on the skins and pressed after the completion of alcoholic fermentation. A small-scale basket press was used, and the skins were pressed up to 1.5 bar. The wine from each treatment was collected after pressing and kept separately in 4.5-L glass bottles. The wines were filtered, bottled and sealed in 750 mL screw-cap bottles. The wines were kept at 15°C.

Analyses of standard parameters in wine

Wines A and B were analysed for pH, volatile acidity (VA), total acidity (TA), malic acid (MA), lactic acid (LA), glucose and fructose, ethanol and glycerol using the Winescan FT120. Volatile flavour compounds were also analysed using GC. Determination of the ethanol content and volatile flavour compounds are as described below.

Determination of ethanol content

The Winescan FT120 mid-infrared spectrometer (Foss Electric, Ltd, Hillerød, Denmark) equipped with a purpose-built Michelson interferometer was used for the quantification of the ethanol content of the wines. The apparatus has ready-to-use calibration models for quantification of the important wine parameters, including ethanol (Gishen & Holdstock, 2000; Nieuwoudt, 2004).

Determination of volatile flavour compounds

The volatile aroma profile of the wines (esters, higher alcohols and volatile acids) was determined by analysing a diethyl ether extract of the wine on the GC-FID (Malherbe, 2007).

The extracts for injection into the GC were prepared by extracting 5 mL of wine with internal standard, 4-Methyl-2-Pentanol (Fluka, ≥ 97%) and 100 µL of 0.5 mg/L solution in wine stimulant (water pH, 3.5; 2.5 g/L tartaric acid and 12% ethanol) with 1 mL of diethyl ether (99.5%, Merck).

The wine/ether mixture was followed by sonication for 5 minutes in an ultrasonic bath to facilitate the mixing of the diethyl ether layer and the wine. This was followed by centrifugation for 3 minutes at 4000 rpm to separate the diethyl ether layer from the wine. The diethyl ether layer was removed from the wine and dried on anhydrous sodium sulphate (Na_2SO_4) (99%, Merck). The dried diethyl ether extract was transferred to a vial insert and capped thereafter injected into the GC-FID. The concentrations of the volatile compounds were calculated by comparing their retention times and peak areas to those of known standards.

RESULTS AND DISCUSSION

Synthetic medium treated with Gluzyme Mono® 10.000 BG: Laboratory-scale fermentation

Enzyme dose

An effective Gluzyme dose was needed for use in winemaking to reduce the glucose content of grape must in order to produce a wine with reduced alcohol content. Several different enzyme

concentrations were investigated using synthetic grape juice. These were as follows: 0, 5, 10 and 20 kU. The experiments were carried out initially in synthetic grape juice (40 g/L sugar), with and without aeration. Fig.1 shows the estimated glucose and gluconic acid concentrations of treated and control samples.

A decrease in glucose and an increase in gluconic acid concentrations were observed. This decrease in glucose concentration was more pronounced in aerated (Fig. 1A) in comparison to non-aerated (Fig. 1B) synthetic grape juice, which could possibly be explained by the fact that the enzymatic reaction requires oxygen for effective conversion of glucose to gluconic acid. The enzymatic reaction seemed very slow and it was assumed that because the enzyme is encapsulated with wheat flour, it might require longer contact time for the enzymatic reaction to occur, considering that these were not at the optimal conditions for the enzyme. Although an extrapolation was made from 9 to 24 hours, clearly gluconic acid was higher at higher concentrations of Gluzyme (20 kU). Therefore, if the concentration of the enzyme is increased, more products will be formed. The initial sugar concentration was 40 g/L (glucose: fructose of 1:1 ratio), which corresponds to 0.1 mol/L glucose. At the end of the nine hours of Gluzyme treatment, 0.04 mol/L gluconic acid was obtained from the 20 kU enzyme concentration. This indicated that 0.06 mol/L glucose was converted resulting in 0.04 mol/L gluconic acid that was formed directly from the action of the enzyme on glucose.

It has been shown that both rate and extent of glucose conversion by pure GOX increased with increasing enzyme dose (Pickering *et al*., 1998), and this is consistent with results reported by Villettaz (1987) and Heresztyn (1987) up to the maximum dose (1 g/L) used. Gluzyme seemed to follow the same pattern in trials carried out in synthetic grape juice with up to 20 kU concentration which led to a 0.5% v/v decrease in ethanol concentration.

