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Novel fining agents to heat stabilise wine



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Executive summary

Bentonite, a type of aluminium clay is a low cost fining agent currently used to remove heat unstable and haze forming proteins from white wines though there are negative aspects to its use, such as quality loss and sustainability challenges. This study began with a screening process to identify the most effective types of polysaccharides to heat stabilise wine. The study also outlined the use of renewable polysaccharides (carrageenans) as an alternative to bentonite for heat stabilising white wine.

Kappa- and kappa- (90%)/iota- (10%)[1] carrageenans were the most effective polysaccharides for heat stabilising white wine.

A range of commercially available polysaccharides was systematically screened in a 2014 Riverland Chardonnay wine for their ability to heat stabilise wine. A total of 28 carrageenans, 14 pectins, and three carboxymethylcelluloses (CMCs) were tested. None of the pectin polysaccharides tested could heat stabilise wine to less than 2 NTU. The CMC polysaccharides did not improve heat stability. Only two types of carrageenan polysaccharides, the kappa- and kappa- (90%)/iota- (10%) type carrageenans[1], were found to consistently heat stabilise white wines. Pure iota- and pure lambda- carrageenan types did not effectively heat stabilise wine and may cause filtration difficulties.

A mechanism has been proposed for heat stabilisation of juice/wine with carrageenans. It is likely to involve the formation of an electrostatic complex between the negatively charged sulfate groups in the kappa-carrageenan and positively charged wine proteins that entrap and precipitate the wine haze proteins through sedimentation. The project has also outlined and tested methods for characterising carrageenans analytically, so that quality control parameters can be defined and checked to ensure winemakers are using the most optimal fining agent.

Polysaccharide analysis has allowed us to define quality control parameters for identifying the most effective carrageenans to use.

A ^{1}H-NMR technique has enabled the identification of different types of carrageenans — e.g. kappa-, iota-, or lambda-, that are present in a carrageenan sample. Polysaccharide linkage analysis can be used to determine the molar % of kappa-, iota- and lambda- carrageenan in one sample. We have been able to provide definitions around the type of carrageenans that are the most efficient at heat stabilising wine, as well as defining several critical parameters to ensure product integrity.

Carrageenans can be added at different stages of the winemaking process to heat stabilise wine.

In pilot-scale winemaking trials, the carrageenans were added either at the juice stage, at the fermentation stage, or to finished wine, and ultimately resulted in a heat stable product. As such, carrageenans can be added with the intention of being removed as part of the lees separation process, such as during flotation, racking or centrifugation. This may provide the benefit of streamlining the winemaking process and reducing the number of tank movements required to produce a finished wine.

[1] The commercial blended described as kappa- (90%)/ iota- (10%) carrageenan by the supplier consisted of 94 % kappa- and 6 % iota- carrageenans by polysaccharide linkage analysis.

Adding carrageenans to juice or wine required vigorous tank mixing and a relatively large volume of carrageenan/juice/wine stock solutions. The carrageenans were added in slightly lower quantities than bentonite, but were still able to heat stabilise the same wine.

Carrageenans need to be added in conjunction with pectolytic enzyme treatment, when added at either the juice or fermentation stage, to avoid filtration difficulties.

The carrageenan fined wines made from enzyme treated juice were found to be compatible with cross-flow filtration.

Wine quality was acceptable for wines fined with carrageenan. Descriptive sensory analysis indicated that wines fined with carrageenan had more tropical fruit characters than wines fined with bentonite.

No taints were observed in any wines fined with carrageenan. In descriptive sensory analysis, the bentonite wines showed lower intensity of most sensory attributes, and were described as 'stripped' compared to the untreated control; several of the carrageenan treatments wines had higher intensity of tropical fruit-related attributes (box hedge, sweaty, passionfruit) and also higher viscosity.

The cold soluble (sodium salt) type kappa-carrageenan increased sodium levels in the wine to three times the levels found in the control wine, which had no fining agent added. Calcium levels, after using pure (potassium salt) carrageenans, were slightly modified. Quantities of potassium, copper, iron, magnesium, and manganese did not appear to be impacted by the carrageenan fining agents to any level that would cause a metal instability or impact current export limits.

At time of publication the use of carrageenans to heat stabilise wines is substantially less cost effective than using bentonite.

Currently, bentonite costs approximately $1.50 - $2 per kilogram, whereas carrageenans can range in cost from $27 - $48 per kilogram. Adding carrageenan at winemaking stages that generate wine solids (lees) — e.g. during juice clarification or fermentation, or prior to cold stabilisation — facilitates the removal of the carrageenan along with the juice or wine lees. We do not currently have conclusive wine recovery data (such as % gross lees) to indicate that the use of carrageenans in wine decreases the proportion of lees.

At the time of publication, carrageenans are not permitted additives in wine.

At present, pectin and carrageenan are not permitted additives in wine, although they are approved for food use. An outcome of this research is that the Winemakers' Federation of Australia (WFA) has submitted an application to Food Standards Australia New Zealand (FSANZ) seeking to amend Food Standards Code – Standard 4.5.1 to approve the use of pectin/carrageenan in commercial Australian winemaking.

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1. Introduction

Achieving heat stability in white or rosé winemaking refers to the process of removing certain grape proteins that persist through fermentation but are at risk of precipitating post bottling and forming an unsightly haze in the wine. These proteins have been identified as grape pathogenesis-related (PR) proteins, thaumatin-like proteins (TLPs), and chitinases (Waters, et al., 2005). They are known to form hazes slowly over time, particularly if the wine is exposed to elevated temperature during either transportation or storage.

At present wineries remove heat unstable protein from wine (fining) using bentonite, a type of aluminium clay (Muhlack, et al., 2016). Bentonite clay is negatively charged. Heat-unstable wine proteins have a slight positive charge at wine pH and hence, bind electrostatically to the negatively charged bentonite. This binding is non-selective and bentonite can have quality impacts, such as flavour stripping, particularly at high dose rates. Studies have shown that bentonite can remove ethyl esters and terpenes at levels that could impact the wine's sensory properties (Sanborn, et al., 2010; Lira, et al., 2015). Bentonite swells in wine, causing a significant quantity of wine to be occluded into the bentonite lees sediment. Barblett (2010) estimated that, for every 1 kg of bentonite used in winemaking, 4.6 L of wine is absorbed into bentonite lees. Methods to extract wine that is absorbed by bentonite lees are oxidative and thereby reduce wine quality. Moreover, these extraction methods do not effect complete recovery of the absorbed wine volume. Reducing the levels of lees generated when using heat stabilising fining agents is therefore a very high priority for efforts to improve wine recovery and reduce waste.

In this investigation, various types of commercially available polysaccharides were systematically tested for their effectiveness and practical ability to heat stabilise white wine. The polysaccharides included pectins, carrageenans, and carboxymethylcelluloses. The study also proposes some theories around structural and functional characteristics that make some of these polysaccharides suitable for winemaking applications.

The aim was to discover a novel fining agent that would achieve heat stability of white wine without negative side effects to either wine sensory, wine processing, or wine physio-chemistry.

Pectin and carrageenan are not legal additives in wine, though they are approved for food use. Following the outcomes of this project, a submission has been made to Food Standards Australia New Zealand (FSANZ) to amend Food Standards Code – Standard 4.5.1 to legalise pectin/carrageenan use in commercial Australian winemaking.

2. Background

Pectins and carrageenans have been used to clarify beer as a replacement for isinglass (animal protein); research into their use in winemaking, in addition to brewing, was initiated by Carlton and United Brewing (CUB), a division of Foster's, in a collaboration with the Cooperative Research Centre (CRC) for Biopolymers. The outcomes of this research were patented in 2006 (Duan, et al., Patent No. WO2008/148156). An ideal 'pectin formulation' was developed for beer, in which specific pectin structures—such as molecular weight (MW) and degree of methylation (DM)— were determined to optimise the beer filtration process and develop quality standards for brewers (Duan, et al., 2006; 2008).

A study conducted by Treasury Wine Estates (TWE) and the Australian Wine Research Institute (AWRI) (Marangon, et al., 2013) showed that some carrageenans could be used to heat stabilise wine in lower quantities than bentonite. The study also found that such carrageenans could be added at the juice, ferment, or wine stage and result in a heat stable wine. However, it was also observed that, if added in excess, and not removed by filtration, certain types of carrageenan appear to cause a haze.

This project aimed to better understand the effectiveness of different types of polysaccharides for heat stabilising wine. Additionally, the project sought to broaden the number and type of polysaccharides tested. Testing to date has been limited in the number of potential fining agents examined. Forty-five commercially available polysaccharides were systematically screened in this study and their ability to heat stabilise wine documented. The commercially available polysaccharides were sourced from four different suppliers.

Expected outcomes from this study include the ability to establish the most efficient polysaccharides for heat stabilising wine and to develop quality control standards for their use in winemaking.

The use of polysaccharides in wine for the purpose of heat stabilisation will perform the technical function of a processing aid.

3. Types of commercially available polysaccharides

3.1. Carrageenan

Carrageenans are naturally occurring polysaccharides extracted from edible red seaweeds of various species of the Rhodophyta, including *Gigiartina, Chondrus crispus*, *Eucheuma*, and *Hypena* (Nayar, et al., 2014). The carrageenan content of commercial seaweeds varies from 30% to 60% of dry weight, depending on the seaweed species and the environmental conditions (Necas, et al., 2013).

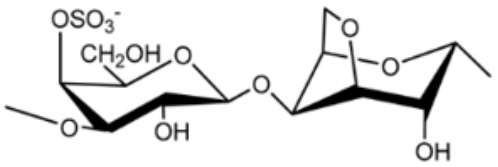
Carrageenans are large, unbranched polysaccharides comprised of a repeating disaccharide unit (carrabiose), with a molecular weight range of 100,000 - 800,000 Dalton (Necas, et al., 2013). These polysaccharides are composed of a galactan backbone of alternating 1,3-linked-β-D-galactopyranosyl (G-units) and 1,4-linked-α-D-galactopyranosyl (D-units) residues, predominantly as 3,6-anhydro-D-galactose (DA-units) (Campo, et al., 2009). These 'carrabiose' repeat units further contain sulfate ester groups.

There are three types of commercially available carrageenans, namely kappa- (κ-), iota- (ι-), and lambda- (λ-), which are differentiated by their number and position of sulfate ester substituents (*Figure 3.0*). These carrageenans are widely used in industrial applications, with kappa- and iota- carrageenans used for their gelling properties, and lambda- carrageenan — a non-gelling form — used as a thickening agent.

