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Cryogenic fractionation as a post-fermentation strategy for partial dealcoholisation of red wine: impacts on acid balance, colour, and phenolics

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ABSTRACT

A rise in popularity of no/low-alcohol beverages and increasing vineyard temperatures associated with climate change has driven research into and the subsequent improvement of dealcoholisation methods. Several studies focused on the effects of post-fermentation dealcoholisation methods have reported no significant effects to basic physio-chemical parameters, but volatile compounds are lost. Cryogenic fractionation is a potential method that preserves volatile compounds. There is limited published research regarding cryogenic fractionation and this study investigated whether it is an effective method for removing ethanol from wine. It also investigated any significant effects to chemical parameters. 750 mL of a commercially produced blend of Touriga Nacional, Touriga Franca, and Tinta Roriz at 12.5 % *v/v* was cryogenically treated at –15 °C. Fractionation of the thawing wine provided a 100 mL liquid fraction and 650 mL ice fraction, of 26.2 % *v/v* and 5.2 % *v/v* alcohol respectively. The chemical parameters of the ice fraction analysed were pH, TA, colour using the modified Somers colour and total phenols by Folin–Ciocalteu assays, and organic acid composition by HPLC. A one-way ANOVA and Kruskall–Wallis tests were performed, with post-hoc analysis using Tukey's HSD and Dunn's tests. Ethanol was significantly reduced to 5.2 % *v/v*. Tartaric acid concentration, TA, total phenolic concentration, and colour density were significantly reduced, however lactic acid concentration was significantly increased in the ice fraction. pH, malic acid concentration, and anthocyanin concentration were not significantly affected. The method implemented to carry out cryogenic fractionation was proven effective at partially but significantly removing alcohol from wine. KHT precipitation and a lack of lactic acid partitioning during the freezing of wine significantly altered TA but did not affect pH. KHT precipitation and the reduced solubility of anthocyanin-tannin co-pigments due to the temperatures of cryogenic fractionation is believed to cause a colour loss. This study demonstrates the potential of cryogenic fractionation to be used as an alternative method to effectively reduce alcohol in wine. Further work is suggested to establish a more comprehensive understanding of the impact on the wine beyond the chemical parameters and explore the viability of this method on a commercial scale.

KEYWORDS: cryogenic fractionation, alcohol removal, climate change, no/low-alcohol, temperature

INTRODUCTION

1. Background

The removal of alcohol from wine is not a novel concept, but in recent years has received growing interest. The interest and research can be attributed to a societal change that has seen a consumer-led drive for no/low-alcohol beverages (Bucher *et al.*, 2018), and as a response to increasing vineyard temperatures associated with climate change (Liguori *et al.*, 2019).

1.1. No/low-alcohol beverages

The negative impact alcohol can have on mental and physical well-being, driving, social behaviour, and pregnancy is widely documented (Pickering, 2000) and consequently, no/low-alcohol beverages are being produced to provide alternatives.

The common methods implemented post-fermentation to reduce a finished wine to a no/low-alcohol wine (Table 1) are providing increasingly positive results. The majority of the published literature have reported no significant change to the physio-chemical parameters of the wine after dealcoholisation (Sam *et al.*, 2021). There are currently, however, limitations to these methods that are often highlighted by the undesirable changes they cause to the wine's flavour and aroma profiles (Varela *et al.*, 2015).

1.1.1. Non-membrane-based methods

Vacuum distillation (VD) and spinning cone column (SCC) are carried out in a continuous vacuum and at low temperatures to preserve the delicate volatile aromas and flavours often lost through heating (Schmidtke *et al.*, 2012). Belisario-Sánchez *et al.* (2009) reported low aroma losses of between 1–18 % using SCC and that the beneficial antioxidant activity and stability of the phenolic compounds present in the wine are not affected by the low temperatures.

TABLE 1. Summary of current methods used to manage alcohol content in wine.

Technique	Time of application	Alcohol reduction potential (% *v/v*)	Limitations/potential impact on wine	Reference(s)
Vineyard cultural practices	Pre-fermentation	0–2	Not applicable for no/low. Individual practice has low alcohol reduction potential. Best results achieved from a combination of practices. Noteworthy effect on ripening date.	Gutiérrez-Gamboa et al. (2021); Palliotti et al. (2014)
Early harvested juice fermented for blending		1–3	Not applicable for no/low. Herbaceous and bitter notes.	Fanzone et al. (2020); Kontoudakis et al. (2011); Martínez de Toda and Balda (2013)
Membrane filtration of must		2–5	Not applicable for no/low. Membrane fouling and replacement of materials. Flavour and colour concentration reduced.	García-Martín et al. (2010); Varela et al. (2015)
Must/juice dilution		Winemaker's choice	Not applicable for no/low. Legal limits apply. Reductive aromas and herbaceous notes.	Schelezki et al. (2020); Teng et al. (2020)
GOX		0–5	Not applicable for no/low. Additional SO_2 required for microbial stability. High volumes of gluconic acid produced.	Pickering et al. (1999); Schmidtke et al. (2012)
Arrested fermentation	Mid-fermentation	Winemaker's choice	Not applicable for no/low. Sweet wine produced, lacking in ethyl esters.	Capece and Romano (2019); Malfeito-Ferreira (2011)
Alternative yeast selection		0–5	Not applicable for no/low. Unpredictable flavours. Increased concentration of acetaldehyde, acetoin and diacetyl. Lack of consumer trust.	Heard (1999); Kutyna et al. (2010); Nevoigt and Stahl (1996); Sam et al. (2021)
Non-membrane-based techniques (VD, SCC)	Post-fermentation	0–100	High capital and operational costs. Reduction of volatile compounds.	Margallo et al. (2015); Motta et al. (2017); Sam et al. (2021); Schmidtke et al. (2012)
Membrane-based techniques (RO, nanofiltration, OD, pervaporation)		0–100	High capital and operational costs. Increased bitterness and astringency. Reduction of volatile compounds. Membrane fouling and replacement of materials.	Catarino and Mendes (2011); Liguori et al. (2013); Margallo et al. (2015); Meillon et al. (2009); Sun et al. (2020)

Both VD and SCC methods do, however, still require some thermal treatment of the wine, 15–20 °C (Sam *et al.*, 2021) and 28–38 °C (Schmidtke *et al.*, 2012) respectively, and therefore will result in volatile compound, such as ethyl and isoamyl ester loses (Motta *et al.*, 2017). Furthermore, these methods require high capital investment and the operational costs often make them unfeasible for small wineries (Margallo *et al.*, 2015), but perhaps more manageable for larger ones. Life cycle assessment has shown that SCC is the least efficient of all alcohol removal methods summarised in Table 1 (Margallo *et al.*, 2015). Although, in larger wineries, efficiencies can be improved by re-using the ethanol wastewater elsewhere.

1.1.2. Membrane-based methods

Reverse osmosis (RO), nanofiltration, osmotic distillation (OD) and pervaporation are all membrane-based methods that are used to remove alcohol from wine indirectly, where RO is the most commonly implemented (Schmidtke *et al.*, 2012).

RO and nanofiltration are both pressure-driven methods that require low operating temperatures and consequently minimal volatile compound losses are observed and energy consumption is low (Schmidtke *et al.*, 2012). RO has produced wines displaying similar organoleptic profiles to their corresponding original alcohol content wines (Bui *et al.*, 1986). Comparatively, nanofiltration has been shown to preserve more of the organoleptic characteristics that arise from the fermentation process (Catarino & Mendes, 2011). Due to a lower pressure differential required across the filter membrane, nanofiltration can be more cost-effective than RO (Margallo *et al.*, 2015). Membrane fouling and other technical requirements associated with nanofiltration, however, can make it a less financially viable method than alternative alcohol removal methods (Varela *et al.*, 2015).

