Towards Continuous Wine Fining: Materials Characterisation and Cross-flow Performance

Testing of Polymer-Bentonite Mixed Matrix Membranes

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Abstract

This study investigates the feasibility of using bentonite-embedded mixed matrix membranes to perform wine fining on white wines to eliminate protein instability. Membranes made using polyethersulphone or polyvinylidene fluoride were fabricated using the wet phase inversion technique and used to filter unfined New Zealand Sauvignon Blanc wine in a cross-flow filtration system.

The morphologies of all the fabricated membranes were characteristic of asymmetric phase inversion membranes. Confocal laser scanning microscopy analysis on the membranes suggests that the two major mechanisms of protein removal are adsorption by the embedded bentonite particles and size exclusion via the membrane morphology. Polyethersulphone membranes were found to produce wine of similar levels of protein stability (around 90% protein removal) and polyphenol content as polyvinylidene fluoride membranes of the same membrane composition and operating conditions but with as much as 80% higher flux. Stabilising wine in one filtration pass was possible with polyethersulphone membrane with 25 wt% bentonite while maintaining high flux. These promising results show that the tested process has the potential to replace the current batch-wise fining process with continuous cross-flow membrane filtration with benefits such as increased processing speed and reduced wine loss.

Keywords: wine fining; mixed matrix membrane; bentonite; protein filtration; cross-flow filtration

1.0 Introduction

In the wine industry, bentonite fining is used to stabilise white wines by removing unwanted proteins before they denaturise and form a white haze \((1, 2, 2, 2, 2, 2, 2, 2)(1, 2)\). It is a simple and reliable batch process but with several problems, such as long processing time and the generation of a high amount of waste known as lees \((1)(1)\). Many studies had been undertaken to improve the efficiency of this process, such as the use of alternative fining agents \((3)(3)\), filtration and centrifugation processes \((4, 5, 5, 5, 5, 5, 5, 5, 4, 5)(4, 5)\), and in-line dosing to decrease processing time \((5, 6, 6, 6, 6, 6, 6, 6)(5, 6)\).

This study proposes another alternative, the use of mixed matrix polymeric membranes in a cross-flow filtration system.

Ultrafiltration membranes are known to be able to separate proteins mainly based on their molecular weights \((7, 8, 8, 8, 8, 8, 8, 8, 9, 9, 9, 9, 9, 9, 9)(7-9)\). While proteins can be removed from white wines using this method, membranes with low molecular weight cut-off (MWCO) are required, and such membranes have been found to also reduce colour and total polyphenol content in wines, which can negatively impact the taste, aroma and experience of the wine \((9, 10, 10, 10, 10, 10, 10, 10, 11, 11, 11, 11, 11, 11, 11, 11, 11, 11, 11, 11, 11)(9-12)\). In addition, the study by Flores et al. \((10)(10)\) found that the use of ultrafiltration membranes did not guarantee the protein stability of the processed wine.

Embedding micro- or nano-sized particles into membranes results in mixed matrix membranes (MMM) \((13)(13)\). Embedding clay particles in polymeric membranes results in membranes that show improved mechanical and thermal stabilities and increased porosity \((14, 15, 15, 15, 15, 15, 15, 15, 16, 16, 16, 16, 16, 16, 16, 17, 17, 17, 17, 17, 17, 17, 17, 17)(14-17)\). Avramescu et al. \((18)(18)\) proposed and described the use of protein adsorber membranes, making use of the embedded clay or ion exchange particles that actively adsorb protein molecules. The study by Anadão et al. \((14)(14)\) demonstrated how to successfully embed montmorillonite particles in a polysulphone membrane. This fabrication method was later adopted by Tran et al. \((15)(15)\) who examined the use of high clay fraction MMMs...
as protein adsorber membranes to remove proteins from a model wine solution and found favourable results: the membranes were capable of removing some bovine serum albumin (BSA) from the model wine solution in a dead-end filtration setup, and that the embedded montmorillonite was responsible for a fraction of the membrane rejection of BSA. MMMs combine the principles of adsorption (and chromatography when in series) and membrane filtration in a single separation step that harness particles with an active surface on which the protein adsorbs as the wine is filtered. The ideal MMM must show specificity onto the encapsulated particles while maintaining an open matrix with well-attached particles that have most of their active surface available for adsorption.

The main function of cross-flow filtration in the wine industry is the clarification of wines, removing any suspended and precipitated solids, or to remove microbiological organisms to prevent spoiling (19, 20, 20, 20, 20, 20, 20, 20). With the advancement in MMM technology, the extension of this process to remove wine proteins can be realised. This work attempts to extend the use of cross-flow filtration from wine clarification to protein stabilisation by making use of protein adsorber membranes as the filter medium and examines different membrane attributes that can affect the effectiveness of the process.