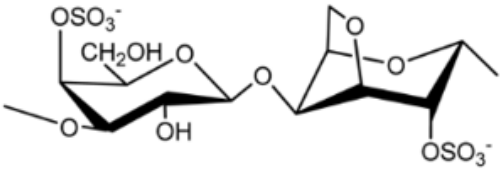
Besides these three major types of carrageenans, two other types, called mu- (μ-) and nu- (ν-) carrageenans, are the biological precursors of kappa- and iota- carrageenans, respectively (Campo, et al., 2009).

The main ionisable cations found in carrageenans include sodium, potassium, calcium, and magnesium. The type of cation salt affects the functional properties of the polysaccharides. For example, only lambda-carrageenan and the sodium salts of kappa- and iota- carrageenan are soluble in cold water. Potassium and calcium salts from kappa- and iota- type carrageenan are not soluble in cold water, but will swell as a function of the concentration and type of cations present, the water temperature, and the condition of dispersion.

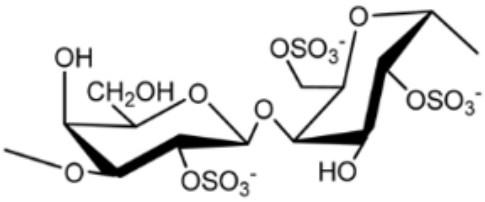
Figure 3.0. Schematic representations of different structures of the three most commercially important carrageenans.



Kappa (κ-)-carrageenan (G4S-DA)



Iota (ι-)-carrageenan (G4S-DA2S)



Lambda (λ-)-carrageenan (G2S-D2S6S)

Major applications of carrageenans in food include their use in dairy and meat products, due to their strong binding to food proteins. Other common examples of their use in foods include as a gelling agents in ice cream, cream, milkshakes, salad dressings, sweetened condensed milks and sauces, to increase product viscosity.

3.2. Pectin

Pectin is a naturally occurring polysaccharide found in grapes, including in the common winemaking grape *Vitis vinifera.* The amount of pectin in ripe grape berries varies from 0.02 - 0.5% w/w (Rankine, 2000). Pectin also occurs naturally in a wide range of edible fruits and vegetables, as part of the plant cell wall structure. In grapes, as in other fruits, pectic substances are structural agents present in the cell wall.

Pectic polysaccharides are comprised of linear (smooth) and branched (hairy) fragments, with linear homogalacturonan (HG) as a dominant structural component. The hairy regions are the rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) regions. HG contains poly-α-(1→4)-D-linked galacturonic acids (1,4-α-D-GalpA) that are partially methyl-esterified and/or partially acetyl-esterified of the carboxylic acid residues. The degree of methyl-esterification (DE) is the ratio of methyl-esterified residues (6-O-methyl-α-D-GalpA) of the HG backbone to the total carboxylic acid units, which strongly influence the functional properties of the pectic polysaccharides (Vorgen, et al., 2009) (*Figure 3.1*). Commercial pectins are sourced from lemon, lime, or apple.

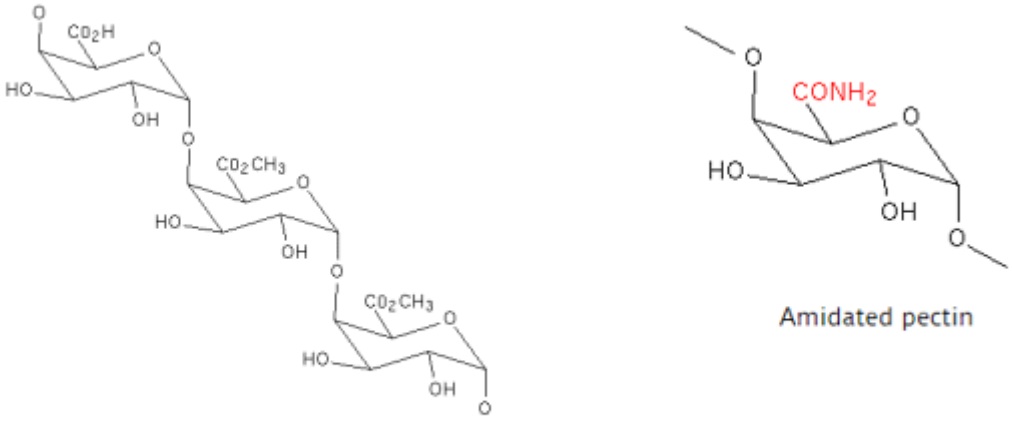
They are categorised according to their methoxy content:

- High methoxy (HM) is >50% esterified (i.e. DE >50%)
- Low methoxy (LM) is <50% esterified (i.e. DE <50%)
- Low methoxy can also be chemically amidated

Pectin is traditionally used as a gelling agent in a wide range of fruit-based products, including jams, marmalades and jellies. HM pectin requires a significant amount of sugar to gel properly, while LM pectin requires less. Consequently, LM pectin tends to be used in low sugar products. LM pectin can also be used as a protein stabiliser in acidic milk beverages, such as drinking yoghurt. LM pectin requires the presence of calcium ions to gel properly. Its viscosity may also be increased in the presence of other cations.

In the brewing industry, a specific HM pectin (DE >70%) is used as a fining agent in place of isinglass (Duan, et al., Patent No. W2008/148156). The HM pectin did not increase the viscosity of the beverage in the presence of calcium. The pectin is a processing aid for increasing beer clarity and is not retained in the final beverage product.

Figure 3.1. Schematic representations of the basic structure of pectin and amidated pectin.



Basic pectin chain of esterified D-galacturonic acid resides in an alpha-(1-4) chain

3.3. Carboxymethylcellulose

Carboxymethylcellulose (CMC) is a cellulose derivative with carboxymethyl groups (-CH2-COOH) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone. It is synthesised by the alkali-catalysed reaction of cellulose with chloroacetic acid. The polar carboxyl groups render the cellulose soluble and chemically reactive. The functional properties of CMC depend on the degree of substitution of the cellulose structure (i.e. the number of hydroxyl groups along the cellulose backbone chain that has been substituted), as well as the chain length of the cellulose backbone structure and the degree of clustering of the carboxymethyl substituents.

In the wine industry, CMC is used as a sodium salt, i.e. sodium carboxymethylcellulose, to achieve potassium tartrate (KHT) stability or to cold stabilise wine. A potential benefit is to save on cooling costs associated with winery operations, as traditional cold stabilisation is achieved by cooling the wine down to 0°C and holding at that temperature for extended periods of time.

It is believed the mechanism of CMCs in promoting cold stability in wine is that in the presence of these molecules, KHT crystals grow more slowly and change their morphology. Their shape becomes flatter because they lose two of the seven faces of the tartrate crystal structure, thus altering their dimensions. This change in tartrate crystallisation forms a more stable structure and prevents KHT precipitation (Bosso, et al., 2010).

4. Materials and methods

4.1. Polysaccharide screening and winemaking trials

4.1.1. Heat test

Juices or wines were filtered (0.45µm) and heated at 80°C for 2 hr, then cooled at ambient temperature for 2 hr. Haze was measured by calculating the difference in turbidity between heated (final) and unheated (initial) samples in nephelometry turbidity units (NTU) by means of a nephelometer. Samples with differences of <2 NTU were considered to be heat stable.

A separate heat test involved extension of the heating period (at 80°C) to 6 hr.

4.1.2. Fining agent trials

The carrageenan and pectin additions required preparing stock solutions at specific concentrations — 1% (10 g/L), and 2% (20 g/L), for carrageenan and pectin, respectively — in juice/wine, and mixing these with a homogeniser to ensure a well-mixed solution.

Fining trials to determine the quantity of either bentonite or polysaccharide required to achieve heat stability were completed by adding increasing doses of the stock fining agent solution to aliquots of juice or wine and mixing well, then holding overnight at room temperature. Filtered samples (0.45µm) were submitted for heat testing (Section 4.1.1).

The effect of polysaccharide over-addition was tested by adding more than three times the concentration of fining agent required to achieve heat stability of the wine.

4.1.3. Viscosity index measurement

The viscosity index measurement was carried out by rating the viscosity of various carrageenan samples in a 1.5% solution (15g/L) of both water and wine. Viscosity index data are based on a 1-6 rating, where 1 = low viscosity (liquid) and 6 = high viscosity (solid) at room temperature.

4.1.4. Standard method for adding carrageenan to juice, ferment and wine

4.1.4.1. Preparing polysaccharide stock solutions

For each 50L vessel, a 6L volume of juice or wine was extracted from the vessels for the preparation of the polysaccharide stock solution. The extracted volume was placed in a 15L container and mixed with the homogeniser while small amounts of polysaccharide were added gradually to achieve the addition rate required for 48L total of juice or wine. Pectin or carrageenan was added slowly, in small portions, while the solution was continuously mixed. Further portions of polysaccharide were not added until the previous portion had been dissolved. Addition of the required quantity of polysaccharide took approximately 10 min, with the mixing speed being varied as necessary. On completion of the polysaccharide addition, the stock solution was mixed for a further 10 min to ensure there were no residual pockets of dried carrageenan. This stock solution was then added back to the same vessel from which it was originally derived.

4.1.4.2. Adding stock solution to the juice ferment or wine

The prepared polysaccharide stock solution (Section 4.1.4.1) was added slowly to the originating juice/wine vessel via a funnel and tube while the vessel was continuously mixed. The vessel was mixed for an additional 10 min after the stock solution had been added.

4.1.5. Winemaking

The Sauvignon Blanc juice (approx. 2000L) was transferred to the pilot winery in a pallet tank and homogenised to the same turbidity. In V15, no pectinase was added; however, in V16 the oenological pectinase, Ultrazym (Novozymes), was added at 3 mL/hL. Juice portions (48L per treatment) were divided into 9 x 50L vessels for the addition of polysaccharide to juice stage. The 9 x 50L polysaccharide (TWE 1 - 9) juice treatments were cold settled at 0°C, and the remaining juice was cold settled in the pallet tank for 36 hr. A 48L portion of clarified juice was added to each of the 24 x 50L vessels (TWE 10 - 33) and moved to the 15°C room. Following cold settling for 24 hr, the juice treatment vessels were racked off juice lees and moved to the 15°C room. Once the clarified juice had reached 15°C, rehydrated yeast was added at 200 mg/L, following which fermentation was conducted at 15°C. When residual sugar reached <2 g/L, SO_2 levels were adjusted and 2g/L KHT was added. The wine was chilled for 3 weeks at 0°C for cold stabilisation.