OD and pervaporation are methods based on the evaporation of wine through a membrane at low temperatures (Sam *et al.*, 2021). Both methods have a low energy demand and it has been shown that OD can be more efficient than RO and SCC (Margallo *et al.*, 2015). Liguori *et al.* (2013) found that OD had no significant effect on the pH, total acidity, composition of organic acids, tartaric esters content, or total phenolic content of red wine. Significant differences, however, were seen in colour intensity when the alcohol removal was greater than 6.1 % *v/v* (Liguori *et al.*, 2013). Furthermore, Liguori *et al.* (2019) reported that at total dealcoholisation the sensory profile of the wine changed, and volatile compounds levels had decreased by 98 % of the measured attributes. Pervaporation has a high selectivity for compounds, such as ethanol or volatile aromas, and Sun *et al.* (2020) reported a 65–70 wt% retention of volatile compounds after dealcoholisation of red wine. Process times for both methods, however, can be lengthy owing to low ethanol flux in osmotic distillation (Schmidtke *et al.*, 2012) and low permeation rates in pervaporation (Sun *et al.*, 2020).

1.2. Climate change

Increasing temperatures associated with climate change are having a direct impact on viticulture (Jones *et al.*, 2005; Parker *et al.*, 2020). Technical ripeness in grapes is achieved earlier as a direct consequence of greater vapour pressure deficit and sugar accumulation (Keller, 2020). This can lead to anthocyanin sugar decoupling, where phenolic development requires the fruit to hang for longer in cooler temperatures seen in later months (Martínez de Toda & Balda, 2015). This causes a problem for both vineyard managers and winemakers. The choice: pick grapes with target potential alcohol and acidity levels, but lacking beneficial phenolic ripeness, or pick grapes with target phenolic ripeness and high potential alcohol levels and low acidity.

Viticultural measures can be taken in an attempt to delay the ripening of the grapes, thus reducing the potential alcohol levels and avoiding anthocyanin sugar decoupling. Alternatively, measures can be taken in the winery throughout the winemaking process to manage the final alcohol level.

1.2.1. Pre-fermentation

1.2.1.1. Vineyard cultural practices

Gale and Poljakoff-Mayber (1967) studied the effect of anti-transpirant spray on the transpiration rate and photosynthetic ability of the vine when applied to the apical leaves. Di Vaio *et al.* (2020) reported a reduction in sugar levels of 2 °Brix in Falanghina at harvest time. Similar results were reported on Sangiovese by Palliotti *et al.* (2013) equating to a 10-day ripening delay, however anthocyanin levels were reduced by up to 15 %. Palliotti *et al.* (2014) suggested that anti-transpirant sprays should only be applied to cultivars in which high levels of anthocyanins develop naturally, thus a potential reduction in anthocyanins is less significant than on the sugar accumulation.

Pruning style and timing can also have an impact on ripening. Gutiérrez-Gamboa *et al.* (2021) reported that late winter pruning and minimal pruning can have a delaying effect on sugar accumulation by up to three weeks.

The advantage of these viticultural practices is that they are financially attractive and often easy to implement. To achieve any long-term mitigation of the increasing temperatures associated with climate change, however, a combination of vineyard cultural practices is required.

1.2.1.2. Early harvested juice fermented for blending

Collecting and fermenting the grape clusters removed during the green harvesting process can mitigate climate change (Fanzone *et al.*, 2020; Kontoudakis *et al.*, 2011; Martínez de Toda & Balda, 2013; Schelezki *et al.*, 2018). Wines produced from this fruit will have low alcohol and high acidity levels, which can be blended with wines made from fully ripened grapes with beneficial phenolics. Moreover, no additional equipment is required. Kontoudakis *et al.* (2011) found that this method produced reduced alcohol wines with similar phenolic and sensory properties to those made without blending. Conversely, Martínez de Toda and Balda (2013) reported wines contained high levels of herbaceous aromas and bitterness.

1.2.1.3. Membrane filtration of must

Nanofiltration of previously ultrafiltered retentate of the must and blending can then be carried out to achieve the desired sugar concentration and corresponding potential alcohol levels (Varela *et al.*, 2015). Whilst García-Martín *et al.* (2010) achieved a 1.8 % *v/v* reduction in alcohol in a red wine, it was negatively impacted by flavour concentration and colour reduction. Furthermore, difficulties with membrane fouling and additional materials costs can be prohibitive (Varela *et al.*, 2015).

1.2.1.4. Must/juice dilution

The addition of water to must is not legal in many winemaking regions. In 2017, however, in response to high sugar levels in grapes, Australia authorised the dilution of must to no lower than 24.4 °Brix (Teng *et al.*, 2020). Teng *et al.* (2020) found that, in small volumes, dilution of must from well-ripened Shiraz fruit produced wines with an increased perception of chocolate and dried fruit characteristics. Reductive aromas, however, developed when large volume dilutions were used (Teng *et al.*, 2020) and wines can be reminiscent of those made from early-harvest fruit (Schelezki *et al.*, 2020).

It is important to note that dilution uses water, a scare resource in these particular regions.

1.2.1.5. Glucose oxidase

Using the enzyme glucose oxidase (GOX) to convert glucose in must to gluconic acid and hydrogen peroxide has been shown to reduce the fermentable sugars, resulting in reduced alcohol wines (Pickering *et al.*, 1999). Studies have found that oxidation efficiency of the enzyme is dependent on many factors, such as must oxygen content, must pH and temperature, and the alcohol reduction can range from less than 4 % up to 40 % (Schmidtke *et al.*, 2012).

The production of gluconic acid can lead to unwanted excessive total acidity and out-of-balance characteristics in the final wine (Schmidtke *et al.*, 2012). More importantly, increased sulphur dioxide (SO_2) levels, close to or in excess of the legal limit, are required to provide microbial stability (Pickering *et al.*, 1999).

1.2.2. Mid-fermentation

1.2.2.1. Arrested fermentation

Preventing the completion of the fermentation by various methods will successfully result in a reduced ethanol content wine. The high levels of residual sugar, however, increase the risk of microbial instability and result in additional winemaking procedures or increased levels of SO_2 (Sam *et al.*, 2021). Furthermore, during longer fermentation periods yeasts produce a large amount of ethyl esters and monoterpenes that are responsible for the volatile compounds associated with a good aroma profile (Capece & Romano, 2019). A short, incomplete fermentation period, therefore, will result in a sweet wine with a poor aroma profile.

1.2.2.2. Alternative yeast selection

Saccharomyces cerevisiae yeasts have long been used in winemaking due to their production of ethanol in fermentation environments that see other strains die. Non-*Saccharomyces cerevisiae* can be used to achieve reduced ethanol levels from 0.2–2 % in wines (Sam *et al.*, 2021), but often produce unpredictable and unwanted flavours (Heard, 1999). Consequently, research into genetically modified yeasts strains as an alternative has increased. Re-routing the carbon from ethanol production to alternatives, often glycerol, has been successfully achieved using genetically modified strains, achieving up to a 45 % reduction in ethanol (Nevoigt & Stahl, 1996). This can, however, lead to increased concentration of unwanted fault-perceived chemicals such as acetaldehyde, acetoin, diacetyl, and acetate (Kutyna *et al.*, 2010).

Furthermore, the consumer's lack of trust in genetically modified organisms used in the food and drink industry has a large impact on the marketability of the product.

1.2.3. Post-fermentation

The aforementioned methods implemented to produce no/low-alcohol wines are all applicable to partially remove alcohol as a tool to mitigate the effects of climate change in viticulture and winemaking. In production of no/low-alcohol wines, partial removal of alcohol up to 2 % has minimal changes to organic acid and phenolic levels (Varela *et al.*, 2015). Furthermore Gil *et al.* (2013) reported that removing ethanol from red wine by up to 2 % *v/v* using RO does not appear to have a large effect on a wine's aroma and taste profile, when tasted by a trained panel. Diban *et al.* (2013), however, reported a 20 % aroma compound loss when a reduction of 2 % *v/v* alcohol was achieved by OD. King *et al.* (2013) reported that the partial removal of alcohol by SCC can decrease the perception of complexity and hotness and Lisanti *et al.* (2013) found that vibrant red fruit flavours become cooked after a reduction of 5 % or more.

2. Cryogenic fractionation

There is limited published literature regarding the use of cryogenic fractionation to reduce the alcohol content of finished wine. Freeze concentration, however, was patented by Vella (1984) as a method to remove alcohol from a finished wine for the production of 'light' wines. Cryogenic fractionation is a form of freeze concentration and can be used to separate out two miscible liquids (Md Zamani *et al.*, 2015). Potentially, it can provide an ice fraction of reduced alcohol wine and liquid fraction of increased alcohol wine. It is this ice fraction that will be the focus of this study. Historically, a similarly simple method, referred to as 'jacking', was used to increase the alcohol percentage of cider (Black, 2010). Sufficient cooling of the cider, resulted in the water content forming easily removable ice blocks, leaving behind a more alcoholic cider (Black, 2010).