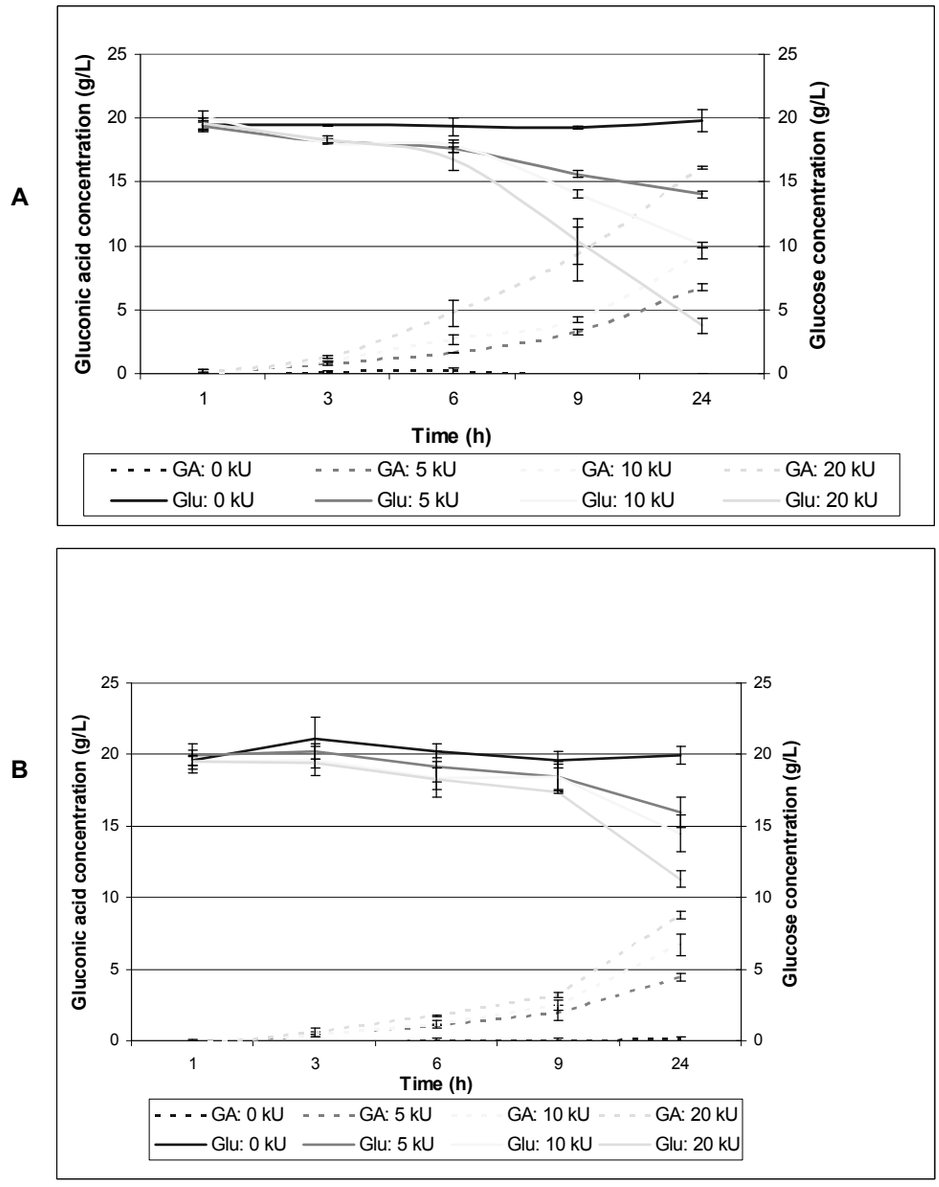

FIGURE 1

Glucose (Glu) and gluconic acid (GA) concentrations of Gluzyme-treated synthetic grape juice (40 g/L sugar) before fermentation at different enzyme concentrations. A: with aeration and B: without aeration.