Polysaccharides were added to individual vessels at three stages — to unclarified juice, prior to cold settling, to fermentation after a 2 Baume drop, and to finished wine (*Table 6.1*). The mass of each vessel, before and after all juice or wine movements, was measured and recorded.

Following cold stabilisation for 21 days on gross lees, the wines were racked, filtered and bottled under tin saran laminate screw caps in 750 mL bottles.

The 2015 wines were filtered for bottling using Z Pad (nominal 0.8µm, IMCD), followed by a 1.0µm and then a 0.45µm nylon cartridge (Pall). The 2016 wines were cross-flow filtered for bottling using a titanium membrane (nominal 0.2µm, AMS Advanced Metallurgical Solutions), then filtered using a nylon cartridge filter of 0.45µm (Pall).

4.1.6. Protein concentration

Protein concentrations were determined using Bio-Rad Protein Assay Kit, following the manufacturer's instructions. Protein standard graphs were made using bovine serum albumin.

4.1.7. Filterability testing

Filterability testing was conducted at the AWRI Commercial Services lab, at Urrbrae, under the conditions of 2 bars of pressure and a 25mm diameter, 0.45µm membrane filter.

4.1.8. Scanning Electron Microscopy (SEM)

The wines and water blank used for the SEM experiment were filtered through 0.45µm filters and prepared as follows. Approximately 1cm square pieces of the filters were cut out and fixed in 2.5% glutaraldehyde in phosphate buffered saline (PBS) for 2 hr at room temperature. The filter pieces were then rinsed for 20 min

in PBS, three times. The rinsed filter pieces were dehydrated in 10%, 30%, 50%, 70%, and 90% v/v of ethanol in water, for 20 min per concentration. The filter samples were then incubated in 100% ethanol three times, for 1 hr each step. The dehydrated filter samples were dried in a Balzers CPD030 critical point dryer, and then mounted flat onto aluminium SEM stubs using double-sided carbon tape. The samples were then gold-coated in a Dynavac Xenosput sputter coater. The samples were imaged in a Philips XL30 field emission SEM at 2.0 kV and a spot size of 2. Digital images were captured at a magnification of 350,000 X with a resolution of 1424 x 1024 pixels.

4.1.9. Sensory assessment

For the descriptive sensory analysis, wines were assessed for sensory attributes at the AWRI sensory and flavour aroma laboratory, Urrbrae. Descriptive sensory analysis was conducted in 2015 on the V15 cold soluble kappa- (Na+) carrageenan-treated wines and the kappa-(90%)/iota-(10%) carrageenan treated wines. In 2016, descriptive sensory analysis was completed only on the V16 pure kappa- (K+) carrageenan wines.

In 2015 a panel of nine assessors (3 male, 6 female) with an average age of 55 years (SD = 9.3) was convened.

In 2016 a panel of eight assessors (1 male, 7 female) with an average age of 54 years (SD = 6.3) was convened.

Assessors of the 2015 and 2016 wines attended three training sessions to determine appropriate descriptors for rating in the formal sessions. During these training sessions, the assessors evaluated wines from the study that represented the full range of sensory properties. The assessors to generate and refine the appropriate descriptive attributes used these wines and definitions through a consensus based approach. Wines were assessed for appearance, aroma and palate. In sessions two and three, standards for aroma attributes were presented and discussed and these standards were available during the booth practice session and the formal assessment sessions.

Following the three training sessions, tasters participated in a practice session in the sensory booths imitating the same conditions as those for the formal sessions. After the practice session, any terms which required adjustment were deliberated and the final list of terms determined.

For the 2015 vintage, 24 wines were presented and for the 2016 vintage, 15 wines were presented to the sensory assessors. Each wine was presented to the assessor twice in a modified Williams Latin Square incomplete random block design, generated by FIZZ sensory acquisition software (version 2.47B, Biosystems, Couternon, France).

The intensity of each attribute was rated using an unstructured 15 cm line scale from 0 to 10, with indented anchor points of ‘low’ and ‘high’ placed at 10% and 90% respectively. Data was acquired using Fizz sensory software (version 2.47B, Biosystemes, Couternon, France).

Panel performance was also measured using Fizz, Senstools (OP&P, The Netherlands) and PanelCheck (Matforsk) software, and included analysis of variance for the effect of judge and presentation replicate and their interactions, degree of agreement with the panel mean and degree of discrimination across samples. All judges were determined to be performing to an acceptable standard.

Analysis of variance (ANOVA) was conducted using Minitab (Minitab Inc., Sydney, NSW). The effects of treatment (T), judge (J), ferment replicate nested in treatment, interaction between judge and treatment nested in ferment replicate and the interaction between replicate (R) and treatment nested in ferment replicate were assessed. Following ANOVA, Tukey’s honestly significant difference (HSD) value was calculated (P=0.05).

Principal component analysis (PCA) was based on the mean values for the treatments averaged over ferment replicates, panellists and replicates, using the correlation matrix.

4.2. Polysaccharide analysis methods

4.2.1. Total polysaccharide analysis

Carrageenans and pectins (200 mg) were dissolved in water and vortexed or sonicated to create a homogeneous solution. Four volumes of absolute ethanol were added to the polysaccharide solution, and the suspension was placed in a cold storage (4°C) overnight to precipitate polysaccharides. Samples were then centrifuged at room temperature (1500 g, 10 min) to remove supernatant. The pellet was washed extensively with absolute ethanol (x2), acetone (x1) and methanol (x1), centrifuging between each wash. The sample was vacuum dried at 40°C overnight.

4.2.2. Sulfate content of carrageenan

The sulfate content of the polysaccharide was determined through the method used by Tabataba (1971), as modified by (Craigie, et al., 1984).

4.2.3. Linkage analysis of pectin and carrageenan

The partially methylated alditol acetates were generated by reductive hydrolysis, and were separated by gas chromatography-mass spectroscopy (GCMS) then identified by their mass spectra and their retention times relative to *myo*-inositol hexa-acetate, as described by Lau, et al. (1993).

Pectic polysaccharide linkage composition and methyl esterification were determined according to the method used by Pettolino, et al. (2012).

Linkage composition of carrageenan samples was investigated by forming the dimethyl sulfoxide (Me_2SO)-soluble triethylammonium salts of the polysaccharides, and methylating as described by Stevenson, et al. (1991).

4.2.4. ^{1}H-NMR spectroscopy

Carrageenan solution (1% w/v) was prepared in D_2O (99.9%, Sigma). ^{1}H spectra were recorded on a Bruker Advance III 600 MHz spectrometer equipped with a cryoprobe (Bruker Daltonics, Germany).

Chemical shifts (δ) were measured in parts per million (ppm) relative to the residual D_2O signal at 4.82 ppm. All spectra were processed and analysed using Topspin (version 3.0, Bruker Biospin).

4.2.5. Molecular size distribution

Size-exclusion chromatography (SEC) was used to measure the molecular size distributions of the different carrageenans. SEC was performed on a PSS (Mainz, Germany) Agilent 1100 series, using a Shimadzu RID-10A refractive index detector. Samples were prepared using 4 mg/mL carrageenan in dimethylsulfoxide (DMSO) with 0.5 % lithium bromide (LiBr). Samples were injected into the column. The injection volume

was 100 µL, the flow rate was 0.3 mL/min, and the temperature was 80°C. SEC separates by hydrodynamic volume, Vh, not by molecular weight. For linear polymers, there is a unique relationship between molecular weight M (or DP) and Vh (Villaplana, et al., 2011).

4.2.6. Sedimentation test

The sedimentation test was carried out at 4°C, using heat unstable wine. Carrageenan stock solutions (1% w/v) were prepared in wine, and mixed with a homogeniser to ensure a well-mixed solution. Carrageenan was added at 1.2 g/L to heat unstable wine in order to achieve a final volume of 250 mL. The solution was mixed using a homogeniser for 2 min, and then transferred to a glass measuring cylinder (250 mL) and allowed to settle for 96 hr. Both a negative (no fining agent) and a positive control (bentonite, 1 g/L) were included in the test.

5. Screening of polysaccharides to heat stabilise wine

5.1. Heat testing of polysaccharides in a 2014 Riverland Chardonnay wine

A systematic approach was taken to test the ability of 45 commercially available polysaccharides to heat stabilise a similar wine. In a commercial wine production scenario, a white or rosé wine is not considered heat stable unless the turbidity difference, following an industry standard heat test (Section 5.2.3), is less than 2 NTU.

The types of polysaccharides tested were pectin, carrageenans and CMCs. *Tables 5.0 - 5.2* show the results of heat testing 14 pectins, 3 CMCs and 28 carrageenans respectively, in the same 2014 Riverland Chardonnay wine. Details of the structure of these polysaccharides are also listed alongside the heat test results.

An important point to note is that some of these commercially available fractions contain buffering agents, such as dextrose or sucrose, at high levels (up to 15%). Buffer agents may be common in the confectionary and sweet food industries, however, they are an undesirable agent in a wine fining agent, as sugars are an illegal additive in the Australian wine industry. As such, it was critical that we specified with the supplier not to include sugar-based buffering agents in the commercial carrageenan samples.

In commercial beer production, pectins are used successfully as a fining agent for removing solids and facilitating filtration. The results from heat testing 14 pectins in the same 2014 Riverland Chardonnay wine indicated that, despite a range of pectin types tested, none was suitable for heat stabilising white wine. Variable DMs did not alter the efficacy of the pectins as a fining agent. While the unheated Chardonnay wine had a heated turbidity of 41.5 NTU, the same wine — treated with the more effective heat stabilising pectins — tended to show a heated turbidity in the range of 5.6 - 15 NTU, with the relatively high addition rates of 1.6 - 1.75 g/L.

While protein analysis (*Figure 5.1*) confirms that pectins go some way toward heat stabilising wine, by removing a limited amount of protein, they were unable to achieve a commercially acceptable heat stable wine.

The best performing pectin was a low methoxy pectin derived from citrus, with the sample code P-D-902, which showed a final heat difference of 5.6 NTU.