In Vella's freeze concentration method the ice fraction that formed as the temperature was lowered to –18 °C was removed and allowed to thaw back to a liquid. The two fractions were analysed and blended to achieve a desired alcohol content and further adjustments made to the blend to improve stability and flavour profile (Vella, 1984). Upon comparison against other reduced-alcohol wines, it was reported that the 'light' wines produced by Vella from a blend of white grape varieties were organoleptically superior and the cooked or bitter flavours found in other reduced-alcohol wines were not present (Vella, 1984).

The concept of freeze concentration is more commonly used on sugar concentration in fruit juice (Deshpande *et al.*, 1984). Zhang *et al.* (2016) increased sugar concentration of grape must from 14 °Brix to 23 °Brix as an alternative to chaptalisation. Wines made from treated juices were judged to be better than those made with chaptalised juice, and furthermore the phenolic concentration was increased (Zhang *et al.*, 2016). Additionally, freeze concentration has been investigated as a means of improving colour in wine made from must of underripe and suboptimal grapes. The results showed the wines had increased alcohol content, improved colour, and improved aromas profiles (Wu *et al.*, 2017).

Miyawaki *et al.* (2016) used progressive freeze concentration to increase sugar concentration in apple juice from 13.7 °Brix to 25.5 °Brix. This method has further been developed to provide an alternative to distillation and is reported to retain a similar flavour profile to that of the original beverage (Miyawaki & Inakuma, 2021).

The use of methods based on the freezing points of liquids are common in the food and drinks industry, but in recent years cryogenic fractionation is yet to be investigated as an alternative method for the removal of alcohol from wine. Unlike the post-fermentation methods summarised in Table 1, it has been proposed that, due to low temperatures involved in cryogenic fractionation, the retention of volatile compounds will be improved (Petzold *et al.*, 2013). Furthermore, if tanks are used to cold stabilise wine at –4 °C, it is possible to use this existing equipment to remove alcohol from wine using cryogenic fractionation, instead of the additional costs associated with the alternative methods. Vella (1984) showed that it is possible to produce 'light' wines in this way, but their work did not investigate the effects it had on the chemical properties of the wine. Additionally, the 'light' wines were produced from undisclosed varietals and it is therefore unknown what effect varietal difference has on the success of cryogenic fractionation.

3. Research objectives

Currently there is limited published literature regarding cryogenic fractionation as a method to remove alcohol from wine. Consequently, the effects of this method on wine are currently unknown.

This study therefore investigated the effect that cryogenic fractionation has on the chemical properties of red wine. Ethanol content, titratable acidity (TA), pH, organic acid concentrations, colour density, total anthocyanin concentration, and phenolic concentration were measured and analysed to determine any significant changes.

The objective of this study was to test the proposed methodology for the removal of alcohol and to obtain initial findings that would form a foundation of knowledge for the impact of cryogenic fractionation of wine. Furthermore, this study would highlight areas of further research to achieve a more comprehensive understanding of the effects of cryogenic fractionation on wine.

MATERIALS AND METHODS

1. Materials

This study was carried out on Qunita de la Rosa, duoROSA Red Wine 2020 with 12.5 % *v/v* alcohol, 4.8 g/L (as tartaric acid) total acidity, pH 3.77, and 1.54 g/L total sugars (Quinta de la Rosa, 2023).

34 × 28 × 5 cm (L × W × D) non-stick carbon steel trays (Chef Aid, 2024) were used to contain the wine when placed into a chest freezer (Vestfrost Solutions, 2024).

A fine cotton butter muslin cloth (Bigger Trading Ltd., 2024a) secured in a 10-inch diameter funnel (Bigger Trading Ltd., 2024b) was used to strain the liquid fraction from the ice fraction.

2. Experimental design

A cryogenic fractionation treatment was carried out in triplicate, where the ice fraction and liquid fraction remained separate, providing samples Sn_{ice}, Sn_{liquid}, where n represents the experimental unit of 750 mL red wine.

A positive control treatment was carried out in triplicate as above. After fractionation, the ice and liquid fractions were recombined, providing samples PCn, where n represents the experimental unit. This was to account for any extraneous changes due to the presence of oxygen.

No cryogenic fractionation was carried out on the negative control, NC.

This sample design is summarised in Figure 1.

3. Methods

Trays S1, S2, and S3 containing 750 mL of red wine were placed overnight into a freezer with a –15 °C set point, Figure 2 images a) and b). Once frozen, the wine was broken up and transferred into a 10-inch diameter, muslin-lined funnel, Figure 2 images c) and d). All fractionation was carried out within the freezer and 100 mL of liquid fraction was collected. The remaining ice fraction was placed back into the tray and allowed to thaw, Figure 2 image e). This treatment was carried out in triplicate providing liquid fraction samples $S1_{liquid}$, $S2_{liquid}$, and $S3_{liquid}$, and ice fraction samples $S1_{ice}$, $S2_{ice}$, and $S3_{ice}$.

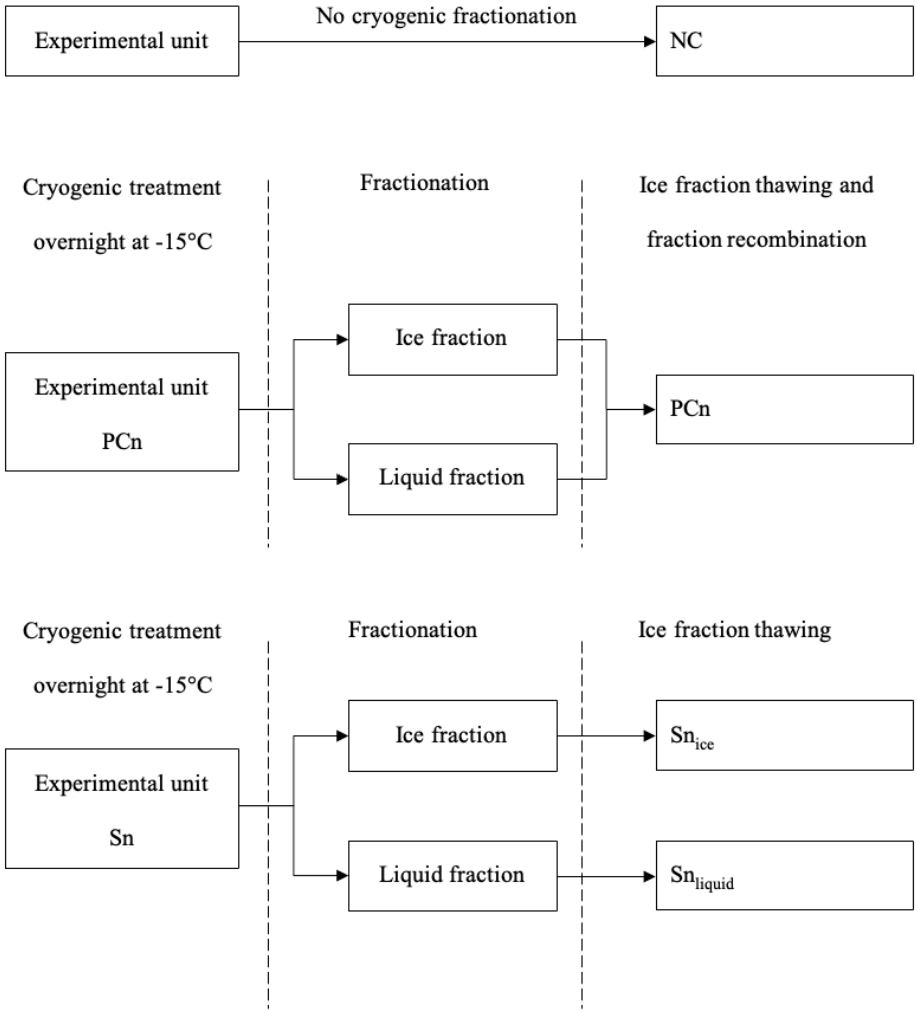


FIGURE 1. Experimental design diagram, where n represents the experimental unit number of 750 mL of red wine. NC – Negative control, no cryogenic treatment carried out on experimental unit. PC – Positive control sample underwent cryogenic fractionation in triplicate, but ice and liquid fractions recombined. Sn – Sample underwent cryogenic fractionation in triplicate, where 650 mL of ice fraction and 100 mL of liquid fraction were achieved.

