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Production Technologies for Reduced Alcoholic Wines

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Technologies for reduced alcoholic wines
1. Abstract

The production and sale of alcohol-reduced wines, and the lowering of ethanol concentration in wines with alcohol levels greater than acceptable for a specific wine style, poses a number of technical and marketing challenges. Several engineering solutions and wine production strategies that focus upon pre or post fermentation technologies have been described and patented for production of wines with lower ethanol concentrations than would naturally arise through normal fermentation and wine production techniques. However, consumer perception and acceptance of the sensory quality of wines manufactured by techniques that utilise thermal distillation for alcohol removal is generally unfavourable. This negative perception from consumers has focused attention on non-thermal production processes and the development or selection of specific yeast strains with down-regulated or modified gene expression for alcohol production. The information presented in this review will allow winemakers to assess the relative technical merits of each of the technologies described and make decisions regarding implementation of novel winemaking techniques for reducing ethanol concentration in wine.

2. Introduction

The manufacture and sale of reduced alcoholic strength beverages is fraught with legal complexities. Definitions of wine and wine products specify minimum ethanol concentrations of varying quantity applicable for different countries. For example, in Australia, products labelled as wine must contain greater than 8% v v\(^{-1}\) ethanol (alcohol) (ANZFA 2002b). Fermented grape products with an alcohol concentration less than this amount may not fit this definition. Thus the use of the term ‘Reduced Alcohol Wine’ to describe a product destined for the Australian market may not be permitted, however the product could be branded as a ‘Reduced Alcohol Wine Product’. The term ‘Low Alcohol’ may be applied to beverages
derived from fermented grape juice that contains less than 1.15% v v⁻¹ ethanol and ‘Non-intoxicating’ implies the beverage contains less than 0.5% v v⁻¹ ethanol (ANZFA 2002a). Further confusion arises from wine production techniques using early harvested grapes with naturally occurring low levels of fermentable sugars, and consequently, naturally low alcohol concentrations. These products may not have undergone any post fermentation process to modify alcohol levels but may still contain less than the minimum specified ethanol concentration to be considered a wine. Some countries have legislative complexity arising from different government authorities regulating wine and reduced alcohol wine. In the United States of America, wine as defined within the Federal Alcohol Administration Act, must contain between 7 and 24% v v⁻¹ alcohol, while the Bureau of Alcohol, Tobacco and Firearms regulates labelling requirements. As de-alcoholised wine products usually contain less than 7% v v⁻¹ ethanol, labelling provisions are covered by a separate federal act administered by the Food and Drug Administration (Anon 2005). The use of the term ‘Partially Fermented Wine Product’ may be suitable for some stock keeping units, however, the use of thermal distillation or membrane processes for reduction of alcohol from wines prepared from grape juice that has been fermented to dryness (<2.0 g L⁻¹ sugar) could render this term unsuitable. Further confusion may also arise according to the technique of reducing the alcohol concentration. The use of glucose oxidase enzymes for the reduction of fermentable sugars may not be permitted as processing aids for wines in some countries or regions (van Oort and Canal-Llaubères 2002), but their use in the manufacture of wine products or food products may be permissible. Such definitions vary according to the specific labelling laws for individual countries or economic trading zones, and exceptions based upon historical production of specific wine styles are notable. Throughout this review, the terms alcohol-reduced, de-alcoholised and low-alcohol are used interchangeably in the context of wine production, although it is acknowledged that specific legal definitions of these terms
may exist. This paper is not intended to be a guide to the legalities or otherwise of the various production techniques that can be employed for reducing ethanol concentrations in fermented beverages, or a discussion of labelling or wine production laws associated with specific countries or economic trading zones in which goods with reduced alcohol are manufactured and exported. Producers who wish to develop a range of wines with lower than normal ethanol concentrations are strongly advised to seek expert opinion from the regulatory bodies and government organisations appropriate for that manufacturer.

Production techniques for manufacturing low or reduced alcoholic strength beverages have been developed over the last 15 to 20 years in order to satisfy a consumer demand for healthier alcoholic products. Decreasing alcohol consumption is a worldwide trend and lower alcohol consumption rates are associated with certain positive health benefits (National Health and Medical Research Council (Australia) 2009). Beverages with reduced ethanol concentrations are also more favourably excised in most countries thereby producing some competitive advantages for wine sales compared to full alcoholic strength wines. Producers may also wish to marginally reduce alcohol concentrations in full strength wines to correct balance and maintain consistency of style between vintages or blends. It has also been reported that alcohol reduction can lead to an alcohol ‘sweet spot’ (Wollan 2006). These sweet spots have been described when the alcohol level in a given wine is varied by as little as ± 0.1%. Significant differences in flavour intensities and balances are purportedly present in the favoured sweet spot alcohol levels (Wollan 2006). This anecdotal evidence should be considered in light of recent investigations that demonstrate ethanol difference thresholds are approximately 1.0 % v v⁻¹ (Yu and Pickering 2008). The production methods that are of most relevance to the wine industry, whilst not exhaustive, are summarised in Table 1 and generally form the basis of discussion in this paper. Technologies have been classified according to the stage of wine production they are typically used; i.e. pre, concurrent or post
alcoholic fermentation. However, some well established ‘post fermentation’ techniques such as low temperature distillation have been applied during alcoholic fermentation for the removal of approximately 2% v v\(^{-1}\) of ethanol without significantly changing the concentration of other wine constituents (Aguera and others 2010). This information will allow winemakers to assess the relative technical merits of each of the technologies described and make decisions regarding implementation of novel winemaking techniques for reducing ethanol concentration in wine.

3. Pre-fermentation Technologies for Limiting Alcohol Production

Limiting alcohol production during fermentation by lowering the concentration of fermentable sugars in juice through early grape harvest, juice dilution, or arresting fermentation while significant levels of unfermented sugars remain in the wine are some of the options that enable wines with reduced alcohol levels to be produced. Early grape harvest may result in wines that are organoleptically undeveloped due to reduced flavour precursor development in the grapes prior to harvest, high acidity levels and lack of yeast-contributed flavour compounds. Comparatively, arrested fermentation leaving high residual sugar levels in wines may dictate that the finished product will require Pasteurisation for microbial stabilisation, thereby leading to potential loss or alteration of volatile flavour and aroma compounds. Adjusting vine leaf area to crop ratio is an interesting viticultural intervention for moderating the concentration of fermentable carbohydrates in harvested wine grapes (Whiting 2010; Stoll and others 2009). This promising and emerging approach to managing grape sugar concentrations attempts to address the imbalance between carbohydrate accumulation and the development of sensorially important grape constituents. However significant research is still required to determine the optimum leaf to crop ratios, timing and location of leaf removal from the vines relative to fruit location and the long term impact upon vine physiology. Juice dilution can only be performed using a low Brix grape adulterate, as
addition of water is not a permitted process for wine production. Low Brix grape juice, a by-
product of grape juice concentrate, is more commonly used to maintain wine concentration
during reverse osmosis or thermal distillation techniques for alcohol removal. Recent
methods that target pre-fermentation production strategies for reducing alcohol in wines have,
therefore, focused upon technologies that minimise loss or alteration of desirable organoleptic
qualities and off flavour development. The use of enzyme technology for lowering the
concentration of fermentable sugars in grape juice thereby limiting alcohol production prior to
fermentation is one such methods and will form the basis of discussion for pre-fermentation
production options in this paper.