One of the apple-derived pectins, P-D-910, tested in a small scale (500mL) bench test, resulted in the ferment turning a browner colour (data not shown) than that of the control ferment (no fining agent). Consultation with the pectin supplier, Herbstreith & Fox, indicated that it is not unusual for apple-derived pectins to cause browning in food products. While it is possible that colour could be restored post-fermentation, using a citrus-derived pectin avoids any pectin-induced browning.

Table 5.0. The composition and heat stability of 14 pectins tested in a 2014 Riverland Chardonnay wine, heat turbidity = 41.56 NTU.

	Code	Pectin type and source	GalA (%)	DE (%)	DA (%)	#Rate (g/L)	Initial haze NTU	Final haze NTU	Heat Test
1.	P-A-373	Low methoxy	87	10	-	1.70	0.5	14.7	14.2
2.	P-A-374	Low methoxy	95	30	-	1.65	0.5	11.0	10.5
3.	P-A-384	High methoxy	94	70	-	1.75	1.0	23.6	22.6
4.	P-D-701	Low methoxy, Apple	82	38	-	1.70	0.7	15.8	15.1
5.	P-D-910	Low methoxy, Apple	75	4.1	-	1.65	0.6	14.0	13.4
6.	P-D-005	Low methoxy, Amidated, Citrus	88	40	10	1.75	0.7	26.9	26.2
7.	P-D-025	Low methoxy, Amidated, Citrus	89	30	20	1.75	0.8	12.2	11.4
8.	P-D-04	High methoxy, Citrus	80	59	-	1.75	0.9	12.5	11.6
9	P-D-902	Low methoxy, Citrus	78	6.1	-	1.75	0.6	6.2	5.6
10.	P-B-1	High methoxy, Orange, Dextrose (15%)	82	68	-	1.6	7.6	17.4	ND
11.	P-B-2	High methoxy, Lime, Dextrose (15%)	85	70	-	1.65		27.0	
12.	P-S-9135	Low methoxy, Citrus	84	9.4	-	1.75	3.5	28.0	ND
13.	P-S-1879	Sodium salt/poly pectate, Orange	85		-	1.75	0.7	12.3	
14.	P-S-2157	Low methoxy, Apple	77	7	-	1.75	0.7	21.9	

P = Pectin; A = CP Kelco; B = Cargill; C = IMCD; D = Herbstreith & Fox; S = Sigma;
GalA = Galacturonic acid; DE = Degree of methyl esterification; DA = Degree of amidation; LM = Low methyl esterification (<50 DE); HM = High methyl esterification (>50 DE); #Addition rate to achieve lowest possible NTU turbidity; ND = Not determined, as initial turbidity less than 1.0 NTU could not be achieved.

The heat testing graphs for the accumulative addition of pectin to 2014 Riverland Chardonnay wine, for six different pectins, are shown in *Figure 5.0*. This likewise illustrates that, while pectins contribute to a decrease in wine turbidity following the heat test, they do not heat stabilise the wine to a commercially acceptable level. *Figure 5.1* illustrates that, for the low methoxy pectin (P-D-902), both the heat turbidity and the protein concentration decrease as the pectin addition rate increases, but the wine is not heat stabilised.

Figure 5.0. Heat stability of Riverland Chardonnay wine after addition of six different types of pectin, expressed as heat haze NTU.

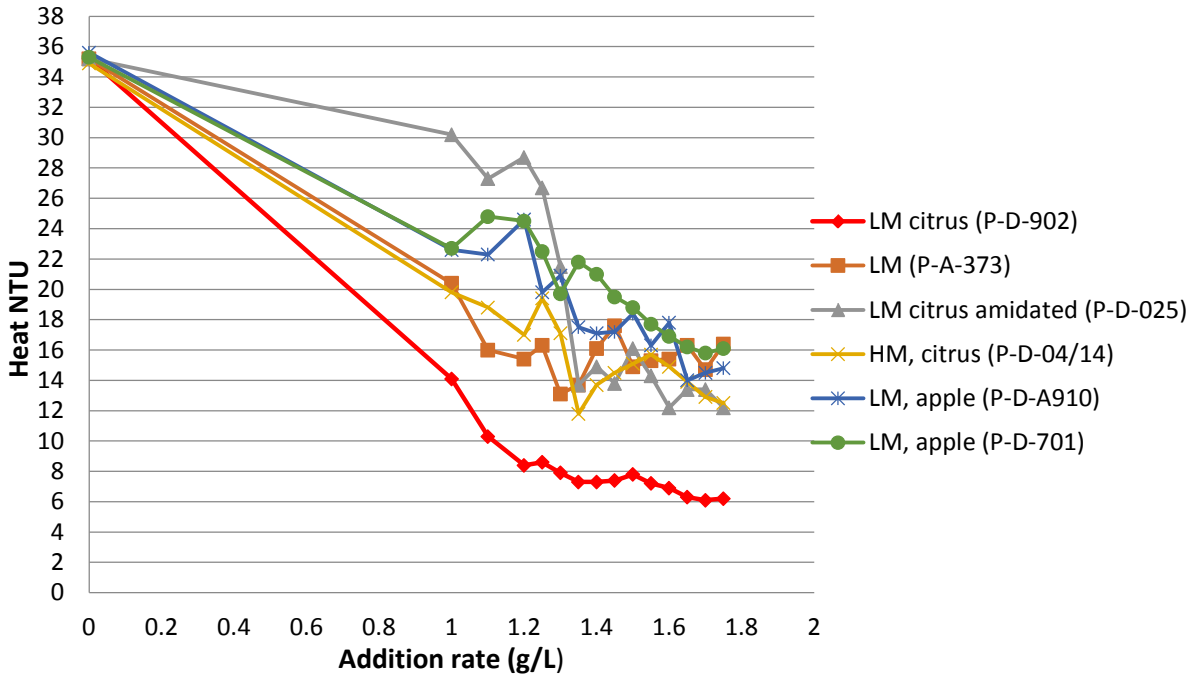
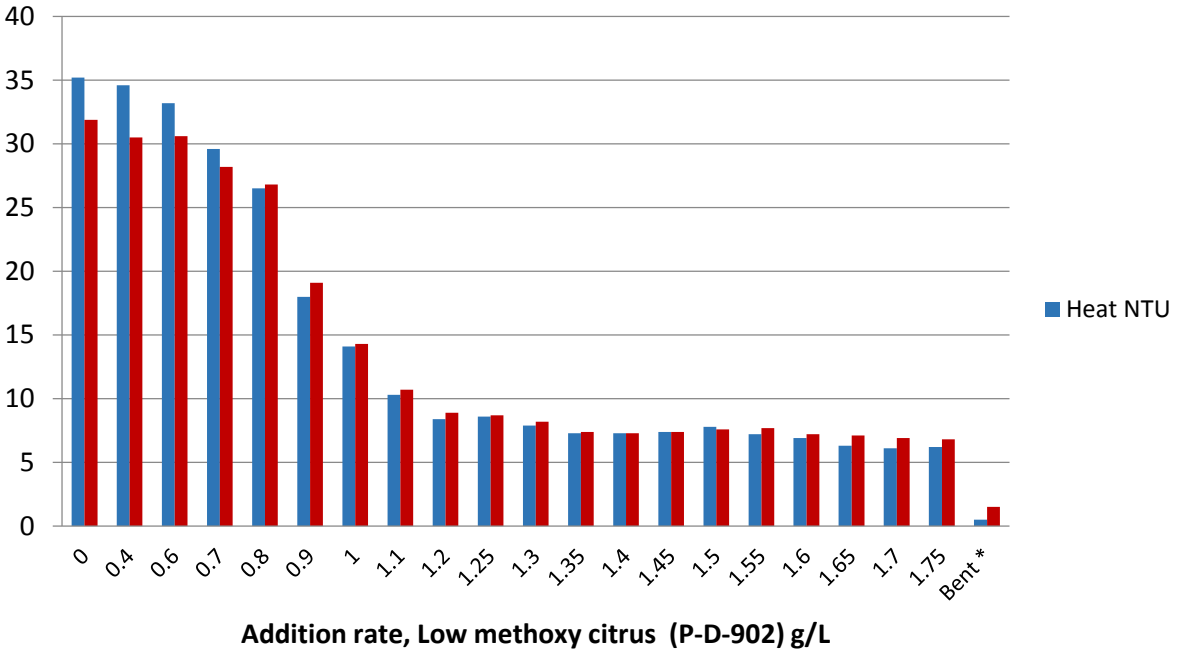


Figure 5.1. Heat turbidity and protein concentration of a 2014 Riverland Chardonnay wine after the addition of low methoxy citrus pectin (P-D-902). Bent = Bentonite addition rate (0.8 g/L).*



A small number of CMCs were included in this study, as CMCs are a type of polysaccharides that is legally permitted for use as a wine additive, and is typically added for the purpose of cold stabilising white wine. *Table 5.1* indicates that CMCs do not have any ability to heat stabilise wine. In fact, CMCs result in a higher turbidity wine under the heat testing conditions of commercial wine production.

Table 5.1. The heat stability of three carboxymethylcellulose (CMC) tested in a 2013 Adelaide Hills Chardonnay wine.

	Code	**Polysaccharide**	**Stock solution (%)**	***Gel strength**	**Addition rate (g/L)**	**Initial NTU**	**Final heat NTU**
1.	CMC-C-Dow	CMC	2.5	1	1.4	0.9	> 99.9
2.	CMC-C Vinostab	CMC	7.5	1	1.6	0.9	> 99.9
3.	CMC-C-Dow-CRT15000PPA	CMC Very fine particles, low viscosity grade	1.25	6	Difficult to dissolve	-	-

*Gel strength data was measured in water and is based on a 1-6 rating of 1.5% solution (15g/L), where 1 = low viscosity (liquid) and 6 = high viscosity (solid) at room temperature; CMC = Carboxymethylcellulose; G = Gum; A = CP Kelco; C = IMCD.

Of the 28 carrageenans tested, the types of commercially available carrageenans that gave the lowest heat test result—between 1.8 - 3.3 NTUs, at an addition rate of 1 - 1.2 g/L—were the cold soluble or pure kappa-carrageenans (*Table 5.2*). Also, a kappa- (90%)/iota- (10%) blended carrageenan was successful, or nearly successful, at heat stabilising wine, with heat test results between 1.4 - 3.4 NTUs.

Table 5.2. The % composition and heat stability of 28 carrageenans tested in a 2014 Riverland Chardonnay wine.