The positive control treatment was carried out using the above fractionation technique and both fractions were recombined to provide samples PC1, PC2, and PC3.

All samples were bottled, sealed and stored at room temperature throughout analysis.

4. Analysis methods

4.1. Ethanol content

A Megazyme Ethanol (liquid ready) enzymatic kit, K-ETOHLQR, was used and all methods were carried out as per the manufacturer's instructions (Megazyme, 2017). All reagents were provided within the enzymatic kit.

A Thermo Fisher Multiskan Sky 1530 microplate reader (Thermo Fisher, 2017) was used with a Thermo Fisher nunc 96 well U bottom plate (Thermo Fisher Scientific, 2021).

Positive and negative control and liquid fraction samples were prepared at a 1:500 dilution with de-ionised water.

Ice fraction samples were adjusted to pH 9.0 with 2 M sodium hydroxide (Fisher Scientific UK, 2023d) to remove carbon dioxide, before being prepared at a 1:25 dilution with de-ionised water.

Ethanol (Fisher Scientific UK, 2023b) standards of 2, 3, 4, 5, and 6 % were prepared for comparisons against the ice fraction samples.

Ethanol standards of 10, 12, 14, and 16 % were prepared for comparison against the negative and positive controls.

Ethanol standards of 30, 40, 50, 60, and 70 % were prepared for comparison against the liquid fractions.

4.2. Acidity by titration

10 mL of wine sample was titrated to pH 7.0 using 0.1 M sodium hydroxide (Fisher Scientific UK, 2023d).

All titrations were carried out using a Schott Instruments TitroLine easy machine, for auto-titrating (Schott Instruments, 2004).

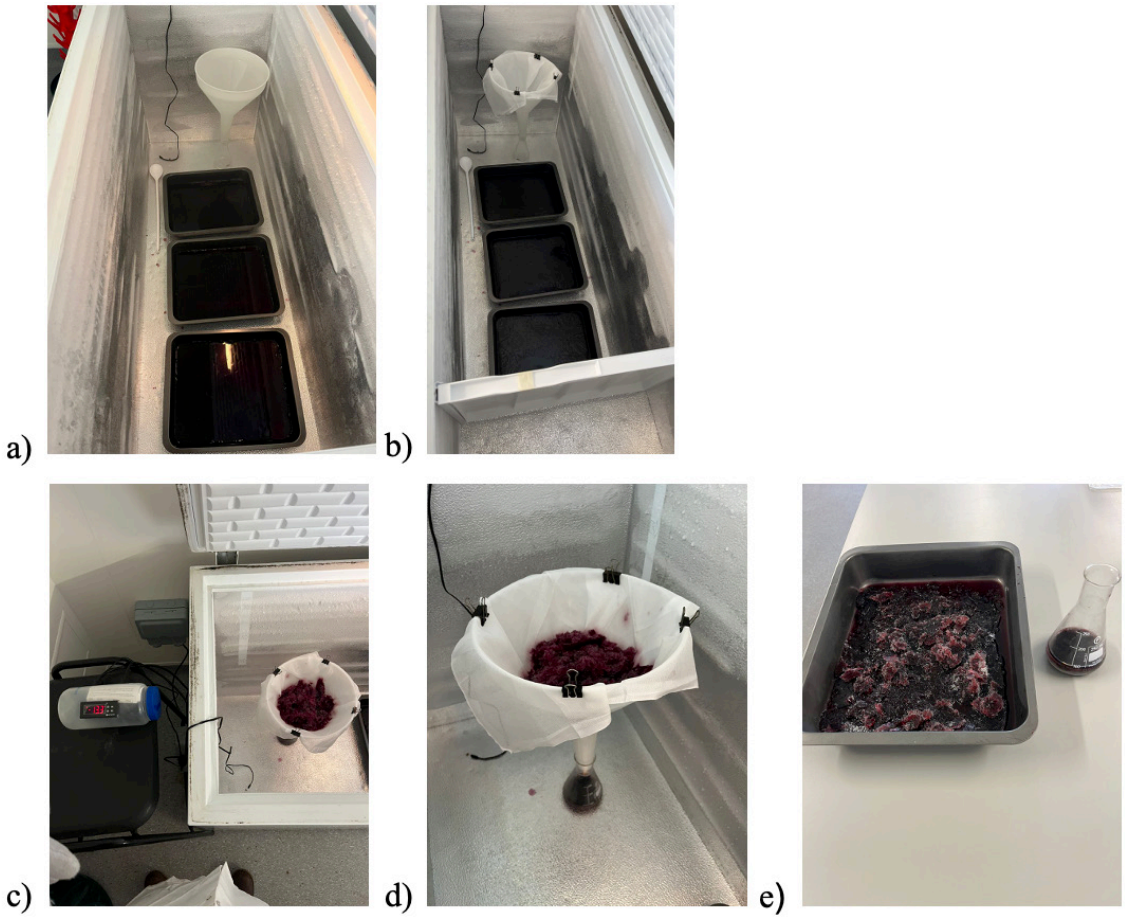


FIGURE 2. Photographs of cryogenic fractionation of red wine. a) and b) cryogenic treatment. c) and d) fractionation process. e) liquid fraction and ice fraction thawing.

TitroLine easy machine was calibrated as per the manufacturer's guidance, using pH 4.0 and pH 7.0 buffer solutions (Reagecon, 2024).

4.3. Acidity by pH

A Jenway 3510 pH meter was used to measure the pH of samples (Jenway, 2009).

Jenway 3510 pH meter was calibrated as per the manufacturer's instruction, using pH 4.0 and pH 7.0 buffer solutions (Reagecon, 2024).

4.4. Total phenols

The Folin–Ciocalteu method was used, as outlined by OIV (2019), but modified as below.

The following reagents were used in the assay: 2 N Folin–Ciocalteu reagent (Sigma Aldrich, 2024a), 200 g/L sodium carbonate solution (Thermo Fisher Scientific, 2024), 5,000 mg/L gallic acid standard solution (Fisher Scientific UK, 2023c), and de-ionised water.

Gallic acid standard solutions were prepared with the following concentrations: 0, 50, 100, 150, 250, 500, and 1,000 mg/L.

In duplicate, 20 µl of each gallic acid standard solution, 1,580 µl of de-ionised water, and 100 µl of Folin–Ciocalteu reagent was added to a semi-micro cuvette (Fisher Scientific UK, 2024b) mixed and left at room temperature for 5 minutes.

All wine samples were diluted by 1:10. In triplicate, 20 µl of wine sample, 1,580 µl of de-ionised water, and 100 µl of Folin–Ciocalteu reagent was added to a semi-micro cuvette, mixed and left at room temperature for 5 minutes.

300 µl of sodium carbonate was added to each cuvette and mixed.

Incubation of samples was carried out at 40 °C for 30 minutes.

The Genesys 6 spectrophotometer (Thermo Spectronic, 2002) was set to 765 nm, blanked with 0 mg/L gallic acid standard solution, and then the absorbance of samples was read.

4.5. Organic acids

Organic acid concentrations were calculated using an Agilent Technologies 1260 Infinity II HPLC High Performance Liquid Chromatography (HPLC) machine with a diode array detector 1A (Agilent Technologies, 2018). The oven temperature was 25 °C and a buffer solution of 25 mM sodium phosphate (pH 2.5) was used at a flow rate of 1 mL/min (Agilent Technologies, 2024b). A Poroshell 120 EC-C18 reversed phase column was used (Agilent Technologies, 2024a).

A standard 1:1 dilution with de-ionised water of 9 g malic acid (Avocado Research Chemicals Ltd., 2024b), 9 g lactic acid (90 % in water) (Avocado Research Chemicals Ltd., 2024a), and 16 g tartaric acid (Fisher Scientific UK, 2023e) was prepared and further diluted to 1:2, 1:4, 1:8 standards.