2.0 Materials and Methods

2.1 Membrane Fabrication

Membranes were made using wet-phase inversion (also known as phase inversion by immersion precipitation and non-solvent induced phase inversion). Two different polymers were tested as the polymer membrane matrix: Polyethersulphone (PES) (Solvay Advanced Polymers, Belgium) and Polyvinylidene fluoride (PVDF) (Sigma-Aldrich, USA, Mw 275,000). These polymers were readily available and are commonly found in the construction of microfiltration membranes used in wine filtration where PES represents the more hydrophilic polymer and PVDF the more hydrophobic polymer (19). The polymer pellets were dissolved in N-methyl-2-pyrrolidone (NMP, Merck Schuchardt, Germany) by mechanical stirring for several hours to create a polymer dope solution. PES can be dissolved at room temperature while PVDF required heating to 45°C in a water bath to dissolve in a similar amount of time. Deionised water was produced using a Thermo Scientific EasyPure II water purification system (Thermo Fisher Scientific, NZ).

Bentonite particles (Sigma-Aldrich, USA, hydrated particle size d(0.5) of 4.49 µm according to analysis by particle size analyser, (Malvern Mastersizer2000, Malvern Instruments Ltd, UK) were then added to the dope followed by several additional hours of stirring to reach a visually homogeneous casting solution. The weight compositions of the polymer, solvent and bentonite are presented in Table 1. These weight compositions were chosen after several fabrication tests on the ease and consistency of the fabrication process and the membranes, except for membranes PES0 and PV0 which are control membranes with similar (rounding error) dope solution composition to the MMM. Prior to casting, the solution was degassed using an ultrasonic bath (Bandelin Sonorex Digital 10P Ultrasonic Bath, Bandelin Electronic GmbH & Co. KG, Germany) for 30 min. An Elcometer 3700 Doctor Blade (Elcometer Ltd, UK), set to a casting thickness of 250µm, was used with an Elcometer 4330 Basic Motorised Film Applicator (Elcometer Ltd, UK) to cast the membranes sheets (membrane length and width variable depending on volume of casting solution prepared) onto a plate of glass at a casting speed of 1 cm/s at room temperature (20 to 22°C). The glass plate was then immersed immediately (to minimise solvent evaporation time) in a bath of deionised water for the
membranes to fully form. The membranes were then washed with deionised water before storage in a bath of deionised water (which also serves to hydrate the embedded bentonite particles).

### 2.2 Cross-Flow Filtration

An in-house-built cross-flow filtration system was used in all experiments. The key components were two 2.5” MET cross-flow filtration cells (Membrane Extraction Technology Ltd, UK) with a Hydra-Cell G03 pump (Wanner Engineering, Inc, USA).

Membranes were cut to size using a surgical blade, so that they could be installed into a cross-flow filtration cell. Each cell has an active membrane area of 14 cm². Membranes were preconditioned by running deionised water through the system at the same pressures as the filtration experiments (4.5 bar) at 16 L/h (pump flowrate setting with pure water and no membranes). A backpressure valve was used to adjust the system pressure to the desired level. The system was kept running for 30 min to complete the preconditioning of the membrane before the water was drained. Experiments were run under the same pump settings at pressures of 2.0, 3.0 and 4.5 bar. These pressure values were chosen in the attempt to cover the level of pressures used in current wine microfiltration system and to achieve meaningful rate of filtration in this setup (ability to collect sufficient sample for analysis from permeate tap in under 30s).

Unfined Sauvignon Blanc wine (Pernod Ricard Winemakers NZ, collected prior to the bentonite fining stage, with a known bentonite dose of 0.8 to 1.0 g/L bentonite solution required to stabilise, as confirmed via Prostab® and heat tests performed at the supplier) was filtered using the same method as above, and the permeate was collected with a clean beaker for analysis. Permeate samples were taken at regular intervals as well as the permeate flowrate. The samples were tested for protein stability using the Prostab® analysis kit. Each filtration experiment used 750 mL of wine and was run three times using new membranes and all experiments were performed at room temperature with a water bath installed to maintain the temperature of the wine to 20°C. Since this new process aims to combine cross-flow filtration and protein stabilisation, no additional filtration or centrifugation steps were performed prior to experiments.

Wine contains many foulants resulting in membrane fouling. The formation of a cake or gel layer on the membrane surface (due to deposition of suspended colloids and particles such as yeast and bacteria), pore blocking (by colloids, polysaccharides and polyphenols), and adsorption of macromolecules onto membrane material were found to be the major contributing factors to flux decline in wine microfiltration (19). There are countered using a combination of blackflushing and washing and chemical cleaning of the membranes (19). The use of more hydrophilic membrane were also found to reduce the rate of fouling since most foulants in wine adsorb more readily onto hydrophobic surfaces. Backflushing was tested in this study using nitrogen gas as the backflush fluid. Nitrogen gas at 1 bar gauge pressure was connected to the permeate outlet controlled by a tri-way valve, and backflush was performed by switching off the pump and initiating the nitrogen gas flow for 30s.

### 2.3 Clean-in-Place (CIP)

A cleaning procedure similar to the CIP procedure in industry was adopted. A laboratory grade alkaline cleaning solution, Decon90 (Decon Laboratories Ltd, UK), was used to create a 5% cleaning solution with tepid tap water. The filtration system, with the membranes removed, was run using the cleaning solution for 1 hr with the water bath controlling the temperature at 40°C for the cleaning process. The system was then drained of the cleaning solution then operated with tepid tap water...
maintained at the same temperature for another hour. The system was then drained completely before rinsing with tap water three times, followed by a final rinse with deionised water.