	Sample code (production year)	Carrageenan type	$CHO(%)	#Rate (g/L)	Initial haze (NTU)	Final haze (NTU)	Heat test (NTU)
1.	Ca-A-367 (2014)	iota- / nu-	98%	0.2	9.1	26.9	ND
2.	Ca-A-368 (2014)	lambda-	96%	0.2	14.0	50.6	ND
3.	Ca-A-369 (2014)	kappa- (semi refined)	88%	0.2	0.6	50.6	50.0
4.	Ca-A-370 (2014)	Iota- (semi refined)	89%	0.2	5.0	41.2	ND
5.	Ca-A-371 (2014)	kappa-	96%	1.0	0.57	2.77	2.2
6.	Ca-A-372 (2014)	iota- / nu- (cold soluble)	97%	0.3	25.2	55.5	ND
7.	Ca-A-CSW (2014)	kappa-, iota-, lambda- (sucrose 12%)	86%	0.95	0.7	4.9	4.2
8.	Ca-A-529 (2014)	lambda- (pure)	96%	0.95	19.9	32.7	ND
9.	Ca-A-532 (2014)	kappa-, iota-, lambda- (cold soluble lambda-)	96%	0.90	0.8	6.7	5.9
10.	Ca-A-533 (2014)	kappa- (cold soluble)	98%	1.0	0.6	2.4	1.8
11.	Ca-A-978 (2015)	kappa-	97%	1.2	0.7	2.7	2.0
12.	Ca-A-979 (2015)	kappa-, iota-, lambda- (cold soluble lambda-)	-	1.2	0.9	8.5	ND
13.	Ca-A-980 (2015)	kappa- (cold soluble)	-	1.2	0.5	3.5	3.0
14.	Ca-B-1 (2014)	kappa- (dextrose 10%)	88%	0.95	0.6	3.5	2.9
15.	Ca-B-2 (2014)	kappa- (90%)/iota- (10%) blended	96%	1.00	0.8	2.2	1.4
16.	Ca-B-1R (2014)	kappa- (pure) (Protex)	-	0.95	0.86	3.5	2.64
17.	Ca-B-2R (2014)	kappa- (dextrose 10%) (Protex)	-	1.00	1.80	3.9	2.1
18.	Ca-B-1R (2015)	kappa- (Pure)	83%	1.2	0.8	3.9	3.1
19.	Ca-B-2R (2015)	kappa- (dextrose 10%)	95%	1.2	0.6	3.9	3.3
20.	Ca-B-3R (2015)	kappa- (90%)/iota- (10%) blended	88%	1.2	0.5	3.9	3.4
21.	Ca-C-359 (2014)	(Viscarin SA)	-	1.00	9.0	35.0	ND
22.	Ca-C-812 (2014)	(Gelcarin GP-812-Prod 303)	-	1.0	1.06	8.31	7.25
23.	Ca-C-209 (2014)	(Viscarin GP-209-Prod 209)	-	0.80	5.42	22.0	ND
24.	Ca-S-3889	lambda-	-	0.7	10.7	37.3	ND
25.	Ca-S-1263	kappa-	-	1.00	0.5	5.0	4.5
26.	Ca-S-4014	iota-	-	1.00	14.4	37.3	ND
27.	Ca-S-Blend-1	lambda- (90%)/iota- (10%)	-	1.00	9.4	53.0	ND
28.	Ca-S-Blend-2	kappa- (90%)/iota- (10%)	-	1.00	0.6	5.0	4.4

Ca = Carrageenan; A = CP Kelco; B = Cargill; C = IMCD; D = Herbstreith & Fox; S = Sigma. $ CHO= carbohydrate as total polysaccharide expressed as a % (w/w) and determined by the method described in *Section 4.2.1*. Cation content is commercial data obtained from suppliers.

#Rate = Highest addition rate. ND = Initial NTU (i.e. NTU post filtration and before heating) greater than 1 NTU hence result could not be determined.

Kappa- and iota- carrageenans can occur in the same carrageenan chain (hybrid) or as a blend of different kappa- and iota- carrageenans. The kappa- (90%)/iota- (10%) carrageenans tested in the study were the blended type only. We were unable to obtain hybrid-type carrageenans, which are a more expensive product (*Table 6.4*).

The pure kappa- and kappa- / iota- (blended) carrageenans must be a refined grade to heat stabilise wine. The pure lambda- and pure iota- carrageenans could not heat stabilise wine. The lambda- and iota- carrageenans were difficult to filter and gave high initial turbidities. In heat testing of commercial wines, the initial turbidity, following 0.45µm filtration, must be less than 1 NTU or the test is invalid. Kappa- / iota- / lambda- blends were less effective at heat stabilising wines, producing heat results between 4 - 6 NTU.

The sigma standard kappa- and kappa- / iota- (blended) carrageenans (sample code beginning Ca-S) did not heat stabilise wine quite as effectively as the similar commercial carrageenans, giving heat turbidity test results of approximately 4 NTU.

Consideration should be given to the type of cation salt of the carrageenans utilised in the testing, as well as to the descriptor used by suppliers for potassium and sodium salt products. Kappa- carrageenans naturally occur as a potassium salt, however, they can be modified to the sodium salt through a patented ion exchange process. Suppliers often refer to the kappa- (K^+) carrageenan as 'pure' carrageenan and the kappa- (Na^+) carrageenan as 'cold soluble' kappa- carrageenan (*Table 5.3*). The sodium salt of kappa- carrageenan is soluble in cold water, unlike the potassium salt of kappa- carrageenan, which, when added to water, is characterised by the formation of gels.

More details on the content of the ionisable cations of the 28 heat tested carrageenans can be found in Appendix 13.11.

Table 5.3. Types of kappa- carrageenans identified as suitable to heat stabilise white wine.

Name	No Batch Tested*	Sample Codes (Production Year)	Carrageenan	Description
Pure kappa- carrageenan	4	Ca-A-371 (2015), Ca-B-1R (2014), Ca-B-1R (2015), Ca-A-978 (2015)	κ-carrageenan	K^+ dominant
Cold soluble kappa- carrageenan	2	Ca-A-533 (2014), Ca-A-980 (2015)	κ-carrageenan	Na^+ dominant
Kappa- (90%)/iota- (10%) carrageenan	2	Ca-B-2 (2014), Ca-B-3 (2015)	ι-carrageenan	K^+ dominant

**Batches of the same type of carrageenan were tested to be compositionally the same regardless of sample code or production year (Chapter 7 – Polysaccharide Analysis).*

Figure 5.2 shows the heat stability graphs for kappa- and kappa- / iota- (blended) carrageenans, which can heat stabilise wine to less than 2 NTU. *Figure 5.3* illustrates that, for the cold soluble kappa- carrageenan and the kappa-/iota carrageenan, the level of protein decreased as the turbidity result of the heat test decreased.

Figure 5.2. Heat turbidity testing of 28 commercial carrageenans in a 2014 Riverland wine consistently found that kappa- and kappa-/iota- carrageenans were capable of reducing haze formation to < 2.0 NTU, whereas carrageenans containing lambda did not heat stabilise the wine to < 2.0 NTU.