All samples were filtered using Whatman 0.45 µm syringe filters (Sigma Aldrich, 2024b) prior to HPLC analysis.

4.6. Colour

The modified Somers colour method was used, as outlined in by the AWRI (2012).

The following reagents were used: model wine buffer – 12 % *v/v* ethanol (Fisher Scientific UK, 2023b) and 5 g/L tartaric acid (Fisher Scientific UK, 2023e) adjusted to pH 3.4 with 5 M sodium hydroxide (Fisher Scientific UK, 2023d), 37.5 % *(w/v)* sodium metabisulphite (Avocado Research Chemicals Ltd., 2024c), 0.1 % *(v/v)* ethanal (Fisher Scientific UK, 2023a), and 1 M hydrochloric acid (Fisher Scientific UK, 2024a).

No samples were clarified by centrifuge, due to the limited volume of liquid fraction available.

Treatments were carried out in triplicate for each sample.

Absorbance readings were taken by a Jenway 6315 Spectrophotometer (Jenway, 2008) through 10 mm UV-capable macro cuvettes (Fisher Scientific UK, 2024b).

After hydrochloric acid treatment of liquid fraction samples, the colour density prevented absorbance readings. A further 1:2 dilution with de-ionised water was required.

5. Statistical analysis

Identification of outliers in the data was carried out using Grubbs tests and removed from the data, where determined, before calculating the observed mean values (Ghosh & Vogt, 2012).

Observed mean sample data for chemical parameters was analysed using one-way analysis of variance (ANOVAs) to determine if there were any significant changes that occurred following cryogenic fractionation. ANOVAs were performed using XLSTAT (Addinsoft, 2023) with a confidence level of 95 %, *p*-value 0.05. All data underwent a Shapiro–Wilk test for normality using XLSTAT as part of the ANOVA (Addinsoft, 2023).

For exponentially distributed data, Kruskall–Wallis analysis was performed using XLSTAT (Addinsoft, 2023) to determine if there were any significant changes that occurred following cryogenic fractionation.

Post-hoc analysis using Tukey's Honest Significant Difference (HSD) and Dunn's tests were performed using XLSTAT (Addinsoft, 2023) to determine where the group means differed within the significant model.

Principal Component Analysis (PCA) was performed using XLSTAT (Addinsoft, 2023) to generate correlation circles, observation plots and biplots, all of which help to visualise any correlation that may occur between variables and observations.

Agglomerative Hierarchical Clustering (AHC) was performed on the data using XLSTAT (Addinsoft, 2023) to clearly identify how the data can be clustered into groups, based on dissimilarities.

The supplementary information contains the full data and statistical analysis output.

RESULTS

Table 2 shows that the treatment of cryogenic fractionation had a significant effect ($p < 0.05$) on the ethanol content of the ice fraction samples, where a reduction to 5.2 % *v/v* was observed. No significant effect ($p > 0.05$) was found in the ethanol content of the positive control samples.

The only significant effect ($p < 0.05$) to the positive control samples was tartaric acid concentration, where a reduction of 1.85 g/L was measured.

PCA analysis carried out on the observed means data is shown in Figure 3. 94.82 % of the variability in the analysis can be explained by the eigenvalues F1 and F2, which represent 83.28 % and 11.54 % variability respectively.

The clear grouping of the observations shown in Figure 3 can be further analysed with AHC analysis and the dendrogram, Figure 4, that shows the data is grouped into two clusters. The positive and negative control samples form one cluster, C1, and the ice fraction samples form a second cluster, C2. The AHC results reflect the results for significance shown in Table 2 and provide further confirmation that the significant effects observed in the ice fraction samples were due to the cryogenic fractionation treatment.

1. Acids and acidity

Table 2 indicates that the treatment of cryogenic fractionation had a significant effect ($p < 0.05$) on titratable acidity, lactic acid, and tartaric acid concentration of the ice fraction. There was, however, no significant effect ($p > 0.05$) on pH or malic acid concentration.

The lactic acid concentration measured in the ice fraction showed a significant increase ($p < 0.05$) of 6.96 g/L. Whilst results for the liquid fraction are not the focus of this thesis, it is important to note that 0.00 g/L lactic acid was measured for each of the three liquid fraction samples.

Figure 3 shows a positive correlation between pH, malic acid concentration, and TA, and that they can both be explained by the variable F1. The separation of the eigenvectors indicates that pH has a strong correlation with malic acid concentration, but a weak correlation with TA. When the observations are overlaid to produce a biplot, they have a general relationship with the negative and positive control samples. However, the negative control sample shows a greater relationship with TA and the positive control shows greater relationship with pH and malic acid concentration.

TABLE 2. Summary of the least squares (LS) significant difference from Tukey's HSD and Dunn's tests performed in XLSTAT (Addinsoft, 2023).

Treatment	Ethanol (% *v/v*)	pH	Titratable acidity (g/L)	Total phenol concentration (mg/L GAE)	Total phenolics (au)	Total anthocyanins (mg/L)	Colour density – SO_2 corrected (au)	Lactic acid concentration (g/L)	Malic acid concentration (g/L)	Tartaric acid concentration (g/L)
Negative control	11.0 ± 0.292 a*	3.70 ± 0.012 a	4.91 ± 0.114 a	2,333 ± 44.98 a	45.42 ± 2.53 a	99.67 ± 13.16 a	8.65 ± 0.212 a	1.28 ± 0.043 b	1.23 a	3.27 ± 0.115 a
Positive control	11.1 ± 0.168 a	3.71 ± 0.007 a	4.79 ± 0.066 a	2,386 ± 25.97 a	46.50 ± 1.46 a	96.18 ± 7.60 a	8.87 ± 0.122 a	1.41 ± 0.025 b	1.27 ± 0.00sd a	1.42 ± 0.067 b
Ice fraction	5.2 ± 0.168 b	3.67 ± 0.007 a	3.87 ± 0.066 b	1,943 ± 25.97 b	39.30 ± 1.46 b	84.42 ± 7.60 a	7.35 ± 0.122 b	8.24 ± 0.025 a	1.08 ± 0.017sd a	1.61 ± 0.067 b

GAE – Gallic acid equivalent.

*LS mean values followed by the same letter show no significant difference. Tukey's test carried out with a 95 % confidence interval identifies difference between the means of pairs. Values are mean ± SE. Ses are model-based and were calculated as √(MSE/n), where MSE is the residual mean square from the one-way ANOVA for each response variable. Malic acid concentration was analysed by Kruskal–Wallis analysis and Dunn's test, performed with 95 % confidence interval, and therefore, a standard deviation (sd) is provided in place of standard error.