2.4 Wine Analysis

It is known that protein concentration is not an accurate representation of the protein stability of wines. Wineries use stability tests in order to determine the protein stability of wine and the amount of bentonite fining required to ensure such stability (21, 22, 22, 22, 22, 22, 22, 22). The Prostab® analysis kit (Laboratoire GRESSER-OEnologie, France, a rapid test procedure and small sample volume designed specifically for use in the wine industry as an alternative to the more commonly used heat test) was used in this work. Since Prostab® precipitates all dissolved proteins and so the level of heat instability can be calibrated against an equivalent protein concentration, allowing for more accurate comparison with model wine solutions. In addition, since Prostab® analysis can be performed in a much shorter time than the heat test, it can be used to monitor the heat stability of wine in a continuous process such as the one proposed by this study.

The Prostab® analysis kit was used according to manufacturer instructions: the two reagents were added to samples in a 1:10 volume ratio. Reagent 1 was first added to a sample in a plastic cuvette and the light absorption at 600 nm was measured with ultraviolet-visible spectroscopy (UV-Vis, Perkin Elmer Lambda-35, Perkin Elmer, USA). Reagent 2 was then added, mixed properly, and left to incubate for 10 min in room temperature before repeating the UV-Vis measurement. The level of increase in light absorption indicates the level of protein instability of the sample and is referred to as the Prostab® stability value in this study. Prostab® recommends an increase of less than 0.010 as an indication of a protein stable wine.

The concentration of polyphenols in wine was measured in gallic acid equivalent, a method optimised by Bajčan et al. (23) which was in turn based on work by Singleton and Rossi (24). The method detailed in the study by Bajčan et al. (23) was used as is, with the exception of the sample and reagents volumes scaled down to 0.4 mL of deionised water, 0.08 mL of sample, 0.02 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, USA), 0.24 mL of 20% w/v Na₂CO₃ (Na₂CO₃ from ECP Ltd, NZ) solution, and a final addition of 3.24 mL of deionised water. A standard curve was prepared in order to convert absorbance readings to polyphenol concentration by performing the analysis on solutions of known concentrations of gallic acid.

2.5 Membrane Characterisation

Membrane cross-sections were prepared by freeze-fracture using liquid nitrogen. Membranes were then mounted on brass blocks in a configuration such that the cross-section, top and bottom surfaces could be analysed by scanning electron microscopy (SEM) with a Philips XL30s Scanning Electron Microscope (Philips, Netherlands). They were coated with a 20 nm platinum coating using a sputter coater (Quorum Q1500RS sputter coater, Quorum Technologies, UK) to increase conductivity prior to SEM analysis. Images in secondary electrons (SE) mode were taken with an accelerating voltage of 5.00 kV and a spot size of 2.0 at various magnifications to analyse the surface topography. Images in backscattered electron (BSE) mode were taken with an accelerating voltage of 20.0 kV and spot size of 4.0 at various magnifications (approximately 1 µm penetration depth) to identify embedded bentonite particles. Energy-dispersive spectroscopy (EDS) (EDAX Genesis, EDAX, USA) was also used to analyse the composition of select regions of the membrane when examined under BSE mode. Image analysis was undertaken using ImageJ (NIH, USA).
Confocal laser scanning microscopy (CLSM) was used to analyse the adsorption of proteins on the membranes after filtration. This technique utilised the near-infrared fluorescence of Coomassie blue (CBB) R250 dye when bonded to protein molecules \((25)\). The 0.01 wt% staining solution was made using CBB R250 dye (Sigma-Aldrich, USA) in a solution of 50 wt% methanol (ECP Ltd, NZ) and 10 wt% acetic acid (Thermo Fisher Scientific, NZ) and the destaining solution of 40 wt% methanol and 7 wt% acetic acid was also prepared. Samples were prepared as detailed by \((25)\) before being mounted for CLSM analysis.

A Zeiss LSM710 Inverted Confocal Microscope was used with an EC Plan-Neofluar M27 objective lens (Carl Zeiss, Germany). The samples were fluoresced with a 633 nm laser. CLSM images are z-stack images with a total penetration depth of 50 μm showing only pixels of highest intensity for maximum transparency. This means that both the dye and the laser are able to penetrate the membrane surface to image the embedded bentonite which should show up as dark dots in pre-filtration membrane images. Porosity in the membranes should also show up as dark dots due to the lack of any material for the dye to bind to. Bright spots seen in pre-filtration membrane images would be a result of insufficient local destaining either from dye molecules being adsorbed by foreign particles or being trapped in membrane pores. The level of fluorescence was then analysed using ImageJ software by measuring the mean grey value of the images. For this set of analysis, model wine solution was used instead of wine to minimise the variables that might interfere with the binding of CBB dye and its fluorescence. The model wine solution contains 0.1 g/L thaumatin (Natex, UK) in a 12% ethanol-in-water solution (ethanol from ECP Ltd, NZ) modified to pH 3.5 with tartaric acid (Ajax Finechem, Australia). A typical batch-wise bentonite fining was performed using a 5 wt% bentonite solution to prepare protein-adsorbed bentonite: bentonite solution was added to the model wine solution, mixed thoroughly, and then left overnight. Centrifuge (10k g for 10 min using Sigma Laboratory 4k15 Laboratory Centrifuge, Sigma-Aldrich, USA) was used to separate the bentonite from solution. Bentonite before and after being used in the fining test were used as samples for CLSM analysis and stained with the above-mentioned method. The same was done for membranes using the same model solution in the cross-flow filtration system mentioned earlier.