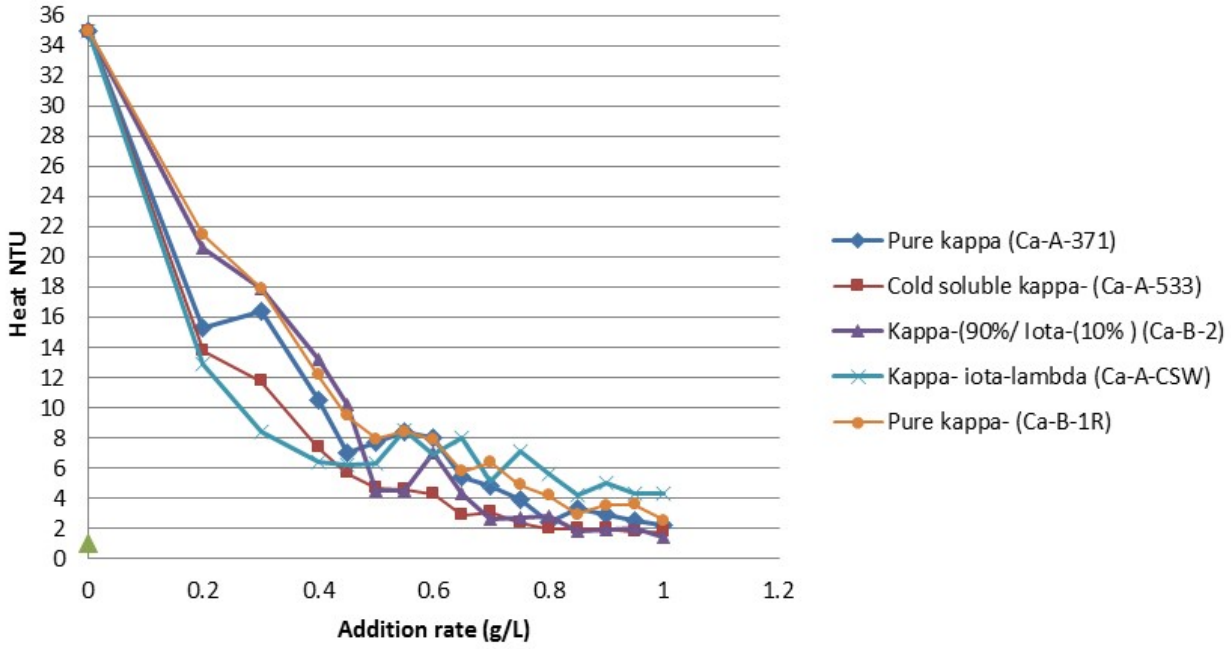
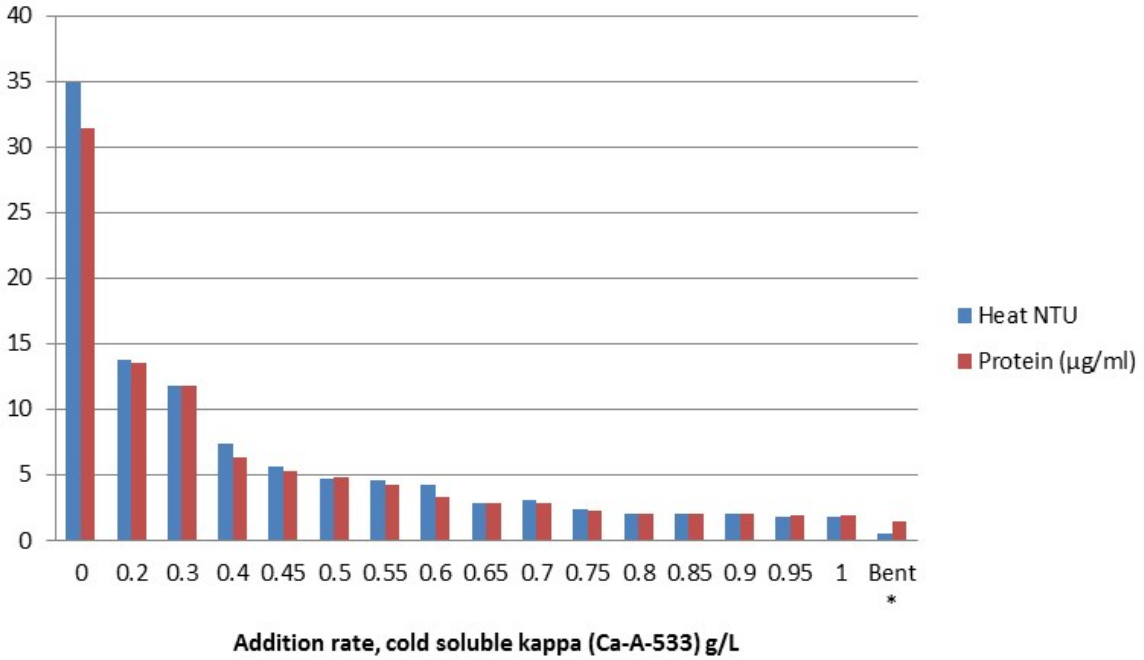
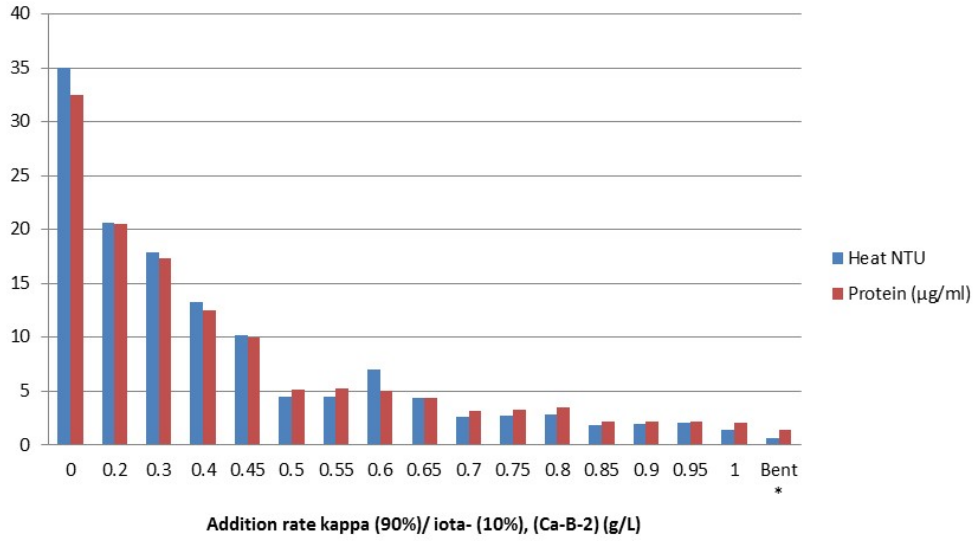


*Figure 5.3. Protein concentration and heat turbidity of a 2014 Riverland Chardonnay wine fined with (a) cold soluble kappa- carrageenan: (b) kappa-/iota- carrageenan and bentonite (Bent) .*Bentonite addition rate = 0.8 g/L.*

a)



b)



5.2. Heat testing of polysaccharides in other wines

In 2014, 45 polysaccharides were bench tested in the Riverland Chardonnay wine and, from this study, it was determined that kappa- carrageenans of either the sodium or potassium salt and a kappa-/iota- blend were the most effective at heat stabilising the white wine. The cold soluble (Na^+) kappa-, the kappa-

(90%)/iota- (10%), and a LM pectin derived from citrus (P-D-902) were selected for the V15 trials based on this testing.

Following the outcomes of the 2014 pilot-scale trials, and to determine whether different types of carrageenans heat stabilise wine at different fining rates, further testing of carrageenans was conducted over a broader range of wine types (*Table 5.4*). Another outcome of this testing was to assist in selecting polysaccharides for the V16 trials. The eight carrageenans determined to be most effective at heat stabilising wine were heat tested with four different wine varieties from four different regions.

When tested across a broader range of wines (*Table 5.4, Appendix 13.0*) of different varieties and region. There did not appear to be any notable difference between the eight kappa- or kappa-/iota- carrageenan products, in terms of their efficiency at heat stabilising wine. Some of the carrageenans sourced in 2015 were the same type of product, procured from the same supplier, as those tested in 2014, but with a different batch code. Further polysaccharide compositional analysis—on successive batch codes from suppliers between 2014 and 2015—indicated that the carrageenan products were essentially the same. The results of this in-depth polysaccharide analysis of successive batch codes and suppliers will be published in a separate research paper by Ratanyake, et al.

Table 5.4. Further heat testing of eight optimal polysaccharides from the original screening of 45 polysaccharides, indicating their heat stabilising wine effects in four different varieties and regions of wine.

		Riesling (Barossa)		**Pinot Gris (Langhorne Creek)**		**Sauvignon Blanc (Robe)**		**Chardonnay (Riverland)**	
Vintage		**2015**		**2015**		**2015**		**2014**	
#Heat haze		**38.9 NTU**		**24.7 NTU**		**40.0 NTU**		**34.6 NTU**	
Total protein (µg/ml)		**27.2**		**21.4**		**27.4**		**31.9**	
Sample code (production year)	**Carrageenan type (%)**	**Rate (g/L)**	**Haze* (NTU)**	**Rate (g/L)**	**Haze* (NTU)**	**Rate (g/L)**	**Haze* (NTU)**	**Rate (g/L)**	**Haze* (NTU)**
Ca-A-371 (2014)	kappa- (pure)	1.4	3.3	1.1	2.9	1.4	3.0	1.0	2.2
Ca-A-533 (2014)	kappa - (cold soluble)	1.4	3.2	1.1	3.2	1.4	3.2	1.0	1.8
Ca-B-2 (2014)	kappa - (90%)/iota- (10%) blended	1.4	2.9	1.1	2.8	1.4	2.8	1.0	1.4
Car-A-978 (2015)	kappa - (pure)	1.4	3.1	1.2	2.8	1.4	2.7	1.1	3.1
Car-A-980 (2015)	kappa - (cold soluble)	1.4	3.2	1.2	3.4	1.4	2.9	1.0	2.9
Car-B-1R (2015)	kappa -(pure)	1.4	2.8	1.1	2.9	1.4	2.8	1.1	2.9
Car-B-2R (2015)	kappa - (90%)/dextrose (10%)	1.4	3.3	1.3	3.9	1.4	3.6	1.1	3.3
Car-B-3R (2015)	kappa - (90%)/iota- (10%) blended	1.4	3.9	1.1	3.5	1.4	3.7	1.0	2.6

** Heat haze without fining agent. Average of two tests.*

The Sauvignon Blanc, Pinot Gris, Riesling, and Chardonnay wines were not completely heat stabilised to a heat turbidity of less than 2 NTU on the bench. This sometimes occurs in bench trials, possibly due to inefficient mixing at the lab scale. These data indicate that kappa- and kappa-/iota- carrageenans, in the wines tested, heat stabilised wines to equal levels at equal rates.

5.3. Selection of polysaccharides for V15 and V16 pilot-scale winemaking

5.3.1. Selection of polysaccharides for the V15 pilot-scale winemaking trial

From the systematic screening of 45 polysaccharides in a Riverland Chardonnay (*Table 5.0*, *Table 5.1*, *Table 5.2*), the polysaccharides selected for V15 pilot-scale winemaking trials included:

Ca-A-533 – Cold soluble kappa- carrageenan

Ca-B-2 – Kappa- (90%)/iota- (10%) carrageenan

P-D-902 – Low methoxy citrus pectin

Cold soluble (Na^+) kappa- carrageenan was selected over the pure kappa- (K^+) carrageenan, as the supplier was of the opinion that the sodium salt kappa- carrageenan would be more soluble in juice or wine. Indeed, the sodium salt kappa- carrageenan is reported in the literature to be more soluble in water than the potassium salt of the kappa- carrageenan (Campo, et al., 2009).

Although the carrageenans were found to be more effective than pectins at heat stabilisation, in the bench studies, it was decided that a pectin polysaccharide would be tested at the winemaking scale. Pectin reduced heat turbidity and protein concentration during bench testing (*Figure 5.1*), and it was unclear whether further improvements could be realised in a scenario more similar to commercial wine production. From a review of the screening trials (*Table 5.0*), the low methoxy pectin derived from citrus P-D-902 gave the lowest heat stability of the 18 pectins tested (heat turbidity = 5.6 NTU), and was thus selected for pilot winemaking trials.

5.3.2. Selection of polysaccharides for the V16 pilot scale winemaking trial

The results of the 2015 pilot winemaking trials were presented to the Industry Reference Group (IRG) (Appendix 13.12), which had input into the pilot winemaking trial plan for 2016 (IRG Meeting Minutes, Oct 2015). It was decided to use juice from the same variety and vineyard as the 2015 trial, but to further test the different types of polysaccharides that were most successful at heat stabilising wine.

The bench screening (*Table 5.2*) trials had clearly demonstrated that the kappa- and kappa-/iota-carrageenans were more effective at heat stabilising wine, without the complications of being difficult to mix or being hard to filter. Further heat testing on a 2015 Barossa Riesling, a 2015 Langhorne Creek Pinot Gris, and a 2015 Robe Sauvignon Blanc did not differentiate kappa- (K^+), cold soluble kappa- (Na^+), and kappa- (90%)/iota- (10%) carrageenans in terms of their respective heat stabilising effects on wine (*Table 5.4*). The pectin P-D-902 was not successful at heat stabilising wine on the bench or at pilot scale and, as such, further testing of pectins was not pursued.