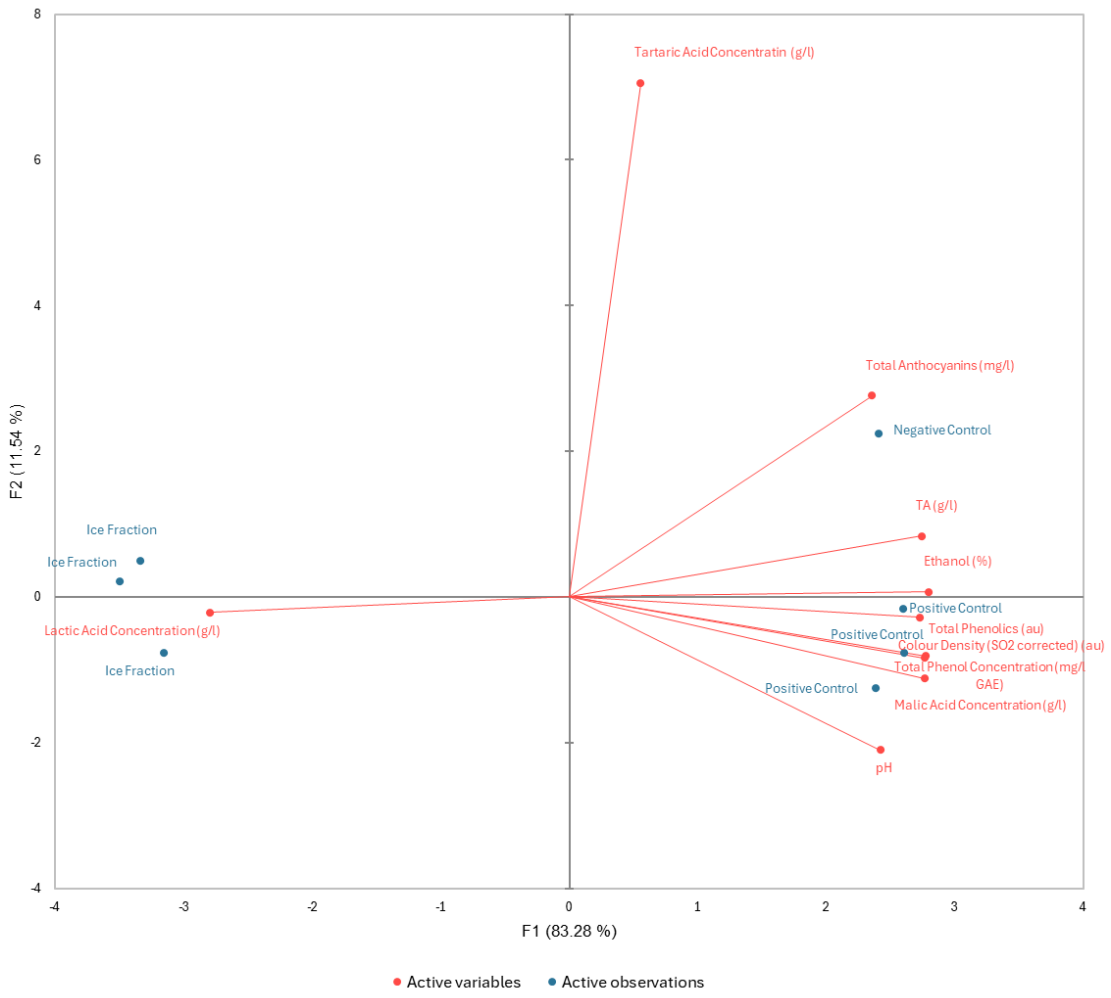


FIGURE 3. PCA biplot of active variables measured in ice fraction, positive and negative control samples following the removal of ethanol from the ice fraction by cryogenic fractionation.

Lactic acid concentration, however, is negatively correlated to the pH, malic acid concentration, and TA, and shows a strong relationship with the ice fraction only. Results with increasingly negative values on the F1 axis have an increasing value for lactic acid concentration, emphasising the results shown in Table 2.

Figure 3 shows that tartaric acid concentration has no correlation to any of the other parameters measured and furthermore that there is no relationship between tartaric acid concentration and any of the samples analysed.

2. Colour and phenolics

Table 2 shows that the treatment of cryogenic fractionation had a significant effect ($p < 0.05$) on total phenol concentration, total phenolics and colour density (SO_2 corrected) in the ice fraction. There was, however, no significant effect ($p > 0.05$) on the total anthocyanins. The effects of oxygen were accounted for by the use of controls (Figure 1).

Variables that are associated with phenolics in Figure 3 can all be explained by increasing values of F1. The eigenvectors for total phenol concentration, total phenols and colour density (SO_2 corrected) are all closely plotted showing a strong correlation and the biplot shows a strong relationship with the positive control sample. Whereas total anthocyanins is less strongly correlated with the other phenolic variables and more strongly related to the negative control.

DISCUSSION

1. Methodology

A statistically significant reduction of 5.8 % *v/v* in ethanol concentration was measured in the ice fraction and no significant change was measured in the positive control. Therefore, the method implemented in this study to reduce alcohol content in red wine by cryogenic fractionation is effective.

The positive control showed no significant effect on the chemical parameters measured, with the exception of tartaric acid concentration and implies that the significant changes measured in the ice fraction are a consequence of the fractionation element of the method.

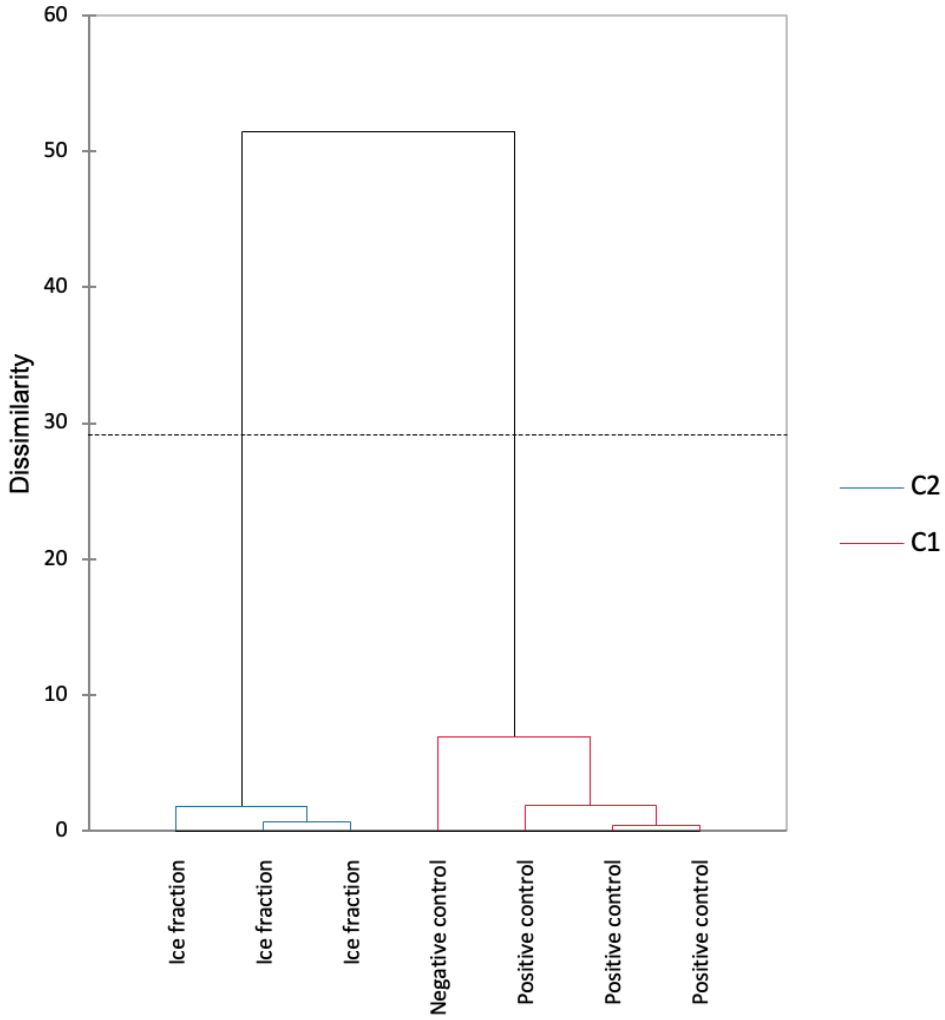


FIGURE 4. AHC dendrogram performed on negative and positive control samples and ice fraction samples, identifying clusters of samples with similar properties and highlighting the significant effect of cryogenic fractionation on the ice fraction. Repetition of samples shown reflections the experiment carried out in triplicate.

2. Acids and acidity

It has been widely reported in published literature that the removal of alcohol from wine, using the post-fermentation methods summarised in Table 1, has no significant effect on the pH, TA, or concentration of organic acids (Sam *et al.*, 2021). The results in Table 2, however, show that there was a significant effect on TA and the concentration of tartaric and lactic acids when alcohol was removed by cryogenic fractionation.

2.1. Tartaric acid

Table 2 shows that a significant decrease in tartaric acid concentration of 1.66 g/L was measured in the ice fraction. In addition, a significant decrease of 1.85 g/L was measured in the positive control. Furthermore, the PCA biplot (Figure 3) shows that tartaric acid concentration has no correlation with any of the other chemical parameters analysed and no relationship with either the ice fraction or the positive control. It is therefore implied that the process of cryogenic fractionation has a significant effect regardless of whether the fractions are recombined or remain separated.