### 3.0 Results

#### 3.1 Membrane Morphology

##### 3.1.1 SEM Imaging of Virgin Membranes

The top and bottom surfaces of several representative virgin membranes are shown in Figure 1. There are several visible differences between PES and PVDF membranes. The top surfaces of PES1 are relatively smooth while PV1 are rough and uneven resulting in micro-folds that are suspected to increase the potential of fouling during filtration. These features are not visible on the pure membranes (no bentonite embedded) likely indicating that they are the result of bentonite particles distorting the membrane matrix.

Embedding bentonite particles changed the porosity of the membranes observable in the bottom surfaces. Comparing membrane PES1 to PES0, the average pore size and the percentage area of the bottom surface covered by pores were increased: in terms of Feret diameter (the longest distance between any two points on the edge of a pore), the average pore diameter increased from \(0.4 ± 0.2 \mu m\) to \(3.3 ± 2.4 \mu m\) and the surface coverage from 3.4% to 10.2%. The presence of embedded particles
increased the porosity and pore sizes, consistent with Yan et al. (26) and Tran et al. (15), who theorised the effect to be the result of interactions between polymer and polymer in solution, such that the interaction between polymer macromolecules are weakened. This increases the spacing between the molecules and increases the diffusion rate of solvent during phase inversion, resulting in larger pores. The bottom surface of the PVDF membranes (nodule clusters) also looks different to the PES membranes and is suspected to be more susceptible than PES membranes to erosion (mass loss) during filtration. Bentonite particles are sometimes visible in the occasional open pores.

The membrane internal structure was visible in the cross-section SEM images which show the internal morphology of the different membranes (Figure 2a to 2e), all of which are characteristic of asymmetric membranes fabricated using the same wet-phase inversion technique dense top layer (no visible open pores) with a porous support layer. The layers at the top surface are made up of micro tubular pores just beneath a very thin skin layer forming the dense, non-porous top surfaces visible in Figure 2a, 2c and 2e, and the bottom layer is of a sponge-like porous structure. The difference between PES and PVDF membranes can also be seen. Specifically, the support layers of PES membranes have a sponge-like structure, while PVDF is that of a sponge-like structure followed by a layer of bonded-nodules that can also be seen in the bottom surface images. PVDF membranes have much thinner micro-tubular pore layer but a much thicker support layer. The micro-tubular layers are expected to provide less resistance to trans-membrane flow but will be more susceptible to deformation from feed pressure.

A slight change to the casting solution concentration resulted in a noticeable change in the cross-section morphology, as seen in Figure 2b and 2c (comparing membranes PES1 and PES2). The images show that a higher bentonite loading resulted in a thinner micro-tubular porous layer. This is likely a result of bentonite particles interacting with the polymer during membrane formation (phase inversion) (26, 27). Bentonite particles act as nucleation points, disrupting the formation of the tubular pores. With increased bentonite loading, there is a larger degree of disruption, resulting in thinner tubular porous layers.

The pores in the support layer develop into large cavities and the sizes of these cavities appear to be related to the solvent content of the casting solution. For example, membrane PES2 with 65% solvent content contains less and smaller cavities than PES1 with 70% solvent content, which in turn were smaller than the pure PES membrane (PES0) with 88% solvent content, where the cavities were so large that the cross-section of the membranes had the appearance of multiple sheets of membranes layered together. These cavities produce structural weaknesses and are generally undesirable.

Figure 2f and 2g are higher magnification images of the cross-section to examine the structure of the membrane matrix around the bentonite particles. It is hypothesised that the ideal MMM must show specificity onto the encapsulated particles, while maintaining an open matrix with well-attached particles that have most of their active surface available for adsorption. For PES membranes, the bentonite particles, while surrounded by the supporting polymer matrix, are held in place at certain points where the bonding between polymer and bentonite is visible. This exposes most of the surface area of the bentonite particles to the fluids flowing through the membrane. PVDF membranes on the other hand show more extensive bonding between bentonite particles and the surrounding polymer matrix. This could result in a lower degree of bentonite surface available for adsorption.

The distribution of bentonite particles in the membranes were analysed using the SEM via BSE mode with EDS. Bentonite particles show up as bright regions in the SEM pictures and are confirmed to be such using compositional analysis using EDS, since bentonite particles contain aluminium and high
quantities of silicon not present in the polymer matrix (which contains much higher quantities of
carbon). Figure 3 shows that the bentonite particles were evenly distributed throughout the
membranes. Trace amounts aluminium were detected in polymer-rich regions, as were trace amounts
of sulphur or fluorine in bentonite-rich regions, due to the depth of analysis of EDS.