The three carrageenans chosen for the 2016 pilot-scale winemaking trials were:

Ca-A-978 – Kappa- carrageenan

(2015 equivalent is supplier code C-A-371 – kappa (pure) K^+ dominant)

Ca-A-980 – Cold soluble kappa- carrageenan

(2015 equivalent is supplier code C-A-533 – cold soluble kappa)

Ca-B-3R – Kappa- (90%)/iota- (10%) carrageenan

(2015 equivalent is supplier code C-B-2 – kappa (90%) /iota (10%))

6. V15 and V16 pilot scale winemaking

6.1. Trial design

Adelaide Hills Sauvignon Blanc juice was used in the V15 and V16 trials (*Table 6.0*).

Table 6.0. Chemical parameters of the Adelaide Hills Sauvignon Blanc juice used in the V15 and V16 trials.

Parameter (data from single replicate)	**Adelaide Hills Sauvignon Blanc juice**	**Adelaide Hills Sauvignon Blanc juice**
Vintage	**2015**	**2016**
Heat test result (without fining agent)	34.8 NTU	45.6 NTU
Alcohol (% v/v)	0	0
Baume	12.0	12.0
pH	3.26	3.16
Total acidity	7.86 g/L	7.95 g/L
Yeast assimilable nitrogen	271 mg/L	199 mg/L
Free SO_2	13 mg/L	14 mg/L
Total SO_2	50 mg/L	54 mg/L

Juice (48 kg) was partitioned in 50L fermenters, and each treatment was repeated in triplicate (*Figure 6.0*, *Table 6.1*). The trial design evaluates whether a polysaccharide can heat stabilise wine when added at the juice, ferment, or wine stage. Over the 2015 and 2016 vintage, the same type of juice (Adelaide Hills Sauvignon Blanc) was used, due to its reputation of requiring a relatively large amount of bentonite to heat stabilise. The cold soluble kappa- (Na+) and the kappa-/iota-carrageenans were repeated in V16.

Figure 6.0. 2015 Adelaide Hills Sauvignon Blanc juice delivered to the Wine Innovation Cluster Winery in Urrbrae, South Australia, for division into 33 treatment and replicate ferments. This process was repeated for the V16 trials.

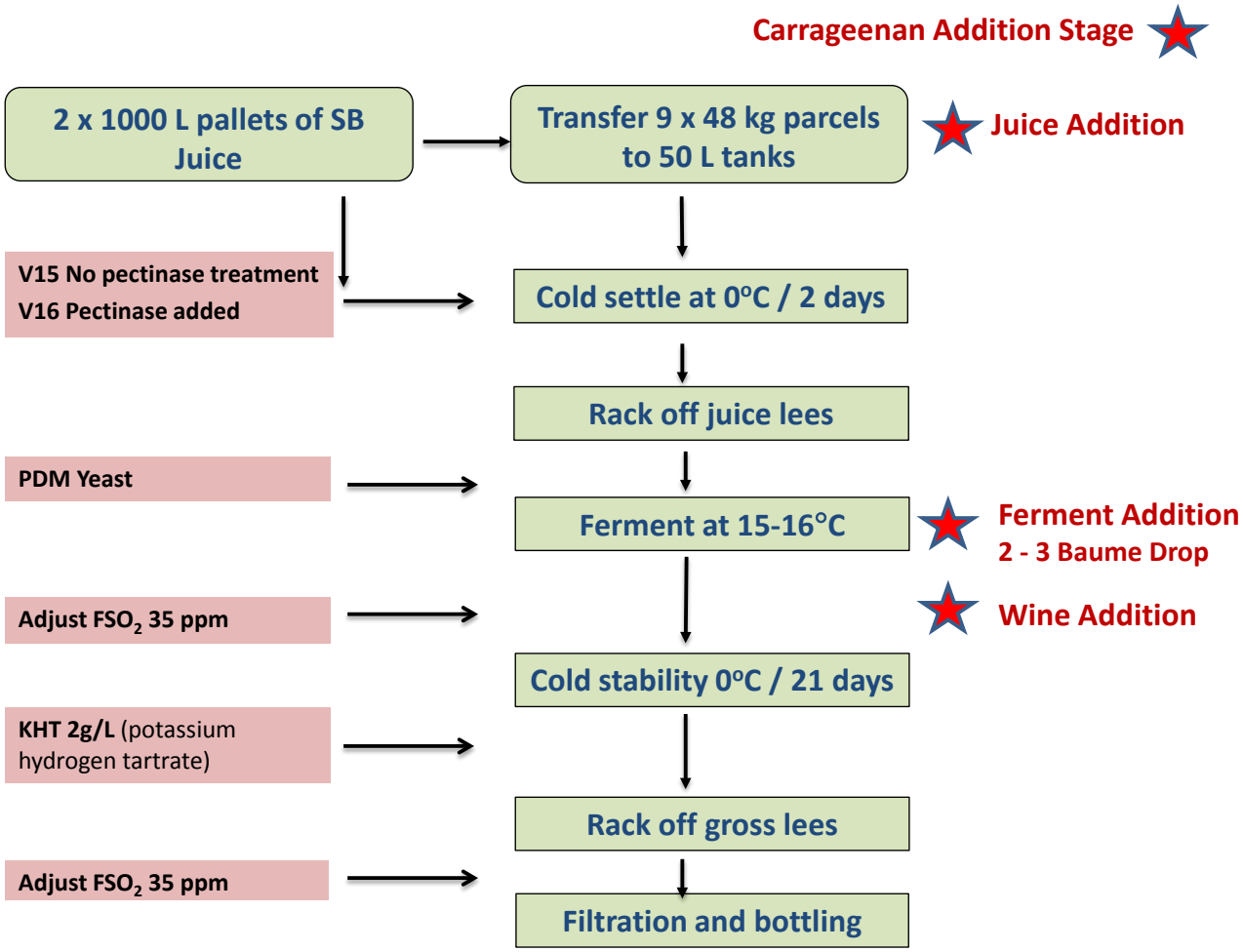


Table 6.1. Experimental design of polysaccharide and treatments for V15 and V16 pilot scale winery trials.

3 Polysaccharides 48 kg Ferments			Addition Stage		Fermentation Number
V15	V16				
Cold soluble kappa-carrageenan (Na+) Ca-A-533	Pure kappa- carrageenan (K+) Ca-A-978	→	Unsettled juice	→	TWE 1 - 3
		→	Fermentation (2 baume drop)	→	TWE 10 - 12
		→	Wine	→	TWE 19 - 21
Kappa- (90%)/iota-(10%) (K+) carrageenan Ca-B-2	Cold soluble kappa carrageenan (Na+) Ca-A-980	→	Unsettled juice	→	TWE 4 - 6
		→	Fermentation (2 baume drop)	→	TWE 13 - 15
		→	Wine	→	TWE 22 - 24
Low methoxy citrus pectin P-D-902	Kappa- (90%)/iota-(10%) (K+) carrageenan Ca-B-3R	→	Unsettled juice	→	TWE 7 - 9
		→	Fermentation (2 baume drop)	→	TWE 16 - 18
		→	Wine	→	TWE 25 - 27
Control (no fining)		→	(No addition)	→	TWE 28 - 30
Control (bentonite)		→	Wine	→	TWE 31 - 33

The white winemaking process chosen for the vintage experiments is a standard process used in production wineries that cold settle their juice (*Figure 6.1*). In V15, no pectolytic enzyme was added to the juice; however, in V16, pectolytic enzyme was used and the polysaccharides were not added until the juice was pectin negative, as indicated by the acidified alcohol test.

Figure 6.1. Pilot-scale winemaking process used in V15 and V16 at the Wine Innovation Cluster Winery, in Urrbrae, with carrageenan addition stages marked. SB = Sauvignon Blanc.



6.2. Determining fining rates for pilot winemaking trial

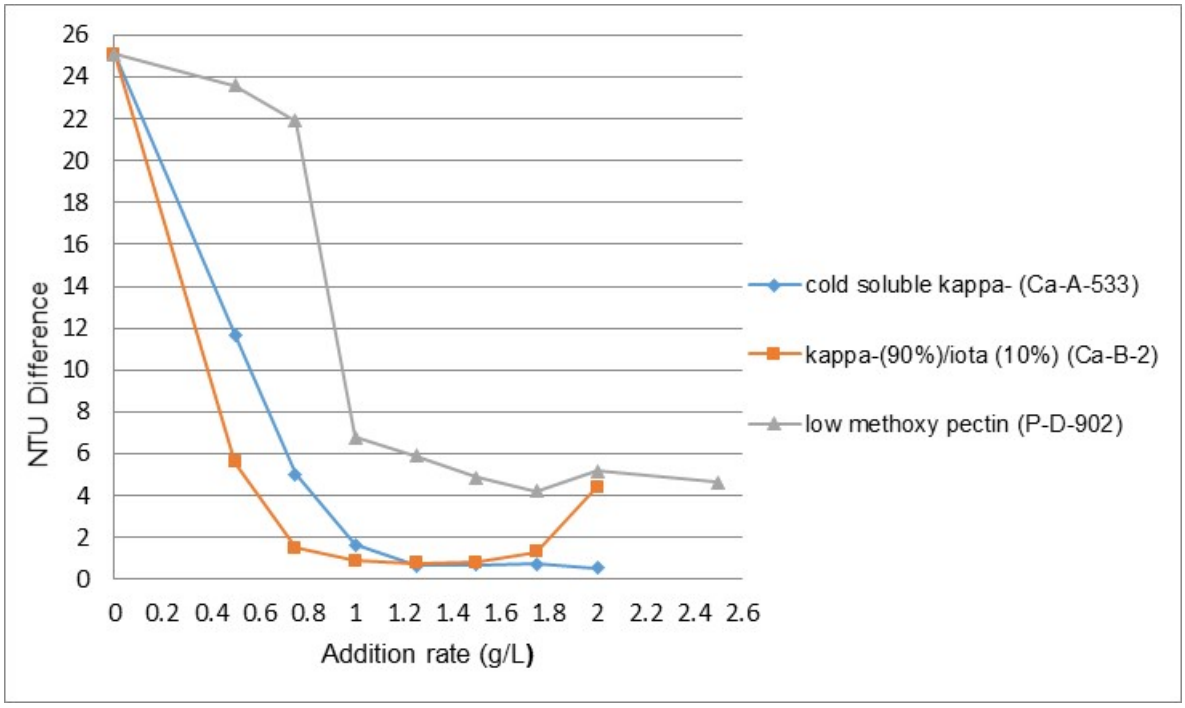
Fining rates for pilot winemaking trials were determined in bench-scale experiments for unclarified juice, ferment or wine, in a similar manner to how a bentonite rate would be determined. Sequential volumes of polysaccharide (0.2 g/L) were added incrementally to separate batches of the juice or wine (Section 4.1.2). Juice must be pectin negative or an initial turbidity reading of less than 1 NTU may not be achieved and, the solution will also be very difficult to filter. If an initial juice/wine turbidity of less than 1 NTU is not achieved, then the heat test may be invalid. Fining rates for fermentation additions were assumed to be identical to those determined for the juice addition. Wine fining rates were determined on a wine sample that had finished fermentation, and following adjustment of SO_2.