Fermentations were also carried out using 200 g/L sugar (glucose: fructose ratio of 1:1) and the accumulated weight loss was used to formulate the fermentation curves as shown in Fig. 2A. The control (without enzyme) fermented slower than the treated samples, however, all samples fermented to dryness.

The wheat flour used in standardisation of this enzyme could act as nutrient to the yeast cell or as yeast solid matter resulting in more rapid fermentation in the treated than in the control samples. Ethanol concentration at different enzyme concentrations after fermentation showed up to 0.5% v/v less ethanol compared to the control at an enzyme concentration of 20 kU (Fig. 2B).

Factors influencing Gluzyme's efficiency

Aeration

The effect of different oxygen levels on the activity of Gluzyme and the resultant ethanol concentration is shown in Fig. 3. Oxygen was introduced into the synthetic grape juice by means of aeration before adding the enzyme, a recommended method by the manufacturer, since pure oxygen can give rise to oxidisation of the enzyme. Aeration alone showed no effect on ethanol concentration whereas the effect of enzyme addition showed significant differences ($p < 0.05$) in ethanol concentration. It has previously been shown that oxygen is essential for optimal conversion of glucose into gluconic acid and hydrogen peroxide by pure GOX enzyme. According to Pickering *et al*. (1998), improved GOX performance is suggested at a higher aeration rate, although this effect becomes negligible by the end of the treatment period.

Temperature and oxygen were also not optimally monitored in our experiments. This could have led to less effective aeration resulting in lower enzymatic activity.

pH

Pure glucose oxidase has an optimum pH of 5.5 and a broad pH range of 4–7 (Pickering *et al*., 1998; Borole *et al*., 2005; Hanft & Koehler, 2006). Gluzyme is known to be stable at pH 5.5. Gluzyme was investigated at the following pH: pH 3, pH 4 (which normally occur in wine) and pH 5.5, the optimum pH for the enzyme. There were no differences observed in ethanol concentration at pH 3 and pH 4 between the control and treated samples, only trends of less ethanol being produced at pH 5.5 were observed ($p = 0.05$) in treated compared to the control sample. A possible explanation could be that the enzyme dose of 30 kU might have been insufficient for optimal glucose conversion at lower pH. Thus a higher enzyme dosage should be evaluated at different pH levels, including the enzyme optimal pH of 5.5 to give sufficient evidence on Gluzyme's efficiency for reducing ethanol content in wine.