3.1. Glucose Oxidase

3.1.1. Biochemical Principle

Glucose oxidase (EC 1.1.3.4) (GOX) is an aerobic glycoprotein with dehydrogenase activity
that catalyses the oxidation of β-D-glucose to D-glucono-1,5-lactone (D-gluconic acid δ-
lactone). This reaction requires the presence of molecular oxygen, and a flavin adenine
dinucleotide (FAD) cofactor to participate in electron donation to form hydrogen peroxide. A
second enzyme, catalase, is frequently present in commercial GOX preparations to degrade
the unwanted peroxide that is formed as a by-product during the oxidation of substrate. D-
gluconic acid δ-lactone spontaneously hydrates to form gluconic acid. The biochemical basis
for these reactions is shown in Figure 1.

3.1.2. Treatment of Grape Juice with GOX

As gluconic acid is not fermented by yeasts, a decrease in alcohol production can be achieved
in wines prepared from GOX treated juice. The use of GOX in wine was first described for
rapid protection of wine from dissolved oxygen (McLeod and Ough 1970; Ough 1975). The
treatment of grape juice with GOX for lowering glucose concentration and consequently alcohol levels in finished wines was explored and further refined (Heresztyn 1987; Pickering and others 1999c; Villettaz 1987). Glucose reduction in grape juice using GOX is presently limited to white grape varieties, as a period of clarification followed by enzyme reaction must first occur prior to yeast inoculation for fermentation to commence. As the glucose fraction in grape juice represents approximately 50% of total fermentable sugars, the theoretical maximum reduction in alcohol production is 50%, compared to wines made from untreated juice. In practice, some inefficiency in glucose oxidation arises and reported alcohol reductions for wines produced using GOX treated juice range from less than 4% to 40%. The efficiency of glucose oxidation is dependent upon enzyme concentration, juice pH, dissolved oxygen concentration, processing time and temperature (Pickering 2000; Pickering and others 1998).

3.1.3. Effect of pH on GOX

The most efficient conversion of glucose to gluconic acid in grape juice is reported to occur in a pH range of 5.5 to 6.0 (Pickering and others 1998), corresponding to the reported pH range for optimum GOX activity (O'Neil 2006). At the considerably lower grape juice pH range, the rate of gluconic acid production is reduced by up to 75% due to acid inhibition of enzyme activity. Deacidification of the juice with calcium carbonate may, therefore, be required to ensure a sufficient reduction in glucose concentration to provide an adequate reduction of alcohol in the finished wine (Pickering and others 1998). Catalase enzyme activity, required for efficient removal of hydrogen peroxide, is present in most glucose oxidase preparations, and is not affected by the high acidity of grape juice. Indirect monitoring of glucose oxidation can be performed by determination of pH and titratable acidity using standard wine laboratory techniques (Heresztyn 1987; Villettaz 1987).
3.1.4. Effect of Oxygen on GOX

Molecular oxygen is an essential requirement for GOX activity and must be sparged into the juice during enzyme treatment. Agitation will assist dispersion of oxygen bubbles, and enhance GOX activity, probably by limiting bubble size and maximising bubble surface area to volume ratios, thereby increasing dissolved oxygen concentrations. Little information is available that describes the optimum dissolved oxygen concentration for GOX treatment of grape juice. Different rates of air sparging, bubble size, sparger design and mixing rates are important considerations that influence the rate of gluconic acid production (Pickering and others 1998). Problems with excessive foaming and evaporation have also been reported with high aeration levels (Heresztyn 1987). The sparging of the juice during glucose oxidation, and peroxide formation during the oxidation of glucose, will also result in oxidation of polyphenolic constituents of the grape juice and development of a brown colour (Villetta 1987). Oxidised phenolics do precipitate during fermentation and consequently the resulting wine is reported to have a more developed golden yellow colour than wines produced with reductively handled juice. GOX wines do have less susceptibility to browning and ‘pinking’ colour reactions during short to medium term storage than control wines (Pickering and others 1999b). Loss of grape volatile precursors and components may also potentially arise from air or oxygen sparging at excessive rates for prolonged periods.

3.1.5. Effect of Temperature on GOX

Reports on optimal temperature for GOX activity are ambiguous. Early experiments with grape juice demonstrated more rapid glucose oxidation occurring at a temperature of 20°C compared to 30°C (Heresztyn 1987). This contrasts with the reported temperature optimum for GOX activity between 30°C to 35°C (O'Neil 2006) while other reports have not demonstrated any significant difference in gluconic acid production rates in GOX treated
juice at temperatures of 20°C and 30°C (Pickering and others 1998). Several advantages are apparent for lower processing temperatures. Higher dissolved oxygen levels in the juice can be achieved at lower temperatures and oxygen concentration is an important rate limiting reactant for GOX activity. Undesirable microbial growth may also be decreased at 20°C, although many wine spoilage organisms are quite capable of growth at this temperature.

3.1.6. GOX Produced Wines and their Composition

A summary of glucose reduction, gluconic acid concentrations, wine composition and resulting alcohol reduction in juice and wines arising from GOX treatment is given in Table 2 and Table 3. The composition of GOX and control wines prepared from deacidified grape juices is also given in Table 4. High levels of gluconic acid produced from conversion of the glucose fraction of fermentable sugars, is a significant problem for finished wine composition. The contribution of gluconic acid to total acidity in wines prepared by GOX treated juice may render the wines out of balance (Pickering and others 1999b). To moderate this problem, and to optimise GOX activity, deacidification of the grape juice with calcium carbonate prior to GOX treatment may be necessary. A typical scheme for the production of reduced alcohol wine by GOX treatment of white grape juice is shown in Figure 2. A particular issue associated with the production of wines from GOX treated grape juice, is the formation of substantial quantities of carbonyl compounds, resulting in significantly higher sulphur dioxide binding than in control wines (Pickering and others 2001). Consequently the total concentration of sulphur dioxide necessary to achieve microbial stabilisation in GOX wines may approach or even exceed legal limits (Pickering and others 1999a). A further deleterious outcome of GOX treatment is an increased susceptibility for these wines to undergo premature browning consistent with increased flavonoid production (Pickering and others 1999c; Pickering and others 1999a; Pickering and others 1999b).
4. Fermentation Technologies for Limiting Alcohol Production