Tartaric acid has a dissociation constant, pK_a, of 3.01 and 4.05 (Ribéreau-Gayon *et al.*, 2006). At the pH of the negative control, 3.7, tartaric acid will therefore dissociate and predominantly be in the conjugate base form, bitartrate, HT^-. The bitartrates will readily bond with potassium, K^+, to form potassium bitartrate salt, KHT. Decreasing temperature and increasing ethanol content of wine decreases the solubility of KHT (Berg & Keefer, 1958). KHT precipitation would be expected as the temperature of the wine reduced to –15 °C, something observed by Zhang *et al.* (2016) when freezing grape juice to –18 °C. Furthermore, the increased ethanol content would reduce solubility into the liquid fraction. As results in Figure S5 ("Summary report of HPLC results – page 2 of 3") show, a reduction of tartaric acid concentration is expected in all samples that have undergone cryogenic treatment and can be seen in PCA biplot, Figure 3.

Tartrate stability of wine will influence the amount of KHT precipitation during cryogenic fractionation. Wine is deemed tartrate stable if no precipitation is observed in wine at room temperature after a cold treatment at –4 °C for 72 hours (Iland *et al.*, 2004). No precipitation should occur in tartrate stable wines until the temperature is reduced beyond –4 °C.

Alternative methods for stability were investigated by Maujean *et al.* (1985), who found that this temperature could be lowered further to –12.8 °C. Greater KHT precipitation would therefore be expected from non-tartrate stable wines during cryogenic fractionation, resulting in a greater reduction in tartaric acid concentration.

It is suggested that, regardless of the temperature to which the wine has been previously stabilised, KHT precipitation should be expected at –15 °C and a reduction in tartaric acid concentration would be measured.

2.2. Malic acid

Table 2 shows that, similar to other post-fermentation alcohol removal techniques, cryogenic fractionation had no significant effect on the malic acid concentration in the ice fraction.

2.3. Lactic acid

Table 2 shows that a significant increase in lactic acid concentration of 6.96 g/L and a 5.2 % *v/v* reduction of alcohol was measured in the ice fraction following cryogenic fractionation. Corona *et al.* (2019) and Pham *et al.* (2020) reported similar findings when using OD and RO-evaporative perstraction methods respectively. Despite these results contradicted published literature, neither study discussed the significant finding in detail, focusing instead on other findings, such as volatile compound losses.

In addition to the significant increase measured in the ice fraction, it is important to note that the HPLC results for the liquid fraction showed 0.00 g/L in all three samples. This implies that lactic acid remained in the ice fraction entirely during cryogenic fractionation, results similar to which could not be found in published literature to date. This is emphasised in Figure 3, which shows a very strong relationship between the ice fraction samples and lactic acid.

The polarity of lactic acid in comparison to malic and tartaric acid is a possible reason why this may have occurred. Lactic, malic, and tartaric acid are carboxylic acids of low molecular weight and highly soluble in water (Waterhouse *et al.*, 2016). Lactic acid is monocarboxylic, whereas malic and tartaric acid are dicarboxylic. Consequently, lactic acid is a more polar molecule that requires less enthalpy than malic and tartaric acid to dissolve into water and therefore more soluble (Waterhouse *et al.*, 2016).

It is well documented that the formation and morphology of ice is very complex, but it has been shown that solutes can be rejected as the ice formation grows, forming a solute concentrated boundary (Petzold & Aguilera, 2009), influencing the structure of the forming ice (Gu *et al.*, 2006). It is suggested that as the water in the wine freezes the less polar, malic and tartaric acid, solutes are rejected, whereas the more polar, lactic acid, solute remains in the water. The rejection of tartaric acid is highlighted in Table 2 and manifested in the precipitation of KHT. Furthermore, the HPLC results for the malic acid show that there is a decrease of malic acid concentration in the ice fraction and an increase in the liquid fraction.

It is therefore hypothesised that cryogenic fractionation will significantly increase the concentration of lactic acid as a consequence of polarity and solubility in water.

2.4. Titratable acidity

Table 2 shows that a significant decrease of 1.04 g/L (as tartaric acid) was measured in the ice fraction. No significant change was measure in the positive control despite a significant decrease in tartaric acid concentration. The significance of any changes to malic acid concentration in the ice fraction could not be analysed.

TA is used to quantify the number of titratable H^+ ions present in a solution, arising from the dissociation of all organic acids (Waterhouse *et al.*, 2016), including those not analysed in this study. The use of post-fermentation methods to remove alcohol generally had no significant effect on the TA of wines (Sam *et al.*, 2021) as would be expected given only a small number of studies reported a significant change to organic acid composition. The organic acid composition of the wine in this study, however, did significantly change and a corresponding change in TA would be expected.

Malic and tartaric acid molecules each contain two carboxylic groups and are di-protic when dissociated, donating two free H^+ ions, whereas lactic acid is monocarboxylic and mono-protic, donating one free H^+ ion. The change in composition of the organic acids in the positive control was not sufficient to have a significant effect on the TA, whereas it did in the ice fraction. Whilst the results for malic acid concentration could not be analysed for significance, the observed means show that there was a decrease. This, combined with a decrease in di-protic tartaric acid and an increase in mono-protic lactic acid concentration was sufficient to significantly decrease the numbers of bound and free H^+ ions in the ice fraction and thus significantly reduce TA.

Cryogenic fractionation will therefore affect the TA of wine as a consequence of the change in the composition of the organic acids. The significance of the effect will be determined by the aforementioned factors influencing the changes in each of the respective organic acids.

2.5. pH

The pH of the wine is determined by $-\log_{10}[H^+]$ ions present in the sample (Waterhouse *et al.*, 2016). Despite the significant changes in the concentration of tartaric and lactic acid and TA, Table 2 shows that cryogenic fractionation had no significant effect on the pH. No significant change in pH has also been reported on most studies investigating the effects of alcohol removal from wine, although this is primarily as a result of no significant change to the composition of organic acids.

Mattick *et al.* (1980) reported that, when investigating different de-acidification methods, it is possible to lower the TA of wine without changing the pH. Furthermore,

Dartiguenave *et al.* (2000) suggested that the organic acids present in wine interact with each other to influence its buffering capacity. The buffering capacity of a wine can be defined as the amount of strong base equivalents needed to raise the pH of one litre of wine by one unit (Ribéreau-Gayon *et al.*, 2006). Each organic acid is in a constant state of equilibrium and compositional changes are compensated for by a shift in that equilibrium. Wine is a complex system containing multiple organic acids, all of which cumulatively provide a buffer capacity for the wine.

It is therefore hypothesised that the buffering capacity of the wine was sufficient to mitigate the impact on pH of the significant changes in organic acid composition after cryogenic fractionation shown in Table 2.

3. Colour and phenolics

Generally, it has been reported that the removal of alcohol from wine has no significant effect on the anthocyanin concentration, colour density, or total phenolics (Sam *et al.*, 2021). There are, however, studies that have reported a significant increase in colour density (Bogianchini *et al.*, 2011; Motta *et al.*, 2017; Pham *et al.*, 2019) and phenolics (Liguori *et al.*, 2010; Russo *et al.*, 2019). Whilst the results in Table 2 show that there was a significant effect on colour density and total phenolics, both parameters were reduced when alcohol was removed by cryogenic fractionation, contradicting the published literature.

3.1. Colour

Table 2 shows that the colour density of the ice fraction was significantly reduced by cryogenic fractionation and a corresponding reduction in anthocyanins would be expected. There was, however, no significant effect on the concentration of anthocyanins in the ice fraction and there must therefore be an alternative explanation for the colour loss.

3.1.1. Anthocyanins

It is understood that the red pigments from anthocyanins are responsible for the majority of colour of young red wines (Somers & Evans, 1974). Anthocyanins can suffer from bleaching in the presence of excess water or bisulfite ions, causing a colour loss (Somers & Evans, 1977). Bleaching is a phenomenon that occurs due to the disruption of double-bond conjunction in the C-ring of anthocyanin molecule and pigmentation is lost (Waterhouse *et al.*, 2016). This commonly occurs when the anthocyanin reacts with water or a bisulfite nucleophile and the double-bond is disrupted at the C2 or C4 position respectively (Berké *et al.*, 1998). The addition of water to a solution will increase the pH and as a result the anthocyanin flavylium cation will equilibrate and react with the water molecule forming a colourless species (Waterhouse *et al.*, 2016). In this study, however, there is no significant change in the pH of the wine after cryogenic fractionation. In the modified Somers colour assay, to mitigate the effect of bleaching caused by the bisulfite ion, excess acetaldehyde is added, to which the bisulfite ions preferentially bind (Iland *et al.*, 2004). The results of the assay show that when the bleaching effect of SO_2 is corrected for, there is still a significant change. The significant colour density change therefore cannot be attributed to the bleaching of anthocyanins.