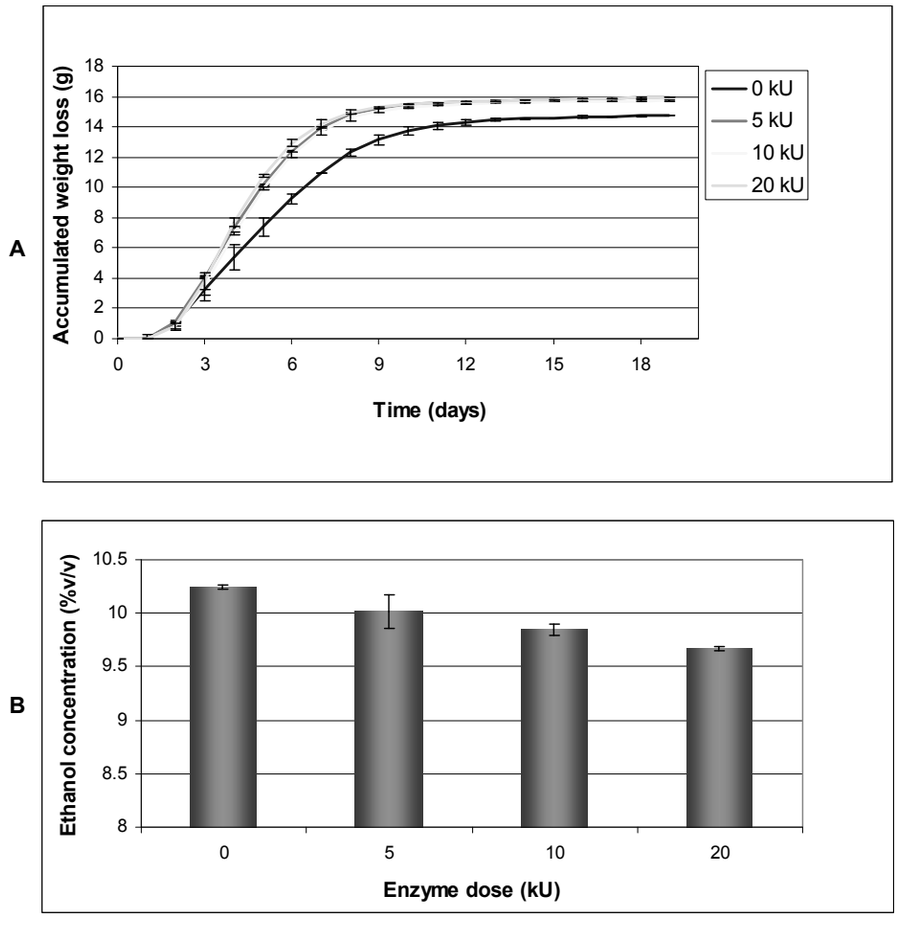

FIGURE 2

Accumulated weight loss of fermented synthetic grape juice (200 g/L sugar) treated with Gluzyme (A). The bars on the graph represent ethanol (EtOH) concentration (%v/v) measured at the end of alcoholic fermentation (B).

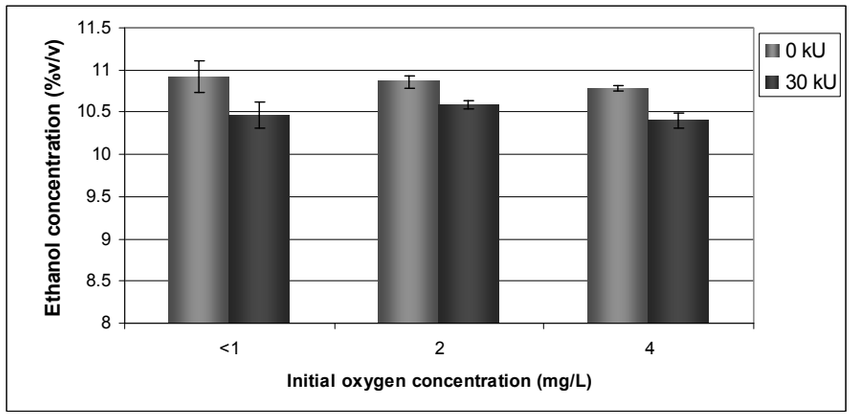

FIGURE 3
Ethanol concentration of fermented synthetic grape juice treated with Gluzyme at different levels of aeration.

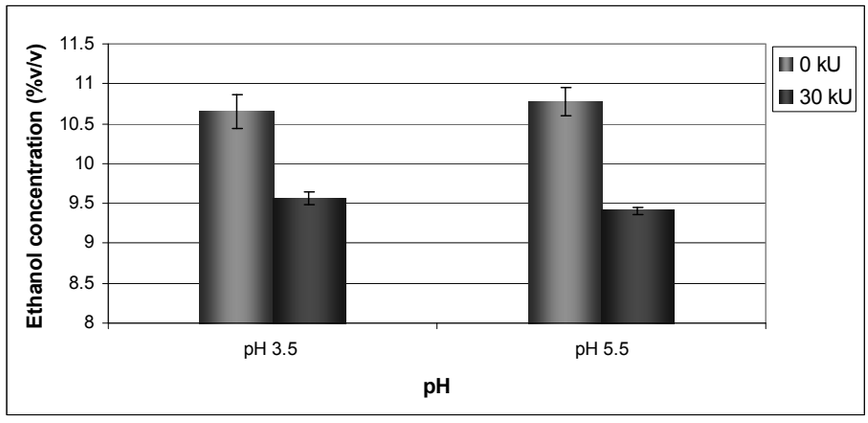

FIGURE 4
Ethanol concentration of fermented synthetic grape juice treated with Gluzyme at different pH levels (pH 3.5 and 5.5) with aeration (8 mg/L O_2).