4.1. Use of Novel Yeast Strains

Selection of specific yeast strains and their use as starter cultures for the consistent manufacture of a wine style is a common winemaking practice. The use of specific yeast strains for wine production also ensures improved fermentation reliability and predictability than reliance upon natural fermentation (Pretorius 2000). A difficulty faced by some winemakers and viticulturists is the production of wine grapes that have a balance between flavour components and accumulated sugar. The requirement for some producers to harvest grapes at high levels of fermentable sugar to achieve typical varietal characters produces wines with excessive alcohol concentrations and undesirable palate hotness (de Barros Lopes and others 2000). One strategy to overcome the excessive production of alcohol in these wines is the selection of yeast strains with lowered ethanol production during fermentation. Some variability in ethanol production by different commercially available wine starter cultures of *Saccharomyces cerevisiae* has been described, however, the difference in final ethanol concentrations determined in one controlled experiment was less than 1% v v⁻¹ (Jenson 1997). The selection of yeasts other than *Saccharomyces cerevisiae* with lower ethanol production rates for grape juice fermentation is possible. Low alcohol wines produced by the fermentation of oxidised grape juice using strains of *Pichia* and *Williopsis* were demonstrated to have an acceptable, albeit different palate structure and organoleptic qualities, than wines produced with *Saccharomyces cerevisiae* (Erten and Campbell 2001). A problem of fermentation using novel or wild yeast species is potential off flavour development and undesirable organoleptic characters (Heard 1999), hence the development of genetically modified *Saccharomyces* strains for wine production.
4.2. Genetically Engineered Saccharomyces cerevisiae

The potential to engineer specific yeast strains of Saccharomyces cerevisiae that divert grape carbon compounds from ethanol to production of other metabolites, or increasing biomass has been identified as a strategy to decrease the final ethanol concentration of wines (Bartowsky and others 1997). A number of gene products that interact with the glycolytic pathway and associated biochemical pathways involved in redox balance have been targeted.

Early work with single gene mutations to isoenzymes of alcohol dehydrogenase (ADH) (Drewke and others 1990) or triose phosphate isomerase (TPI) at low glucose concentrations in aerated cultures (Compagno and others 2001; Compagno and others 1996) enabled higher levels of glycerol to be formed. However, culture conditions in these experiments were significantly different to a typical grape juice or must fermentation, and therefore results should be interpreted with caution. Nonetheless, these early experiments were important in identifying specific metabolic pathways with potential to redirect carbon flux from ethanol synthesis, and the development of methods for genetic manipulations of the genes encoding specific enzymes of interest in wine yeast strains.

More recently, cofactor engineering has been used to modify redox balance within the yeast cell and thereby altering the flux of carbon through a suite of pathways (Heux and others 2006a; Heux and others 2006b; Hou and others 2009; Geertman and others 2006). Transgenic incorporation of water soluble (cytosolic) oxygen dependant nicotinamide adenine dinucleotide hydride (NADH) oxidase enzymes expressed during yeast growth show some promise in modulating ethanol production whilst maintaining redox balance and avoiding unwanted accumulation of sensorially active compounds as carbon flux is redirected through multiple pathways. A significant disadvantage of cofactor engineering is the requirement for
soluble oxygen to be supplied during fermentation and current investigations involve the utilisation of relatively low carbohydrate concentrations relative to grape juice or must.

Most effort in recombinant technologies for manipulation of wine yeasts has targeted increasing glycerol production at the expense of ethanol and has required multiple gene modifications (de Barros Lopes and others 2000; Michnick and others 1997; Nevoigt and Stahl 1996; Geertman and others 2006). Between four and ten percent of grape juice carbon is normally directed to glycerol production during fermentation by *Saccharomyces cerevisiae* with the majority being produced during the initial stages of biomass formation (Ribéreau-Gayon and others 2006; Scanes and others 1998). The final concentration of glycerol in finished dry wines normally ranges between four and nine grams per litre and is dependent upon the yeast strain and a range of environmental signals including temperature, pH, sugar concentration, nitrogen source and sulphur dioxide levels (Remize and others 1999; Scanes and others 1998). The major benefits of glycerol formation for yeast cells during fermentation are twofold. Glycerol is normally produced by *Saccharomyces cerevisiae* during biomass formation at commencement of fermentation to protect cells from the high osmolar concentrations of sugars, thereby preventing cellular dehydration. Also, as the reactions for glycerol formation involve oxidation of NADH, the redox imbalance that arises from anaerobic glycolysis and glucose repression of respiration is corrected (Scanes and others 1998). It is the correction of redox balance that is considered the most important biological function of glycerol formation (Michnick and others 1997).

Glycerol formation arises from reduction of the glycolytic intermediate dihydroxyacetone-phosphate to glycerol-3-phosphate and subsequent dephosphorylation. These two reactions are catalysed by a NADH dependent glycerol-3-phosphate dehydrogenase (GPDH) and a specific glycerol-3-phosphatase (GPP) respectively. Two isoforms of GPDH have been described and designated GPDH-1 and GPDH-2 with expression of the genes encoding these
isoenzymes regulated by the yeast requirement for osmoprotection and redox balance respectively (Michnick and others 1997). Osmoprotection in the early stages of fermentation by up regulated GPDH-1 expression has been shown to have a more significant role in glycerol production than correction of redox imbalance by GPDH-2 during anaerobic fermentation (Remize and others 2003).

As glycerol and ethanol production by yeasts during fermentation are important regulators of cellular redox balance through the regeneration of NAD⁺, any influence upon the flux of these compounds will alter the concentration of a range of other metabolites that are also involved with redox balance. Acetaldehyde, acetate, succinate, acetoain, diacetyl and 2,3-butandiol appear to be the most important of these metabolites (Michnick and others 1997; Remize and others 2000; Taherzadeh and others 2002; Ehsani and others 2009; Cambon and others 2006). The presence of these compounds in wine at levels exceeding their sensory threshold may be detrimental to perceived wine quality (Etiévant 1991; Boidron and others 1988).

Genetic manipulation of yeast strains to over express either GPDH-1 or GPDH-2 has resulted in diminished ethanol of between 19 and 22% in model solutions but significantly less in grape juice fermentations. Concomitant with increased glycerol formation and decreased ethanol production was increases in acetate, succinate, acetoain, acetaldehyde and 2,3-butandiol (de Barros Lopes and others 2000; Michnick and others 1997; Remize and others 1999; Cambon and others 2006). Acetate formation by yeasts during fermentation may occur either by hydrolysis of acetyl-CoA or the pyruvate dehydrogenase (PDH) by-pass pathway in which pyruvate is decarboxylated to acetaldehyde followed by oxidation to acetate (Remize and others 2000; Ribéreau-Gayon and others 2006). The enzymes involved in the PDH by-pass are pyruvate decarboxylase (PDC) and an acetaldehyde dehydrogenase that belongs to the aldehyde dehydrogenase (ALD) group of enzymes. The ALD group of enzymes in Saccharomyces cerevisiae has been extensively characterised with five isoforms (ALD2-6).
being designated, and the most important of these during fermentation are ALD-5 (mitochondrial) and ALD-6 (cytosolic) as both are constitutive enzymes. The expression of isoforms ALD2-4 is glucose repressed and therefore these enzymes do not play any role in cellular redox balance during grape juice fermentation (Navarro-Aviño and others 1999).

*Saccharomyces cerevisiae* strains with a down regulated pyruvate decarboxylase (PDC) gene and over expressed GPDH-1 were developed to enhance glycerol production at the expense of ethanol (Nevoigt and Stahl 1996). PDC activity was reduced to less than one fifth of wild type and GPD-1 over expression resulted in a 45% reduction in ethanol, however the end products, acetate and acetaldehyde derived from alternate redox reactions, were subsequently increased (Nevoigt and Stahl 1996).