3.1.2 SEM Imaging of Post-Filtration Membranes

The post-filtration membranes were imaged using SEM and can be seen in Figure 4. Generally, the
top surfaces, due to their higher densities, showed little noticeable change before and after being used
in filtration for PES MMM but a much more noticeable change in PVDF MMM. The bottom surfaces
showed noticeable increases in pore size (Figure 4b and 4d) or have additional eroded appearances
(Figure 4d) indicating loss of material due to the lower density of the bottom layers. Since the bottom
surfaces were not exposed to tangential flow, the only erosion was from transmembrane flow. PVDF
membranes appear to be less robust than PES membranes, showing increased porosity, roughness and
erosion. The cross-section images after filtration showed that irreversible membrane compaction
occurred, as shown in Figure 4e and 4f. As expected, the slanting micro-tubular pores or cavities were
the most affected by pressure and the sponge-like structure and the perpendicular micro-tubular pores
just under the skin layer appear to be largely unchanged.

3.1.3 CLSM Imaging of Virgin and Post-Filtration Membranes

CLSM analysis was performed on the membranes before and after wine filtration in order to identify
the role of the bentonite particles in the removal of proteins. Pure bentonite was used as a control to
test the fluorescence of bentonite particles before and after being used in fining and the results are
shown in Figure 5a and 5b. Protein-bound bentonite showed significantly higher levels of
fluorescence than unbound bentonite particles under the same CLSM setting. PVDF membranes were
found to have much lower levels of CBB dye staining on the polymer than PES, resulting in much
lower signal-to-background ratios, hence the CLSM analysis focused on the PVDF membranes.

The membranes showed increases in levels of fluorescence after filtration (Figures 5c to 5h) similar to
pure bentonite. The mean grey values of the images (Figure 6) measure the increase in fluorescence
and further show the protein removal by the membranes. Comparing pre- and post-filtration
membranes for pure PVDF membranes (Figure 5c and 5d) to MMM (Figure 5e and 5f), it can be seen
that PVDF by itself can adsorb a certain amount of protein resulting in increased fluorescence after
dye staining, but the amount was much less than when bentonite was embedded, showing that the
bentonite in the MMM is crucial to effective protein removal desired in wine fining. Comparatively,
the difference in fluorescence at the bottom surface was much smaller between MMM and pure
membrane than on the top surface. This means that the embedding of bentonite significantly increased
the adsorption of proteins on the top surface. The protein removal therefore is due to the combined
effect of bentonite’s selective protein adsorption and the intrinsic separation produced by a higher
density top layer of the membranes, both combining to make the top layer responsible for the majority
of the protein separation. This indicates further that these membranes act as membrane adsorbers.
This means that a filtration/loading stage and a regeneration stage are needed to enable the use of
these membranes in continuous wine fining – optimising membrane system operation and membrane
performance is therefore crucial.
3.2 Membrane Performance

3.2.1 Flux and Selectivity in Continuous Wine Fining

The membranes were used in a cross-flow filtration system to process unfined Sauvignon Blanc wine. Filtration results are shown in Figure 7. Flux decline was observed for all membrane cases as shown in Figure 7a. This profile appears to be typical of cross-flow filtration systems processing fouling liquids like wine, where a rapid drop of flux occurred at the start of filtration followed by a gradual decline until a terminal (steady state) flux is reached. While this decline can often be attributed to multiple causes, evidence of bulk filter cake formation and pore blocking by fouling was not found on post-filtration membranes. Therefore, smaller scale fouling via protein adsorption on the membrane and on the bentonite (as per the CLSM results in Section 3.1.3), elastic compression and concentration polarisation were likely the major contributing factors to the observed flux decline. Regeneration of the membrane will therefore be important for continuous processing and membrane reuse after protein loading.

The flux results for different membranes and compositions are shown in Figure 7b. The pure membranes were found to produce the highest flux when compared to MMM due to their lower solid content resulting in lower density (higher porosity) and having no bentonite particles that swell to deform the membrane and blocking pores. PES membranes were also found to produce higher flux than PVDF membranes at the same membrane composition operated under the same filtration conditions, as evident from comparing membranes PV1 with PES1 in Figure 7b. This is expected due to PVDF being inherently more hydrophobic.

Composition of the membranes was found to have an impact on flux, as can be seen comparing membrane PES1 with PES2. Membrane PES2 was found to have a higher flux than PES1 despite having a lower solvent percentage in the casting solution and therefore a lower free volume. The difference is most likely due to the difference in morphology of the two membranes, where PES2 contained less large cavities, a thinner micro-tubular layer and more bentonite particles making the membrane less susceptible to compression by pressure.

The effect of filtration pressure on flux can be seen in Figure 7c. The correlation between pressure and flux across the pressure range of 2.0 to 4.5 bar was inverse parabolic with the optimal pressure at around 3.0 bar. This type of flux-pressure relationship has been observed before and is typically attributed to increased compaction and concentration polarisation cancelling out the effect of increasing driving force (pressure). As a result, the permeance of the membrane reduced with increasing pressure, although the decrease from 2.0 to 3.0 bar was much smaller than from 3.0 to 4.5 bar.

Membrane selectivity was analysed by measuring the rejection of proteins and polyphenols. With protein rejection, since the Prostab® analysis kit returns results as protein stability values of wine samples rather than protein concentration, these results would be more accurately defined as the membrane rejection of protein instability causing species, shortened to protein rejection since proteins are the major factor causing protein instability. In general, the ideal membrane for wine fining will have a good protein rejection and low polyphenol rejection.