3.1.2. Copigmentation

Whilst anthocyanins are responsible for the colour of young red wines, within two years of aging this can change and the copigmented forms, can be responsible for as much as 50 % of colour density (Somers, 1971). Copigmentation occurs in wine when coloured pigments, anthocyanins, form associations with non-coloured organic molecules, such as tannins, and can lead to a bathochromic shift and a colour enhancement (Boulton, 2001). Somers and Evans (1979), however, showed that an increase in non-aqueous solvent concentration can lead to a significant loss of colour, despite no change to anthocyanin concentrations. Brouillard *et al.* (1991) suggested that the loss of colour could be as much as 15 % for the level of ethanol found in table wines. It could therefore be expected that the reduction of alcohol from wine would increase the colour density. Table 2 shows that this did not happen in this study.

Boulton (2001) suggested that the reduction of bitartrates during the method of cold stabilisation can destabilise the copigmentation stacks, resulting in a colour loss. As previously discussed, the temperatures involved with cryogenic fractionation caused KHT precipitation. Furthermore, the decreasing solubility of copigmented forms with decreasing temperature could also cause a significant colour loss (Boulton, 2001).

It is therefore hypothesised that cryogenic fractionation will significantly decrease the colour intensity of red wine as a result of the temperature associated with the method reducing the solubility of KHT and anthocyanin-tannin copigments.

3.2. Tannins

Table 2 shows that the total phenolics measure by the modified Somers colour assay were significantly reduced in the ice fraction, which is also reflected by the results of the Folin–Ciocalteu method assay for total phenol concentration. A loss of phenolics in dealcoholised wine has not been found in published literature.

Tannins can be categorised broadly into two groups, condensed tannins and hydrolysable tannins. Condensed tannins (proanthocyanidin) are oligomeric and polymeric chains of flavan-3-ol units (*e.g.*, catechin and epicatechin) linked by the interflavan C-C bonds, (Khanbabaee & van Ree, 2001) and are naturally occurring in grapes. Hydrolysable tannins, in contrast, are comprised of esterified gallic or ellagic acid with a hexose, commonly glucose core, forming gallotannins and ellagitannins (Khanbabaee & van Ree, 2001) and are extracted from products such as oak in the vinification process (Puech *et al.*, 1999). They are commonly susceptible to cleavage by acid, or enzyme mediated hydrolysis, yielding gallotannins and ellagitannins (or gallic acid, and ellagic acid respectively).

Under mild acid conditions the ester linkage in the gallotannins and ellagitannins can be broken down by hydrolysis into their respective constituents; gallic or ellagic acid (Waterhouse *et al.*, 2016). Conversely, condensed tannins containing interflavan linkages are less susceptible to hydrolysis, hence the original categorisation of the two groups (Khanbabaee & van Ree, 2001). It is therefore possible that the significant reduction in phenolics is caused by hydrolysis. The lack of barrel-aging of the duoROSA 2020, however, would suggest that the hydrolysed tannin content would initially be low, and the effect of any hydrolysis would be small.

It is possible that the difference in polarity between hydrolysable and condensed tannins has caused a significant loss in phenolics after cryogenic fractionation. Hydrolysable tannins contain hydroxyl groups, increasing polarity, and can form hydrogen bonds with water molecules and increasing solubility. Whereas condensed tannins have a higher molecular weight and are less polar, thus less soluble. It was previously discussed with regards to the solubility of anthocyanin-tannin copigments that the solubility decreases as temperature decreases.

It is therefore possible that the reduced solubility of the condensed tannin form of copigmented anthocyanins at low temperatures has resulted in a significant loss of phenolics and colour after cryogenic fractionation.

CONCLUSION AND FURTHER WORK

1. Effects of cryogenic fractionation

The cryogenic fractionation methodology implemented produced a significant reduction in ethanol content of the ice fraction from 11.0 % *v/v* to 5.2 % *v/v*, proving this method effective for the partial removal of ethanol from red wine. It is possible that, with refinement of the method, the extent of ethanol removed from wine could be increased. The majority of published literature regarding post-fermentation methods to remove alcohol from wine do not report any significant changes to the chemical parameters investigated in this study. In contrast, this study shows that cryogenic fractionation had a significant effect on some of these parameters.

A significant change in TA, tartaric and lactic acid concentration, total phenol concentration, total phenolics, and colour density was measured in the ice fraction samples. There was, however, no significant change to pH, malic acid concentration, or total anthocyanins of the ice fraction samples. Consequently, cryogenic fractionation did not have a significant effect on all the parameters measured and therefore the null hypothesis must be rejected.

Various methods, summarised in Table 1, are commonly implemented at different stages of viticulture and winemaking to produce wines with lower alcohol content in response to climate change and/or a market drive for no/low alcohol wine. Many of the pre- and mid-fermentation methods can be limited by the amount of alcohol that can be reduced, and the post-fermentation methods can be financially prohibitive and cause unwanted sensory changes to the wines. It is therefore possible that cryogenic fractionation can provide a possible alternative. The nature of this study was to demonstrate the concept of fractionation of wine cryogenically, and the authors acknowledge that further testing, such as sensory analysis, volatile composition, and mineral composition, would have substantiated the methodology. However, the success of this pilot study provides a grounding for the allocation of resources for further testing.

1.1. Acids and acidity

The polarity and the associated solubility of the organic acids influence the partitioning into either the water or ethanol solvent as the temperature is reduced to –15 °C during cryogenic fractionation. While the buffering capacity of the wine was sufficient to mitigate the compositional change of organic acids, the TA was significantly affected.

Whilst the organic acid composition of the wine was found to be significantly affected by cryogenic fractionation, the partitioning of the individual acids requires further research. Investigating the behaviour and solubility of lactic acid during the cryogenic treatment of the wine may find a maximum concentration, above which lactic acid will partition into both the ice and liquid fraction.

The tartrate stability of wine before cryogenic fractionation will potentially influence the degree of KHT precipitation at –15 °C and therefore influence the reduction in tartaric acid concentration. A comparison of tartrate stability methods and their effect on KHT precipitation during cryogenic fractionation is recommended.

The buffering capacity of wine is unique to each wine, and it is recommended that further research is carried out to investigate the effect of cryogenic fractionation on wines of varying pH and buffering capacity.

1.2. Colour and phenolics

The solubility of condensed tannins was reduced as the temperature of cryogenic fractionation was decreased to –15 °C. The loss of condensed tannins and KHT precipitation, lead to a reduction of anthocyanin-tannin copigments and thus a reduction in colour density following cryogenic fractionation.

Whilst the wine in this study was not barrel-aged, it is unknown whether oak-based additives were used in the winemaking process. It is therefore possible that hydrolysis of hydrolysable tannins present in the wine could have resulted in the reduction in phenolics. It is therefore recommended that, in addition to the modified Somers colour assay and the Folin–Ciocalteu method, a methyl cellulose precipitation assay is carried out to determine the levels of condensed tannin in the wine, pre and post cryogenic fractionation. This will give an indication of the reduction of condensed tannins in comparison with the total phenolic reduction.

2. Further work to explore commercial viability

Following this study into the impact of cryogenic fractionation on the chemical properties of red wine, further research into its impact on organoleptic characteristics would be beneficial. It has been suggested that that the very low temperatures associated with cryogenic fractionation may not have a significant impact to volatile compounds (Petzold *et al.*, 2013). It is recommended that the effect of cryogenic fractionation on the flavour and aroma profiles is analysed by gas chromatography and a trained tasting panel.

The economic viability of cryogenic fractionation has not been investigated in this study. It is recommended that a life cycle assessment is carried out to provide a comparison with the common methods used currently in alcohol removal. Minimal investment in new winery equipment is a financially attractive aspect to cryogenic fractionation, and it is possible that the liquid fraction by-product could be used for fortification or colour improvement within the winery.

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