A novel method to overcome the deleterious effects of high acetate levels in fermentation conducted by GPDH-2 over-expressing yeasts has been developed. By using a yeast strain with an acetaldehyde dehydrogenase-6 (ALD-6) gene deletion, a substantially lower ethanol and acceptable acetate concentration was demonstrated (Eglinton and others 2002). However, the concentration of other sensorially important metabolites such as acetaldehyde and acetoin were significantly in excess of concentrations considered to be acceptable for wine products. Further gene manipulations of *Saccharomyces cerevisiae* were developed that included over-expressed 2,3-butanediol dehydrogenase (BDH) (Ehsani and others 2009), an NADH dependant reductase responsible for the conversion of (3R)-acetoin and (3S)-acetoin to (2R,3R)-2,3-butanediol and meso-2-3, butanediol respectively (González and others 2000). A summary of metabolite concentrations involved in cellular redox balance using genetically modified yeast strains targeting GPDH, ALD and BDH expression is presented in Table 5 and important biochemicals involved in their formation is shown in Figure 3. Clearly, any attempt to redirect carbon flux by gene manipulations causes numerous biochemical pathway interactions. Consequently redox imbalance within the cell must be corrected, and arising
from alternate biochemical pathway modulation is the production of a suite of sensorially
important compounds that may exceed desirable organoleptic concentrations in wine.

4.3. Further Limitations of Gene Technology for Yeast Strain Manipulations

Whilst genetic engineering has shown significant promise as a technology to enable selection
of yeast strains specifically tailored for expression of desirable traits, a number of barriers to
the uptake of this technology for regular wine production are apparent. Controlling the flux
of metabolic intermediates and end products not specifically targeted by genetic
manipulations in yeast strains remains an important barrier that, while not insurmountable,
must be controlled in order to achieve commercial products that are organoleptically
acceptable. Deletion or over-expression of one gene product can significantly alter a suite of
metabolic intermediates and end products during fermentation as the manipulated yeast cells
attempt to compensate altered metabolism. Some metabolic traits are encoded by a suite of
genes or the interaction of several gene systems on different chromosomes within the yeast
genome (Pretorius 2000). Achieving desirable oenological outcomes by genetic engineering
may therefore not be possible with approaches targeting a limited number of genes. A
complicating factor in genetic manipulations of wine yeast is the failure of some transgenic
strains to complete fermentation in a timely manner (Heux and others 2006b; Remize and
others 2000; Remize and others 2003).

Some of the gene inserts used to induce over-expressed gene products may not be stable
within the altered strain. A significant disadvantage is the loss of the plasmid insert from
yeast cells during fermentation (de Barros Lopes and others 2000; Remize and others 1999).
Thus, incorporation of stable plasmid inserts containing over-expressed genes, or gene
deletions limiting the production of specific enzymes that alter metabolic flux, must be
achieved in order to produce consistent and reliable fermentations with these yeasts. Most
importantly however, is the attitude of consumers to foods that contain or produced using genetically modified organisms (GMOs). The use of GMOs in food products is a major cause of concern and mistrust among consumers and has led to the introduction of some trading restrictions of these goods between economic zones, labelling requirements stating the use of GMOs in food production, and, in some instances, the refusal by retailers to stock products that cannot be proven to be free of GMOs or their metabolic products (Burton and others 2001). A possible future use of gene technology in the wine industry will be the identification and comparison of fermentation performance of yeast strains traits bred by selective breeding techniques.

5. Post Fermentation Technologies for Removing Alcohol

5.1. Membrane Transport Processes

The removal of ethanol from wine following the completion of fermentation can be achieved either through the application of thermal distillation processes, with or without vacuum, or the transport of ethanol across a semipermeable barrier or membrane. Various technologies in which a membrane is used for the selective removal of ethanol from beverages have been developed that rely upon molecular permeation of ethanol from the feed stock with high concentration, to a stripping phase with low concentration. The most prevalent membrane based technology for the removal of organic constituents from beverages is reverse osmosis, and emerging technologies such as osmotic distillation and pervaporation are still non-mainstream.

5.1.1. Reverse osmosis

The separation of two solutions of unequal solute concentration by a semi permeable membrane establishes a concentration or pressure gradient between them known as osmotic
pressure. In such a system, water will move by a process of osmosis from the solution of low concentration across the membrane in order to restore equilibrium. However, if sufficient pressure (greater than the osmotic pressure) is applied on the high concentration side, solvent can move out of this solution across the membrane to the low concentration solution, in a phenomenon known as reverse osmosis. Effectively, the concentration of the dilute solution reduces while that of the concentrated solution increases.

From the explanation outlined above, it should be easy to see how this phenomenon could be exploited as a filtration process. In fact, simply adjusting the pore size of the membrane material and the applied pressure give rise to a spectrum of membrane filtration processes with increasing solute permeability (and reducing operating pressure) such as reverse osmosis, nanofiltration, ultrafiltration and microfiltration respectively (Figure 4). Thus, not only water or substances with comparably low molecular weight may pass through the membrane as in reverse osmosis but other solutes and solvents in gradation of size and molecular weights.

During membrane processing, the feed is separated into two streams: retentate (concentrate) and permeate (filtrate). The volumetric throughput of the membrane surface per hour, per metre-squared (L h\(^{-1}\) m\(^{-2}\)) is called the flux and is dependent on the applied pressure and total membrane resistance. The membrane resistance is a composite of factors that limit permeation such as viscosity of permeate, pore size, extent of fouling and interactions between membrane material and feed. The larger the membrane area, the greater the filtration capacity.

The first patent for the application of reverse osmosis in alcoholic beverages was obtained by the West German brewing company Lowenbrau in 1975 for the de-alcoholisation of beer and wine (Meier 1992). Other applications of reverse osmosis in wine production include
removal of colours and flavours, must concentration (as an alternative to chaptalisation),
development of new products such as aperitifs, wine stabilisation against tartrate precipitation
and deacidification (removal of volatile acids) of grape juices (Baldwin 1998; Smith 2002).

As a membrane technology, reverse osmosis requires low energy input, operates at ambient
temperatures, allows reproducible control over separations, requires no disposable filtration
media or other additions and is easily automated for continuous operation (Gibson 1986).
Specifically, in comparison to other methods of producing low alcohol wines such as
distillation, spinning cone technology or arrested fermentation, the reduced alcohol wines
produced by reverse osmosis usually have flavour and aroma profile comparable to the
regular wines from which they were obtained (Bui and others 1986) as no phase change is
associated with the process, and water and alcohol are largely the only components removed
from the feedstock.

5.1.1.1. Membrane types and configurations

Reverse osmosis membranes can be made of several different materials including cellulose
acetate, regenerated cellulose, synthetic polymers and ceramics (Heldman and Hartel 1997;
Westbrook 1989). The cellulosic materials are not as durable and give low flux rates
compared to the synthetic polymers, which are also more selective. Ceramics, while very
strong and durable, are also expensive, being designed originally for separation of uranium
isotopes (Gibson 1986). The most successful membranes are the asymmetric (heterogenous)
types, which are thin skins of membrane material bonded to one or more layers of polymeric
support material to create a composite membrane configuration (Figure 5). Thin film
composite (TFC) membranes are very commonly used where a polymer with high strength
and porous structure is chemically bonded to a very thin film of polymer (membrane material)
with the required permeation selectivity. Such membranes give good flux characteristics and
are very durable under the high-pressure application of reverse osmosis. These membranes are also cleanable and allow back flushing to restore initial flux rates by destabilising any build up of materials on the membrane surface.