In later trials using synthetic grape juice, pH was further tested with aeration at 8 mg/L at pH 3.5 and pH 5.5. A reduction in alcohol content of about 1 and 1.3% v/v respectively was obtained using 30 kU enzyme (Fig. 4). This indicated that both aeration and pH could play a major role on Gluzyme activity under winemaking conditions. Low pH has been shown to be the dominant factor limiting the rate and extent of glucose conversion by pure GOX (Pickering *et al*., 1998). It seemed that Gluzyme in this trial followed the same pattern, although, more work still needs to be done to verify these results in grape juice, considering that this was performed in synthetic grape juice. A significant advantage may be obtained if the juice pH is first adjusted to the enzyme optimal pH before treatment.

Temperature

According to the manufacturer, Gluzyme has 100% activity at 60°C whereas a pure GOX optimum temperature range has been reported to be between 30 and 40°C (Whitaker, 1972). For use in grape juice, a desirable temperature (pure GOX) range of between 15 and 20°C has been suggested (Heresztyn, 1987; Villettaz, 1986, 1987). There were no differences observed in GOX performance by Pickering *et al*. (1998) between 20 and 30°C. In this study, Gluzyme was investigated at 15 and 25°C using synthetic grape juice. No differences in Gluzyme performance were observed. As a result, no differences in ethanol concentration were obtained (data not shown). The manufacturer commented that about 10 to 20% Gluzyme activity could be obtained at these temperatures in comparison to the 100% activity at 60°C. In GOX, authors have noted diminished enzyme activity at higher temperatures (Heresztyn, 1987). A change in temperature means a change in one of the reactants such as oxygen and decreased oxygen solubility at high temperatures may be offsetting the expected benefits of temperature rise (Scott, 1975), although this does not seem to have been the case in this study.

Small-scale wine vinification using Gluzyme -treated grape juice

Treatment of grape juice with Gluzyme

During fermentation, the sugar content of wine A was monitored by recording a decrease in °Brix of the fermenting must. Gluzyme-treated samples fermented between 1 and 1.5 °Brix faster than the control (Table 1). The fermentation process of wine A was completed within three days. It should also be considered that other factors could play a role, such as the yeast strain that was used as well as fermentation temperature.

The grape juice analyses at the end of the enzyme treatment (wine B) are shown in Table 2. A reduction in sugar content of about 11 g/L, which corresponded to approximately 0.68% v/v less ethanol, was obtained at the end of the alcoholic fermentation compared to the control wine. This corresponded with the lower ethanol levels obtained in the final wine. The pH of the enzyme-treated wine dropped with an increase in total acidity (Table 3). This can be attributed to the production of gluconic acid, which has also been observed in pure GOX-treated wines (Pickering *et al*., 1998, 1999a). Although gluconic acid concentration was not determined from these wines, the increase in total acidity, decrease in pH as well as the reduction in sugar content indicates that Gluzyme has the potential of reducing the glucose content of wine, as shown in Table 3 (wine B). The six-hour treatment (wine A) did not show differences in ethanol concentration whereas a 48-hour treatment (wine B) was about 0.68% v/v less ethanol compared to the control wines.

Longer processing time could have been necessary in the case of wine A, considering the low pH of the grape juice as compared to the optimal pH of the enzyme as well as the complex composition of the red grape juice that could possibly influence the enzyme efficiency. However, ethanol concentration of wine A after fermentation did not differ from the control although the pH and acidity of the wine seemed to have been slightly affected by the enzyme treatment.

Since wine A was treated with Gluzyme for six hours, it was assumed that the complex medium of red grape could possibly have delayed the enzymatic reaction, considering that the conditions were not optimal for the enzyme. In addition, the enzyme is encapsulated with wheat flour, which could mean that longer contact time might be necessary for effective enzymatic performance.

Volatile flavour compounds

A decrease in acetic acid concentration was observed in the enzyme treated wine B (Table 4) compared to the control wines with an increase in isoamyl alcohol and ethyl lactate. Propanol showed a decrease in the treated compared to the control wines. No differences were observed in the case of Wine A (data not shown).

The lower acetic acid concentration has been observed in pure GOX wines (Pickering *et al*., 1999a). This decrease in acetic acid concentration could possibly result from the antimicrobial activity of the H_2O_2 that is produced by the GOX reaction. The antimicrobial activity of the GOX system is due to the cytotoxicity of the H_2O_2 that is formed, although lowering of the pH by the gluconic acid that is formed may influence the growth of some microorganisms (Fugelsang *et al*., 1995).