Like flux, protein rejection was not constant during the course of filtration, but rather, decreased gradually, except for pure membranes where the correlation was reversed (Figure 8a). In theory, for common membrane filtrations, membrane rejection increases gradually during the process without
any disruptions, due to the increases in resistances to mass transfer from factors such as filtration cake
and pore blocking (31)(31). This trend can be seen in the results for pure membranes (PV0 and PES0)
which are functionally common filtration membranes. The rejection results for pure membranes also
showed that the membrane morphology is capable of removing a portion of the proteins. The
correlation between rejection and time for MMM appears to be logarithmic in pattern, similar to that
of flux against time profile, with rejection decreasing with time. These results from the MMM
filtrations thus indicate that the embedding of bentonite particles altered the mechanisms of membrane
rejection: in addition to rejection due to structural properties, adsorption of instability causing
components onto bentonite occurs. The inverse correlation shown in Figure 8a can thus be understood
as the loss of adsorption capacity of the bentonite particles (adsorption sites are filled) as the filtration
progressed. A further mechanism that can produce a reduction in rejection with time is the increase in
flux of molecules which can occur during concentration polarisation, where the build-up of molecules
near the surface of the membrane forms a boundary layer of higher concentration, which can
gradually provide a higher driving force for solution diffusion (32)(32). This however may be a lesser
mechanism in this case since the tangential flow stream usually reduces the effects of concentration
polarisation through pushing material away from the membrane surface (31)(31). It has also been
previously reported that concentration polarisation is an unlikely flux-altering mechanism in wine
filtration (19, 33, 33, 33, 33, 33, 33, 33)(19, 33).

The inverse correlation between rejection and time was not observed for polyphenols, as can be seen
in Figure 8b. Phenolic content removal during bentonite fining had been previously studied with the
results showing that while polyphenols and other sensory compounds were removed, the amount
removed and its impact on the sensory qualities of the processed wine varied depending on the type of
wine (34-36). A study by Salazar et al. (36) reported polyphenol removal of 20.6% via bentonite
fining, and 10% via packed column treatment with similar impacts on sensory qualities, and both
processes negatively impacted the sensory qualities of the Macabeu wine. The removal of polyphenol
content in a protein removal process such as a filtration is inevitable, even though polyphenols are
smaller in molecular size than proteins due to the fact that some polyphenol molecules bind to
proteins (1, 35, 35, 35, 35, 35, 35, 35, 37, 37, 37, 37, 37, 37, 37, 37)(1, 34, 35) and that polyphenols are
known foulants in polymeric membranes in wine filtration due to adsorptive interaction between
polyphenols and the membrane material (38, 39, 39, 39, 39, 39, 39, 39, 40, 40, 40, 40, 40, 40, 40)(36-
38). A previous study by Arriagada-Carrazana et al. (38), who filtered wine using 1.2 and 0.65 µm
filters, reported comparable rejection numbers (10% reduction in phenolic content) which the authors
attributed to adsorption by the membranes. In general, there were no common trends noticeable and
points towards normal (and hard to control) experimental variation. This therefore demonstrates that
the selectivity of the bentonite towards proteins is preserved in the MMMs. This also further
demonstrates that a major protein removal mechanisms of these MMMs is via adsorption, which is
desired since this allows removal of proteins that is selective to the adsorptive properties of the
protein rather than on a less selective size-based separation (which is more likely to strip out
molecules such as polyphenols and other compounds that contribute to the flavour and aroma of wine).
This result could also mean that polyphenol rejection is not as affected by fouling and any pore
closures that happen during protein adsorption and membrane compaction that affected the flux and
protein rejection. The rejection of polyphenols is also very low compared to proteins, which is a
favourable result and its impact on the sensory qualities of wine will be tested in a future study
although a loss of taste, aroma and colour will be expected based on results of previous studies.
Membrane composition was found to have a more noticeable effect on protein rejection than the polymer material, as shown in Figure 8a. Membrane morphology is again suspected to be the main cause for this difference, where a slight change in composition from PES1 to PES2 resulted in higher levels of sponge-like structure and less large cavities which allows for more contact between liquid and bentonite particles, increasing adsorption. Membranes PES1 and PV1 displayed almost identical rejection, but the differences in morphology and flux discussed in previous sections, and the difference in rejection between PES0 and PV0 suggest that identical compositions of PES and PVDF membranes do not necessarily produce identical rejection results but should show similar trends of changing flux and rejection due to membrane morphology changes caused by changing membrane compositions. The pure membranes, PV0 and PES0, had the lowest rejection of proteins as expected, due to the lack of bentonite particles for adsorption.

Overall, a high bentonite composition results in a more favourable morphology, where a higher volume fraction of the membrane is of the sponge-like structure with fewer cavities in the microstructure. The best membrane composition tested was PES2 (10% polymer, 65% solvent, 25% bentonite). This membrane composition offered the highest flux and protein rejection and was the only composition capable of fully stabilising the sample wine in a single filtration pass according to the Prostab® results. Attempts to increase the bentonite composition resulted in difficulties in casting solution preparation and membrane casting. Other fabrication methods may be required to increase bentonite composition even further without negatively impacting the robustness of the membrane.