Reverse osmosis, like other membrane techniques, operates under the principle of cross or tangential flow, whereby the liquid flows parallel or tangential to the membrane surface at high velocity under pressure. Some liquid passes through the membrane but the solids or materials with molecular weight higher than the nominal molecular weight cut off (NMWCO) of the membrane will be swept along in the stream of feed across the membrane. Recycling will ensure that more permeate will pass through the membrane during each cycle until the desired concentration of the feed is achieved. In order to effectively do this, several module configurations have been developed. These include the flat sheet (also known as plate and frame), tubular, hollow fibre and spiral wound configurations. The spiral wound configuration makes the most economic use of space for a given membrane area, being flat membranes rolled up together like a cigar (Pretorius 2000). The original space between the membranes serve as permeate collection channels and the new space generated from winding the membranes becomes the feed channel.

5.1.1.2. Applications and limitations

In a reverse osmosis process for alcohol reduction in wines, the feed is the regular wine with normal alcohol content. This wine is pumped at pressures up to 4 MPa (40 atm) through a membrane module and such pressures can result in elevated temperatures at the membrane surface. To avoid excessive temperature arising from high pressures, heat exchangers are typically a component of the apparatus with operating temperatures around 20-22°C (Smith 1996). Operating conditions must be balanced between gaining efficiencies in permeate flux at higher pressures and aroma retention, which is improved at lower temperatures (Catarino
A membrane is selected with a low NMWCO, typically < 200 Da (Catarino and others 2007) so that water and ethanol, being small molecules, pass through the membrane into the permeate stream. The retentate is redirected to the feed tank and the wine is continuously de-alcoholised and concentrated (Duerr and Cuenat 1988; Meier 1992). Wine is restored to the original water content by the addition of low Brix juice, a by-product of grape juice concentrate. Alternatively, low Brix juice may be continuously added to the feed (wine) to keep the volume constant during the process. Basically, the more low Brix juice added, the lower the alcohol content in the feed tank. This also has the effect of increasing permeation since the osmotic pressure is also reduced. The simultaneous production of low Brix juice or alcohol enriched wine, with de-alcoholised wine is possible by using two reverse osmosis units in parallel, one with an ethanol impermeable membrane and redirecting the filtrate from this unit to the feed supplying the ethanol permeable unit (Bui and others 1986). More commonly, rectification of the permeate, to separate ethanol and the water content is possible using thermal distillation processes and redirecting the water component back to the feed tank in a closed loop system maintains wine volume without the requirement for low Brix juice addition. Such apparatus are now commonly used for ethanol removal from wine products (Smith 1996) and a typical process within a closed loop system is shown in Figure 6. Reverse osmosis can be used to reduce alcohol content in wine from about 12-15% v/v to less than 0.5 % v/v, producing a wide range of low alcohol wines, thereby allowing production flexibility.

In comparison to other conventional methods of de-alcoholisation or preparation of low alcohol wines, the capital cost of reverse osmosis is higher and at removal of ethanol to below 0.45% v/v consumes more electricity per litre of ethanol removed (Pilipovik and Riverol 2005). However, labour and other operating costs are low, especially since energy is used more efficiently. Savings on operating costs would also depend on the plant capacity.
Reverse osmosis, as part of the family of membrane filtration techniques, is already being employed for improving efficiency and maintaining viability of the wine industry. Apart from de-alcoholisation, other possible uses in the wine industry include amelioration of wine, treatment of saline water for irrigation and treatment of waste water to reduce costs of waste disposal. Each process requires slightly different configuration of equipment and various changes to the processing of permeate.

5.1.2. Emerging Membrane Technologies: Osmotic Distillation and Pervaporation

Osmotic distillation, also referred to as evaporative pertraction or isothermal membrane distillation (Diban and others 2008) and pervaporation share similar processes in that selective removal of ethanol arises from the establishment of a vapour pressure differential across a membrane, which usually has hydrophobic properties. Thus ethanol removal occurs as a process of evaporation at the wine membrane interface, diffusion of the vapour across the membrane and condensation into the stripping phase.

The nature of the stripping phase determines the process; osmotic distillation employs degassed pure water (Hogan and others 1998; Diban and others 2008) whereas pervaporation makes use of an inert gas containing water vapour (Karlsson and Tragardh 1996; Takács and others 2007). The basic principles of ethanol removal from wine by establishing a vapour differential is illustrated in Figure 7. Osmotic distillation membranes have hydrophobic, apolar, properties, which is essential as this critical factor determines the flux of ethanol from retentate to permeate. Aroma and flavour components have lower vapour pressures in ethanolic solutions than ethanol itself, thus the flux ratios of these compounds are considerably lower and are mostly retained within the wine. Membranes used for osmotic distillation are usually constructed from polyethylene, polypropylene, polytetrafluoroethylene
or polyvinyl difluorides in varying pore sizes. Compared to reverse osmosis, ethanol flux is
considerably lower and thus longer times are required for the equivalent ethanol removal,
however energy savings arise from the considerably lower pressures and product integrity
improved from lower operating temperatures (Varavuth and others 2009). As the vapour
pressure differential across the membrane for aroma compounds is generally much lower than
ethanol, the flux and subsequent aroma losses of important flavour compounds are minimised.
In a pilot scale operation 2 % v v⁻¹ of ethanol was removed from a merlot wine and the loss of
compounds, considered important to wine flavour, to the permeate varied from 0.9% to 98%
(Diban and others 2008). Compound losses were attributed to the polarity and volatility of
the compound and therefore increased with residency time for treatment, thus potentially
limiting ethanol removal. Difference testing with untrained panels could not detect significant
differences between the wines at 13.35 % v v⁻¹ and 11.3% v v⁻¹ illustrating the potential for
the use of osmotic distillation for the removal of small fractions of alcohol from beverages
Pervaporation technologies for ethanol removal has not been as widely adopted as osmotic
distillation or reverse osmosis, and few reports in the literature describe the use of this
technology for the treatment of wine. This may possibly be attributed to higher temperatures
required to achieve effective ethanol permeation compared to osmotic distillation as
temperature elevation will also increase the flux of aroma compounds to the permeate (Takács
and others 2007). One report in which a hydrophilic membrane was employed for ethanol
reduction to a final concentration of 0.5% v v⁻¹ in chardonnay resulted in the retention of 80%
of the concentration of most aroma compounds (Karlsson and Tragardh 1996). Clearly,
further research is required and the retention of aroma compounds improved, before
pervaporation becomes more widely adopted for moderation of ethanol in beverages.
5.2. Spinning Cone Columns

The spinning cone column (SCC) is a device used to extract volatile flavour components from a liquid or slurry. The column consists of a vertical shaft rotating at approximately 350 rpm, supporting up to 22 inverted (pointing downwards) cones. Between each pair of cones there is a fixed inverted cone, attached to the casing of the column (Figure 8). The liquid feed is fed to the top of the column, into the first spinning cone. A film of liquid is flung outwards by centrifugal action onto the inside surface of the casing. The liquid will then drop onto a fixed cone, and migrate as a thin film downwards and towards the centre of the cone under the influence of gravity. The liquid then passes onto the second spinning cone, and the movement is repeated several times until the liquid reaches the bottom of the column. As the liquid film is quite thin, the liquid hold up volume is low and the resident time is typically around 20 seconds. The SCC can handle a range of different materials as feed, from low viscosity products (e.g., wine) to more viscous materials such as coffee extract.