CONCLUSIONS

This study was the first investigation into using Gluzyme in winemaking to reduce the ethanol content. A number of trials were conducted in synthetic grape juice and grape must using Gluzyme to determine the effective dose of the enzyme that would significantly reduce the glucose content of the grape must in order to produce a reduced-alcohol wine. Furthermore, researchers determined certain factors that may affect the enzyme efficiency, and how to optimise these factors for the winemaking process. All trials carried out with Gluzyme in synthetic grape juice and

TABLE 1

Mean values of °Brix (in triplicate), monitored during fermentation of wine A.

Fermentation	0 kU	30 kU
Day 1	24.46	23.16
Day 2	10.80	8.96
Day 3	0.60	<0

The standard deviation of control and Gluzyme-treated wines is ≤ 0.5.

TABLE 2

Analyses of Pinotage grape juice at the end of Gluzyme treatment using Foss Winescan (average of duplicate samples) (wine B).

Parameters	0 kU	30 kU
Glucose-Fructose (g/L)	235.50	224
°Brix	23.70	23.25
Density	1.10	1.10
TA (g/L)	3.84	4.80
pH	3.56	3.43

TABLE 3

Analyses performed after fermentation of Gluzyme-treated grape juice on the Foss Winescan. Wine A is the 6-hour Gluzyme treatment and Wine B is a 48-hour treatment of separate batches.

	Wine A		Wine B	
Parameters	0 kU	30 kU	0 kU	30 kU
pH	3.95	3.84	3.77	3.63
VA (g/L)	0.36	0.30	0.42	0.33
TA (g/L)	5.73	6.32	6.21	6.93
MA (g/L)	2.27	2.38	2.06	1.79
LA (g/L)	0.17	0.20	0.32	0.65
Glucose (g/L)	0.97	0.95	0.81	0.91
Fructose (g/L)	1.0	0.94	0.74	0.66
Ethanol (% v/v)	14.34	14.30	13.30	12.62
Glycerol (g/L)	10.33	11.46	10.78	10.92

VA: Volatile acidity; TA: Total acidity; MA: Malic acid; LA: Lactic acid

TABLE 4

Volatile flavour compounds of wines made from Gluzyme-treated grape juice.

	Wine B	
Volatile compounds (mg/L)	0 kU	30 kU
Ethyl acetate	121.07	90.0
Propanol	122.17	107.91
Isoamyl alcohol	216.29	220.14
Ethyl lactate	109.36	136.52
Acetic acid	640.42	592.25

The relative standard deviations of volatile compounds are lower than 5%.

grape must in our experimental conditions resulted in complete alcoholic fermentations.

The results showed up to 0.5% v/v less ethanol was obtained in enzyme treated synthetic grape juice at an enzyme concentration of 20 kU. The effects of aeration in combination with pH seemed to have improved the reduction in ethanol concentration of the treated than control samples. Raising the pH of the synthetic grape juice at high aeration rate prior to treatment with Gluzyme appeared to be most effective in reducing the glucose content of the must. Different temperatures did not lead to major differences in ethanol concentration under the conditions tested.

The results presented here are an indication of how Gluzyme efficiency would be influenced by these factors. However, more work is required to perform specific enzyme activities for each factor and to optimise these factors for use in winemaking to produce a reduced-alcohol wine. A six–hour Gluzyme treatment did not show differences in ethanol concentration while a 48 – hour treatment showed up to 0.68% v/v less ethanol compared to the control wines using 30 kU enzyme concentration. An increased total acidity and concurrent but slight decrease in pH were observed in treated compared to the control wines. This was attributed to the gluconic acid production. It seemed that Gluzyme treatment also led to a decrease in concentration of acetic acid.

As a preliminary study, the researchers aimed at investigating the effect of Gluzyme on the glucose content under winemaking conditions. The enzyme in its current form is however, not ideal for winemaking; other forms such as liquid or powder should be considered if the enzyme is to be used in winemaking. The effect of Gluzyme treatment on stability and ageing potential of the wines as well as its impact on the overall quality of the wines still need to be investigated further. In these future investigations, the potential antioxidant property of Gluzyme should also be tested by monitoring the dissolved oxygen concentration.

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