The effect of pressure on protein rejection can be seen in Figure 9. Since an increase in pressure resulted in a decrease in flux, and that flux was found to be correlated to rejection, increases in pressure will also cause decreases in rejection. Using membrane PV1 as an example, a pressure increase from 3.0 to 4.5 bar resulted in a flux decrease of 25% and a rejection increase of 27% (taking filtration results at 30 min, average flux decreased from 55.2 to 41.5 L/m²h and average rejection increased from 0.718 to 0.912). Membrane PES1, on the other hand, had a flux decrease of 14% (average flux decreased from 86.7 to 74.6 L/m²h) with no significant change to rejection, under the same conditions. This means that the mechanics of separation of instability causing components is different for the two types of membranes. SEM images in Figure 2f and 2g showed that bentonite particles are more exposed in PES membrane than in PVDF ones. This restricts the access to the bentonite adsorption sites in PVDF membranes. With a lower flux, the protein molecules have more time to diffuse through the PVDF matrix to reach the adsorption sites, and with a higher TMP, the driving force for diffusion is increased, allowing faster diffusion of the protein particles. For PES membranes, the bentonite particles are bonded to the surrounding matrix only at a few points on the particles, leaving most of the bentonite surface exposed for adsorption. Protein molecules do not have to diffuse through a layer of surrounding polymer and thus partly negating the effects of flux and TMP on rejection.

The effect of pressure on polyphenol rejection is much more noticeable, as shown in Table 2. All membranes tested on both 3.0 and 4.5 bar pressure show that operation at 3.0 bar is less detrimental to the quality of the filtered wine. Dropping the pressure to 2.0 bar resulted in an increase in rejection. This is the same trend as observed with the effect of pressure on flux and so the reason for this correlation should be the same: the balance between TMP (driving force) and mass transport resistances (membrane compaction and fouling).
Taking into account all of the results together, it can be seen that multiple factors affected the performance of this process of using MMM to stabilise wine and these factors can be optimised further in future studies. From the factors tested in this study, the more hydrophilic PES was shown to be a superior membrane material than PVDF for this purpose, allowing for higher flux with similar selectivity and is thus the recommended polymer to use. In terms of operating parameters, a 3.0 bar pressure showed clear advantage over higher or lower pressures, offering higher flux and higher protein rejection.

### 3.2.4 Flux Recovery

Due to the retention of proteins on the membrane, the flux can also be further improved by backflushing, which is a commonly used method for a highly fouling liquids such as wine \((19)(19)\). This was tested with membrane PES2 at 3.0 bar and the results shown in Figure 10, which showed that the flux profile can be almost fully reversed with 30s of nitrogen backflush. A 30 min backflush cycle was employed here, although in industrial situations the cycle may be as short as 5 min. With the largest flux decline occurring within the first 10 min of filtration operation, the shorter the backflush cycle, the higher the average flux over a long filtration operation. Membrane thickness measurements before and after filtration with backflush also showed no detrimental reduction of thickness after six 30 min cycles.

With a backflush cycle, membrane PES2 had an average flux of 72.4 L/m²h. Considering the scaling up of the process, a quick calculation would reveal that a membrane cartridge with 10 m² of membrane surface area will be able to process wine at a rate of 790 L/h. Further studies will be required to test the scaling up of these MMM.

### 4.0 Conclusions

Overall, this process showed promising results, capable of producing heat stable wine with low loss in polyphenols. The biggest benefits of this process are the increased processing speed (hours compared to days), and reduction of waste produces (lees generation in bentonite fining removed). This process may even be combined with the current filtration process in wine production, further improving the overall production efficiency. In addition, this shows the potential of particle-embedded MMM as a filter medium and the process can be further expanded to the filtration of other liquid products where the manufacturers wish to selectively remove certain undesirable components.

Current studies on bentonite regeneration using alkali and ethanol are ongoing and its use in regenerating MMM will be a subject for further studies and not explored here. As this study focused primarily on the fabrication and viability of bentonite-embedded MMM, future work involving alternative materials, scale-up designs (larger membranes and cross-flow systems), integration into current wineries and detailed sensory analysis on different types of wines will be required before commercialisation of the new process.

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Table 1

Percentage compositions of casting solutions used to fabricate the membranes.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Polymer composition (wt%)</th>
<th>NMP composition (wt%)</th>
<th>Bentonite composition (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV0</td>
<td>12</td>
<td>88</td>
<td>0</td>
</tr>
<tr>
<td>PV1</td>
<td>10</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td>PES0</td>
<td>12</td>
<td>88</td>
<td>0</td>
</tr>
<tr>
<td>PES1</td>
<td>10</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td>PES2</td>
<td>10</td>
<td>65</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 2