A stripping gas, such as nitrogen, is admitted to the base of the column and passes through the voids between the rotating and fixed cones. An alternative stripping vapour can be generated from redirecting a portion of the product discharge through a heater prior to re-injection. The gas, along with volatile components it has picked up, is collected at the top of the column. Along its tortuous path upward, the stripping gas is exposed to considerable turbulence caused by fins attached to the underside of the spinning cones (Figure 9). It is this turbulence and the fact that the liquid is spread out as a thin film on the upper surfaces of the rotating and fixed cones that enhances a mass transfer of volatile product into the stripping gas. The considerable number of cones also ensures an adequate path length for both liquid and stripping gas within the column.
The column operates under a negative pressure, so volatile components will be evaporated off at a reduced temperature. Typical feed and column temperatures are approximately 30°C. Reasonable clearances between rotating and fixed cones ensures that pressure drops are minimised, and this in turn enables the mass transfer process to occur at almost constant pressure (and hence constant temperature) within the column (Harders and Sykes 1999). Other ancillary items are required for the process to function efficiently (Figure 10). After leaving the feed tank (1), the product is warmed in a regenerative heat exchanger (3) and fed into the spinning cone column (5). Stripping gas is obtained from treatment of a portion of the product discharge through a re-injection heater (7), with the remainder of the product discharge recovered once passed back through the heat exchanger (3). On leaving the column, the stripping gas vapours are fed to a condensate cyclone (8) and volatile components are recovered separate to the treated product. The SCC can be sealed to operate under aseptic conditions, and can be a component of a pasteurising or sterilising process. The column and ancillary components can be cleaned using a ‘clean in place’ (CIP) system.

### 5.2.1. SCC Role in Winemaking

The SCC has numerous applications in the wine industry, including recovery of delicate aromas, removal of sulphur dioxide from grape juice, alcohol reduction of wines and production of grape juice concentrates. The recovery of delicate aromas is an important feature of the equipment, as aromas can be lost in certain winemaking operations such as filtration, fining and oxidation during storage. Flavours can be recovered from juice or from wine before a critical processing operation and then added back to the wine at the blending stage. Since the spinning cone column can operate at low temperatures, delicate flavours are able to remain ‘fresh’ and are unlikely to be heat affected.
The SCC has an important role in the removal of alcohol from wine. The general process for adjusting alcohol concentration in finished wine using SCC technology consists of a two-stage process. The first pass of wine through the SCC occurs at low temperature (~28°C) and vacuum to recover volatile wine aromas in approximately 1% of total product volume. The second pass of product occurs with the de-aromatised wine at a slightly higher temperature (~38°C) and vacuum conditions to remove the alcohol. The final de-alcoholised wine is constructed by re-blending the recovered aroma with the de-alcoholised and de-aromatised base. Blending with full strength wine, juice or juice concentrates to final product specifications prior to filtration and packaging enables a range of product styles to be developed.

An investigation of the effects of the SCC on wine phenolic composition arising from the de-alcoholisation process was undertaken with several Spanish red and white wines. Differences in the free radical scavenging activities, resveratrol, total phenolics, flavonols, tartaric esters, flavonoid and non-flavonoids of the base and de-alcoholised wines were attributed to the presence of varying concentrations of sulfur dioxide, which is removed with the ethanol, and volumetric changes leading to the concentration of constituents in the lowered ethanol wine (Belisario-Sánchez and others 2009).

The SCC has been used to investigate continuous ethanol removal from a fermenting yeast broth (Wright and Pyle 1996). The broth was circulated between the SCC and a fermentation vessel, and ethanol removal efficiency was found to be quite high at 85%. It was observed, however, that the vacuum applied to the SCC affected the viability of the yeast cells, and the cells became smaller in size and had a different shape. This limitation should not be an issue for finished wines, where fermentation has been completed.
5.3. SuperCritical Solvent Extraction

Compression of a gas at temperatures above its critical point will result in formation of a supercritical fluid with increased solvent properties that can be exploited for separation or liquid extraction. The use of carbon dioxide for supercritical extraction in the food industry is gaining popularity and offers several advantages as the critical temperature for this gas is relatively low at 31°C, no toxic substances are required for use, it is relatively inexpensive and easily handled (Rizvi and others 1994). Further, the use of carbon dioxide in wine production does not pose any legal difficulties and is ideally suited for extraction of alcohol from either wine or beer (Marignetti and others 1992). A patented process for the removal of alcohol from wine or beer using supercritical carbon dioxide extraction, and the production of low alcohol beverages subjects the high alcoholic strength beverage to low temperature high vacuum distillation. The captured volatile fraction containing alcohol and aroma compounds is then subjected to supercritical extraction at 80 to 100 bars pressure. Partial expansion of the supercritical fluid by pressure drop to 18 to 25 bars extracts the aroma portion of the distillate, which after carbon dioxide scrubbing is returned by sparging into the de-aromatised wine base remaining after distillation (Sieidlitz and others 1991). A flow diagram illustrating the production of low alcohol wine via supercritical carbon dioxide extraction is shown in Figure 11. The critical extraction process is conducted in a counter current column in which the solvent (carbon dioxide) is pumped as a liquid into bottom of the column and the volatile alcohol mixture fed into the top. The extracted volatile aromas and carbon dioxide gas are recovered from the column head and ethanol-water component drained away as a liquid from the bottom.

Pilot scale experiments for the production of dealcoholised cider have shown the technical feasibility for producing low alcohol products using sequential supercritical extraction of aroma compounds, followed by ethanol from the base wine. Re-blending of the aroma and
base wine components produced a product with some similarity to the original cider. However, removal of some aroma compounds and lack of ethanol, presumably to provide palate weight, produced a beverage with considerable differences from the original (Medina and Martínez 1997). Whilst technically feasible, supercritical extraction using carbon dioxide for the production of low alcohol beverages is not a commonly employed process within the beverage industry. High capital costs, a requirement for high vacuum distillation and the inflexibility of plant remain significant barriers for the uptake of this technology within the wine industry. However, supercritical extractive processes are routinely used for the manufacture of a range of high value foods.

6. Sensory Quality of Low Alcohol Wines

Very little published information is available that describes controlled sensory evaluation of reduced alcohol wines, produced by most of the procedures described in this paper, with the exception of wines manufactured from GOX treated juice. A general consumer perception is that reduced alcoholic wines lack body and flavour (d'Hauteville 1993). The removal of ethanol will have an obvious effect upon the sweetness and palate weight of a wine, due to the sensory characteristics of alcohol. Increased ethanol concentrations enhances the perception of bitterness (Fischer and Noble 1994; Nurgel and Pickering 2006), sweetness (Fischer and Noble 1994), body (Gawel and others 2007; Nurgel and Pickering 2005) and hotness (Gawel and others 2007; Nurgel and Pickering 2006). Conversely lowering ethanol decreases acuity of acidity and astringency (Fischer and Noble 1994). The volatility of aroma compounds is reduced in the presence of ethanol due to their non-polar nature and these compounds have increased solubility in full alcoholic strength wines (Voilley and Lubbers 1998). Thus volatile components may be more easily lost from de-alcoholised wines than full strength wines during processing. Loss of aroma compounds is likely to be greater in thermal distillation techniques than low temperature processes (Pickering 2000). The removal of
ethanol from the wine may also increase the binding of aroma compounds to proteinaceous materials in wines, a process that leads to diminished volatility and thus sensory perception of these compounds (Voilley and Lubbers 1998). The changes in flavour profile due to ethanol removal are therefore a complex interaction of altered volatility and concentration of aroma compounds, loss of alcohol related sweetness and changes to the perception of mouth feel characteristics. The magnitude of sensory changes associated with de-alcoholised wine is dependent upon the quantity of ethanol remaining in the product.

7. Future Potential of Ethanol Modified Wines

The anticipated increase in sales of reduced alcoholic beverages arising from increased consumer awareness of the risks associated with alcohol consumption in the early 1990’s, has not necessarily been transcribed from marketing publicity to commercial reality (Howley and Young 1993). A consumer perception that de-alcoholised wines are organoleptically inferior products is one of the most significant barriers for sales of these wine styles (d'Hauteville 1993). The relative changes in mouth feel and decreased aromas in reduced alcohol wines may arise from chemical changes in volatile compound structure, and reduced volatility of esters and higher alcohols due to the absence of ethanol. The chemical change in volatile compounds associated with thermal distillation has led to the development of low temperature distillation processes. However, the capital costs associated with this type of plant and equipment is relatively high. Recent improvements in membrane technology, portability and flexibility of application for treatment of some wine faults ensured that reverse osmosis has become a widely used production process with rapid uptake by wine producers. Technologies such as a spinning cone column require a significant volume of product in order to become economically feasible. Regardless of the technology employed for production of reduced alcoholic strength wines, a very real issue faced by manufactures is aseptic packaging and timely transport of the product to market. Removal of ethanol from the beverage creates a
highly susceptible product for microbial growth and these products must be packaged in highly controlled conditions in order to preserve product integrity. Blending of ethanol-reduced wines with grape juice concentrates to enhance varietal composition and improve palate weight associated with low sugar levels further exacerbates potential contaminant growth. In spite of these challenges, several commercial reduced alcoholic strength products have been marketed with success. Although the future for such wine products remains unclear, the reduction of ethanol concentration in wines to acceptable levels to maintain style consistency between vintages is, however, likely to remain an important wine production process for many manufacturers.

8. Acknowledgments

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9. References


Remize F, Andrieu E & Dequin S. 2000. Engineering of the Pyruvate Dehydrogenase Bypass in Saccharomyces cerevisiae: Role of the Cytosolic Mg2+ and Mitochondrial K+

Remize F, Cambon B, Barnavon L & Dequin S. 2003. Glycerol formation during wine fermentation is mainly linked to Gpd1p and is only partially controlled by the HOG pathway. Yeast 20(15):1243-1253.


Table 1. Technologies for reducing ethanol concentration in wine and fermented beverages (Duerr and Cuenat 1988; Pickering 2000; Smith 2002; Aguera and others 2010; Takács and others 2007).

<table>
<thead>
<tr>
<th>Stage of Wine Production</th>
<th>Principle</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Fermentation</td>
<td>Reduced fermentable sugars</td>
<td>Early fruit harvest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Juice dilution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose oxidase enzyme</td>
</tr>
<tr>
<td>Concurrent with Fermentation</td>
<td>Reduced alcohol production</td>
<td>Modified yeast strains</td>
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<tr>
<td></td>
<td></td>
<td>Arrested fermentation</td>
</tr>
<tr>
<td>Post Fermentation</td>
<td>Membrane Separation</td>
<td>Reverse osmosis</td>
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<tr>
<td></td>
<td></td>
<td>Pervaporation</td>
</tr>
<tr>
<td></td>
<td>Non-Membrane Extraction</td>
<td>Solvent extraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ion exchange</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinning cone column</td>
</tr>
</tbody>
</table>
Table 2. Glucose oxidation rates in grape juice and alcohol reduction percentages in corresponding wine made from glucose oxidase treated grape juice (Heresztyn 1987; Pickering and others 1999c; Villettaz 1987).

<table>
<thead>
<tr>
<th>Grape Variety</th>
<th>Glucose Oxidase/Catalase Concentration mg L(^{-1})</th>
<th>Glucose Reduction %</th>
<th>Gluconic Acid g L(^{-1})</th>
<th>Alcohol Reduction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chasselas</td>
<td>500</td>
<td>NS</td>
<td>NS</td>
<td>23</td>
</tr>
<tr>
<td>Müller Thurgau</td>
<td>2000</td>
<td>87</td>
<td>73</td>
<td>40</td>
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<tr>
<td>Muscat Gordo Blanco</td>
<td>50</td>
<td>13</td>
<td>11</td>
<td>3.6</td>
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<tr>
<td>Muscat Gordo Blanco</td>
<td>200</td>
<td>31</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Muscat Gordo Blanco</td>
<td>1000</td>
<td>56</td>
<td>53</td>
<td>33</td>
</tr>
<tr>
<td>Riesling</td>
<td>500</td>
<td>36</td>
<td>10-52</td>
<td>23</td>
</tr>
<tr>
<td>Riesling</td>
<td>2000</td>
<td>NS</td>
<td>NS</td>
<td>36</td>
</tr>
</tbody>
</table>

NS: not stated
Table 3. Glucose oxidase/catalase treated Chasselas juice and reduced alcohol wine production (Villettaz 1987).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Juice Analysis</th>
<th>Wine analysis after alcoholic fermentation</th>
<th>Wine analysis after malolactic fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Oxidase/Catalase Concentration mg/L</td>
<td>Contact Time hours</td>
<td>pH g L$^{-1}$†</td>
<td>pH g L$^{-1}$†</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>3.63</td>
<td>3.57</td>
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<td>50</td>
<td>2.0</td>
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<tr>
<td>500</td>
<td>15.0</td>
<td>2.84</td>
<td>3.00</td>
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</tbody>
</table>

† as tartaric acid
Table 4. Composition of Müller-Thurgau and Riesling wines prepared from GOX treated deacidified juice (Pickering and others 1999a; Pickering and others 1999b).