Table showing the average permeance and membrane rejection of polyphenols under various operating conditions.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>System Pressure (bar)</th>
<th>Average Permeance at 30 min (L/m²h.bar)</th>
<th>Polyphenol Rejection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PES0</td>
<td>4.5</td>
<td>21.4</td>
<td>4.7</td>
</tr>
<tr>
<td>PES1</td>
<td>2.0</td>
<td>35.4</td>
<td>13.3</td>
</tr>
<tr>
<td>PES1</td>
<td>3.0</td>
<td>28.9</td>
<td>3.4</td>
</tr>
<tr>
<td>PES1</td>
<td>4.5</td>
<td>16.6</td>
<td>8.3</td>
</tr>
<tr>
<td>PES2</td>
<td>3.0</td>
<td>25.0</td>
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<tr>
<td>PES2</td>
<td>4.5</td>
<td>19.4</td>
<td>8.2</td>
</tr>
<tr>
<td>PV0</td>
<td>4.5</td>
<td>12.1</td>
<td>6.2</td>
</tr>
<tr>
<td>PV1</td>
<td>2.0</td>
<td>23.6</td>
<td>10.4</td>
</tr>
<tr>
<td>PV1</td>
<td>3.0</td>
<td>18.4</td>
<td>2.3</td>
</tr>
<tr>
<td>PV1</td>
<td>4.5</td>
<td>8.5</td>
<td>9.5</td>
</tr>
</tbody>
</table>
Fig. 1. Representative SEM images of top and bottom surfaces of virgin membranes: (a) top surface of membrane PES0 showing a dense and smooth surface; (b) bottom surface of membrane PES0 showing small open pores; (c) top surface of membrane PES1 showing a dense surface; (d) bottom surface of membrane PES1 showing a large amount of larger pores; (e) top surface of membrane PV1 showing a wrinkled appearance; (f) bottom surface of membrane PV1 showing pores and the bonded-nodule structure.
Fig. 2. Representative SEM cross-section images of virgin membranes showing typical layered asymmetric structure of phase-inversion membranes where large cavities were found between the tubular porous layer and the supporting sponge-like layer and within the sponge-like layer: (a) PES0; (b) PES1; (c) PES2; (d) PV0; (e) PV1. Higher magnification images show the connection between bentonite particles to the polymer matrix where in PES membranes, the bentonite particles were more exposed than in PVDF membranes: (f) PES MMM; (g) PVDF MMM.
Fig. 3. SEM images of virgin membranes taken using backscattered electrons showing the uniform distribution of bentonite particles within the polymer matrix: (a) PES0 top surface showing no bentonite particles; (b) PES1 top surface; (c) PES2 top surface; (d) PES2 bottom surface showing bentonite particles and open pores; (e) PV0 top surface showing no bentonite particles; (f) PV1 top surface; (g) PES1 cross-section; (h) PES2 cross-section; (i) PV1 cross-section showing two regions analysed using EDS: region (1) 44.7% C, 7.8% N, 5.5% F, 21.8% O, 4.3% Al, 14.3% Si, region (2) 63.4% C, 5.1% N, 11.8% F, 13.7% O, 1.6% Al, 3.8% Si.
Fig. 4. Representative SEM images of membranes after filtration (compare to Figure 2 and 3 for virgin membranes): (a) top surface of PES MMM showing little change to structure; (b) bottom surface of PES MMM showing increased pore size; (c) top surface of PVDF MMM showing surface deformation; (d) bottom surface of PVDF MMM showing surface deformation and increased pore size; (e) cross-section of PES MMM showing membrane compaction; (f) cross-section of PVDF MMM showing membrane compaction.
Fig. 5. CLSM images showing the fluorescence contrast before and after binding with protein and dyed with CBB: (a) pure bentonite without protein; (b) pure bentonite with protein; (c) PV0 top surface pre-filtration; (d) PV0 top surface post-filtration; (e) PV1 top surface pre-filtration; (f) PV1 top surface post-filtration; (g) PV1 bottom surface pre-filtration; (h) PV1 bottom surface post-filtration.
Fig. 6. The difference in fluorescence between pre- and post-filtration membranes measured via mean grey value in ImageJ of PVDF membranes shown in Figure 6.
Fig. 7. The effects of various factors on flux: (a) an example of flux decline over a period of 120 min of filtration using membrane PES2 operating at a pressure of 4.5 bar. The majority of the flux decline occurred within the first 30 min; (b) the flux profiles for both PES and PVDF membranes used to filter Sauvignon Blanc wine under 4.5 bar pressure; (c) the effect of pressure on average flux and permeance filtering Sauvignon Blanc wine at 30 min after initiating filtration.
Fig. 8. The effects of membrane composition on rejection: (a) changes in average membrane rejection of heat instability causing component in wine from 30 to 120 min of filtration time at 4.5 bar pressure. The pure membranes (PES0 and PV0) showed increasing rejection over time, while the MMM (PES1, and PV1) showed the reverse correlation except for PES2 with near constant rejection over time; (b) the average membrane rejection of polyphenols during the course of 120 min filtration runs on Sauvignon Blanc wine at 4.5 bar pressure.
Fig. 9. Average flux and rejection of instability causing components at 30 min of filtration of two membranes of same composition but different polymer material (PES1 and PV1) at two different pressures (3.0 and 4.5 bar). Both membranes show an increase in flux but a decrease in rejection (barely noticeable with the PES membrane) with a lower pressure.
Fig. 10. Average change in normalised flux across time with membrane PES2 at 3.0 bar pressure on Sauvignon Blanc wine with blackflush cycles employed at 30 min and 60 min using nitrogen gas at 1.0 bar.