<table>
<thead>
<tr>
<th>Component</th>
<th>Deacidified Juice</th>
<th>GOX Treated Juice</th>
<th>Control Juice</th>
<th>Decreased Alcohol Wine§</th>
<th>Control Wine§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Müllner-Thurgau</td>
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<td></td>
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<tr>
<td>Ethanol</td>
<td>%v/v</td>
<td></td>
<td>6.2</td>
<td>10.5</td>
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<tr>
<td>Glucose</td>
<td>g L⁻¹</td>
<td>84.7</td>
<td>10.7</td>
<td>84.7</td>
<td>&lt;1.0</td>
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<tr>
<td>Fructose</td>
<td>g L⁻¹</td>
<td>89.8</td>
<td>87.2</td>
<td>89.8</td>
<td>&lt;1.0</td>
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<tr>
<td>Gluconic Acid</td>
<td>g L⁻¹</td>
<td>&lt;0.3</td>
<td>72.7</td>
<td>&lt;0.3</td>
<td>66.7</td>
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<tr>
<td>Tartaric Acid</td>
<td>g L⁻¹</td>
<td>1.9</td>
<td>1.7</td>
<td>4.3</td>
<td>1.8</td>
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<tr>
<td>Titratable Acidity</td>
<td>g L⁻¹</td>
<td>3.2</td>
<td>26.7</td>
<td>7.1</td>
<td>27.8</td>
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<tr>
<td>pH</td>
<td></td>
<td>4.89</td>
<td>2.93</td>
<td>3.25</td>
<td>3.05</td>
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<td>Riesling</td>
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<tr>
<td>Ethanol</td>
<td>%v/v</td>
<td></td>
<td>6.5</td>
<td>10.2</td>
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<tr>
<td>Brix</td>
<td>°</td>
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<td>18.1</td>
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<tr>
<td>Titratable Acidity</td>
<td>g L⁻¹</td>
<td>2.7</td>
<td>22.7</td>
<td>10.8</td>
<td>22.6</td>
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<tr>
<td>pH</td>
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<td>5.49</td>
<td>3.07</td>
<td>3.08</td>
<td>3.05</td>
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<td>Total Flavonoids</td>
<td>a.u.</td>
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<td>3.89</td>
<td>1.93</td>
<td>2.49</td>
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<td>Total Hydroxycinnamates</td>
<td>mg L⁻¹</td>
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<td>A420</td>
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</tr>
<tr>
<td>Free SO₂</td>
<td>43</td>
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<tr>
<td>Bound SO₂</td>
<td>241</td>
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<tr>
<td>Total SO₂</td>
<td>284</td>
<td>209</td>
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</table>

5. §Analysis at time of bottling
6. †As tartaric acid
7. ‡Corrected for gluconic acid/lactone
8. ¶Caffeic acid equivalents
<table>
<thead>
<tr>
<th>Yeast strain designation</th>
<th>Modified gene product expression</th>
<th>Growth media</th>
<th>Concentration of metabolites</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>(Nevoigt and Stahl 1996)†</td>
<td>glucose 18 g L⁻¹</td>
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<tr>
<td>Wild type</td>
<td>Control</td>
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<tr>
<td>GPD-1</td>
<td>GPDH-1 over expressed</td>
<td></td>
<td>35</td>
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<tr>
<td>pdc</td>
<td>PDC down regulated</td>
<td></td>
<td>29</td>
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<tr>
<td>pdc GPD-1</td>
<td>PDC down regulated  GPDH-1 over expressed</td>
<td></td>
<td>45</td>
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<tr>
<td>(Michnick and others 1997)</td>
<td>glucose 100 g L⁻¹, pH 3.3</td>
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<td>V5/pVTU</td>
<td>Control strain</td>
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<td>GPDH-1 over expressed</td>
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<tr>
<td>Strain / Condition</td>
<td>Glucose Concentration / pH</td>
<td>GPDH Activity 1</td>
<td>GPDH Activity 2</td>
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<tr>
<td>--------------------</td>
<td>--------------------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>V5/pVTU Control strain</td>
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<td>89.2</td>
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<td>GPD1 V5/GPD1 GPDH-1 over expressed</td>
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<td>19</td>
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<td>(Remize and others 1999) glucose 200 g L(^{-1})</td>
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(Cambon and others 2006)  glucose 200 g L⁻¹; malic & citric acid 6 g/L; YAN 460 mg/L

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(Ehsani and others 2009) glucose 240 g L⁻¹
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† converted from mmol L⁻¹
PDC: pyruvate decarboxylase gene
GPDH-1: glycerol-3-phosphate dehydrogenase 1 gene
GPDH-2: glycerol-3-phosphate dehydrogenase 2 gene
ALD6: acetaldehyde dehydrogenase 6 gene
BDH1:(2R,3R)- 2,3-butanediol dehydrogenase
NS: not stated
Figure 1. Biochemical oxidation of β-D-glucose to D-glucono-1,5-lactone (gluconic acid δ-lactone) by glucose oxidase and subsequent hydration to gluconic acid. Catalase enzyme degrades the hydrogen peroxide formed during glucose oxidation (Pickering and others 1998).
Figure 2. Processing scheme for production of reduced alcohol wines using glucose oxidase.
Figure 3  Biochemical pathways and specific enzymes targeted for modulating carbon flux and ethanol production in Saccharomyces cerevisiae. TPI: triose phosphate isomerase; GPDH: glycerol-3-phosphate dehydrogenase; GPP: glycerol-3-phosphatase; ADH: alcohol dehydrogenase; PDC: pyruvate decarboxylase; ALS: acetolactate synthase; ALD: acetaldehyde dehydrogenase; PDH: pyruvate dehydrogenase; DS: diacetyl synthase; BDH: 2,3-butanediol dehydrogenase; DR: diacetyl reductase. Modified from (Cordier and others 2007; Ehsani and others 2009; Remize and others 2000; Ribéreau-Gayon and others 2006).
Figure 4. Separation capabilities of different membrane systems showing typical operating pressure (in parenthesis). RO = Reverse Osmosis; NF = Nanofiltration; UF = Ultrafiltration; MF = Microfiltration.
Figure 5. Schematic of a typical asymmetric composite membrane showing: (1) thin skin of porous membrane; (2) polymeric micro-porous support and (3) polyester support.
Figure 6. Schematic for de-alcoholisation of wine using a closed loop reverse osmosis process. Wine is pumped under high pressure (1) through a semi-permeable membrane (2), and is separated into two streams, permeate (3) and retentate (4). A rectification column (5) is used to thermally distil the permeate with the water (6) added back to the wine and ethanol (7) collected as a by-product.
Figure 7. Basic principle of ethanol removal by vapour pressure differential across a semi permeable membrane. Osmotic distillation employs a stripping phase of degassed pure water: pervaporation uses an inert gas with water vapour. Ethanol migrates through the membrane in a gaseous phase and recondenses within the stripping phase as permeate. Adapted from (Hogan and others 1998).
Figure 8. Mechanical layout of the Spinning Cone Column (SCC). 1: Product in; 2: Product out; 3: Gas in; 4: Gas out; 5: Rotating shaft; 6: Stationary cones; 7: Rotating cones. Courtesy Flavourtech, Lenehan Rd, Griffith, Australia.
Figure 9. Cross section of cone showing fins to create turbulence. 1: Stationary cone; 2: Rotating cone; 3: Fin; 4: Rotating shaft. Courtesy Flavourtech, Lenehan Rd, Griffith, Australia.
Figure 11. Processing scheme for production of reduced alcohol wines using supercritical carbon dioxide extraction (Sieidlitz and others 1991)