Figure 6.2 illustrates the determination of the fining rate for juice and ferment additions in the 2015 Adelaide Hills Sauvignon Blanc juice. Typically, in production, the fining rate would be considered as the point at which the addition rate achieves a heat test result of less than 2 NTU, plus another 0.2 g/L to account for any variation in the method. Hence, in the 2015 fining trials, the addition rate to juice and ferment would be 1.0 g/L cold soluble carrageenan and 1.2 g/L kappa-/iota- carrageenan. Unfortunately,

due to a lab error, the cold soluble kappa- and the kappa-/iota- carrageenan juice and ferments were both given a 1.2 g/L fining.

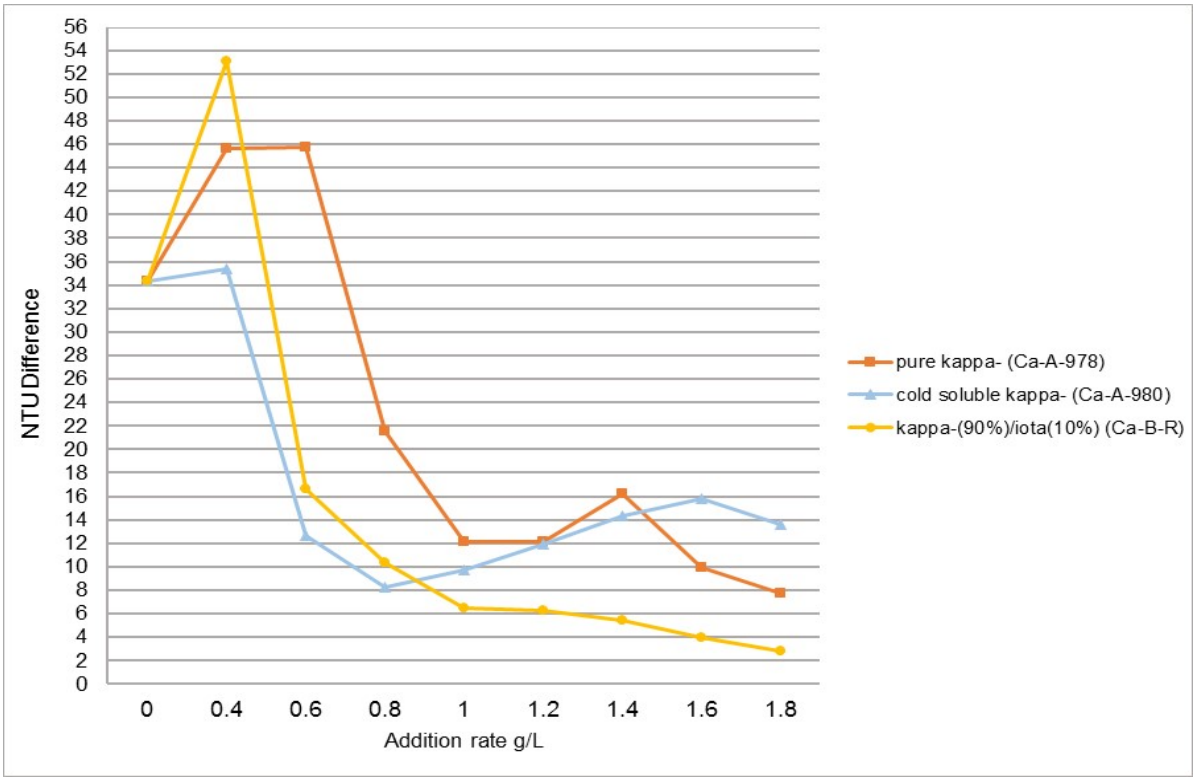
In the case of the pectin, where a heat test result of less than 2 NTU was not achieved, the fining rate was determined by the trend in the graph curve reaching a minimum point (1.5 g/L addition rate).

Figure 6.2. Determining the fining rate for V15 pilot-scale winemaking trials for Adelaide Hills Sauvignon Blanc juice.



In 2016, the fining trials of the carrageenan did not reach less than 2 NTU in the Sauvignon Blanc juice sample (*Figure 6.3*). Hence, the minimum part of the graph plus 0.2 g/L was chosen, which was 1.2 g/L for the cold soluble kappa- and the kappa-/iota- and 1.4 g/L for the pure kappa- carrageenan.

Figure 6.3. Determining the fining rate for V16 pilot-scale winemaking trials in 2016 Adelaide Hills Sauvignon Blanc juice.



6.3. Adding either pectin or carrageenan to juice, ferment and wine

The standard operating method used for adding and mixing polysaccharide to juice and wine was important, due to the strong potential gelling properties of pectin and carrageenans in wine. The rapid formation of gels can potentially make the carrageenan difficult to disperse in the liquid. Grape juice and wine are relatively high in the potassium cation, which is reported to promote the formation of hard gels for kappa-carrageenans (Campo, 2009) (*Table 6.2*).

Table 6.2 describes the common constituents of juice and wine. In particular, it shows the high concentration of potassium, as well as sodium, magnesium and calcium, which vary significantly across varieties and seasons, and would be likely to influence the rheology of carrageenans in wine. The ionic composition of juice and other solutes may affect the solubility of the carrageenan fining agent.

*Table 6.2. Composition of white wine. Juice will have higher levels of potassium and tartaric acid, as these compounds precipitate during fermentation and storage. Glucose/fructose levels in juice range from 150 - 240 g/L. Source: (*Rankine *2002).*

Ethanol	8 - 16% v/v
Tartaric acid	2 - 5 g/L
Other acids (malic, citric, lactic, succinic, acetic)	1 - 12 g/L
Potassium	700 - 2500 mg/L
Sodium	20 - 300 mg/L
Calcium	15 - 150 mg/L
Magnesium	10 - 200 mg/l
Iron	trace - 1.5 mg/L
Copper	trace - 0.5 mg/L
Zinc	trace - 1.5 mg/L

Very thorough mixing of the polysaccharide stock solution was required; this method is described in Section 4.1.4.1. Carrageenans can only be prepared in up to a 2% w/w solution as, beyond that, the solution becomes saturated. Therefore, a relatively large volume of stock solution was required (12.5%) from the total wine volume. The mixing of the solutions in the vessels was carried out using a drill mixer (*Figure 6.4*). Imitating this level of mixing in a commercial setting may require specialised agitators in the winery tanks.

Figure 6.4. Drill mixing device used for pilot-scale winemaking trials.



6.4. Fermentation

Adding carrageenan or pectin to a juice or ferment increased the time taken to complete fermentation by an average of 1.6 days (V15) and 1.0 days (V16) (*Table 6.3*). The reason for this is not clear, although it is possible that the polysaccharides are binding to a nutrient that facilitates fermentation, thereby slightly slowing the process.

The number of days to complete fermentation could be important, as during vintage it is useful to have a faster turnaround of tanks between fermentations to facilitate tank utilisation. However, following consultation with winemakers in the Industry Reference Group (IRG), it was determined that the difference in fermentation time is relatively small and would not be a hindrance.

Table 6.3. Days to complete fermentation for V15 and V16 pilot winery trials on Adelaide Hills Sauvignon Blanc.

Addition Stage	Polysaccharide	V15 Days[#] to complete fermentation	V16 Days[#] to complete fermentation
-	Control - no fining agent	6.0	10
-	Bentonite control	6.0	10
Juice	Low methoxy citrus pectin	7.0	-
	Kappa- (K^+) carrageenan	-	11.3
	Cold soluble kappa- (Na^+) carrageenan	7.0	10.6
	Kappa-/iota- carrageenan	7.3	10.0
Ferment	Low methoxy citrus pectin	8	-
	Kappa- (K^+) carrageenan	-	11.3
	Cold soluble kappa- (Na^+) carrageenan	8	11.6
	Kappa-/iota- carrageenan	8	11.0

[#]Average of replicates.

6.5. Heat stability and protein tests

In V15, the kappa- and kappa-/iota- carrageenans heat stabilised the wine when added at the juice, ferment and wine stages. In V16, the juice additions were slightly unstable for both of the kappa type carrageenans; the average heat test result was 2.5 NTU. The amount of polysaccharide to heat stabilise wine is summarised in *Table 6.4*.

Table 6.4. V15 and V16 fining rates used in the pilot winemaking trials.

Polysaccharides	Addition Stage	V15 Addition Rate	V16 Addition Rate
Cold soluble kappa- carrageenan	Unsettled Juice	1.2	1.0
Kappa- (90%)/iota- (10%) carrageenan	Unsettled Juice	1.2 (1.0)	1.0
Low methoxy citrus pectin	Unsettled Juice	1.5	-
Pure kappa- carrageenan	Unsettled Juice	-	1.2
Cold soluble kappa- carrageenan	Fermentation (2 B° Drop)	1.2	1.2
Kappa- (90%)/iota- (10%) carrageenan	Fermentation (2 B° Drop)	1.2 (1.0)	1.0
Low methoxy citrus pectin	Fermentation (2 B° Drop)	1.5	-
Pure kappa- carrageenan	Fermentation (2 B° Drop)	-	1.0
Cold soluble kappa- carrageenan	Wine	1.4	1.4
Kappa- (90%)/iota- (10%) carrageenan	Wine	1.0	1.4
Low methoxy citrus pectin	Wine	1.8	-
Pure kappa- carrageenan	Wine	-	1.4
Control (bentonite)	Wine	1.4	1.5
Control (no fining)	Wine	0.0	0.0

For the kappa-/iota- carrageenan, only 1.0 g/L was required to heat stabilise the juice in the bench test (*Figure 6.2*). It was due to a lab error that 1.2 g/L of carrageenan was added at the juice and ferment stage.

6.6. Recovery from gross lees

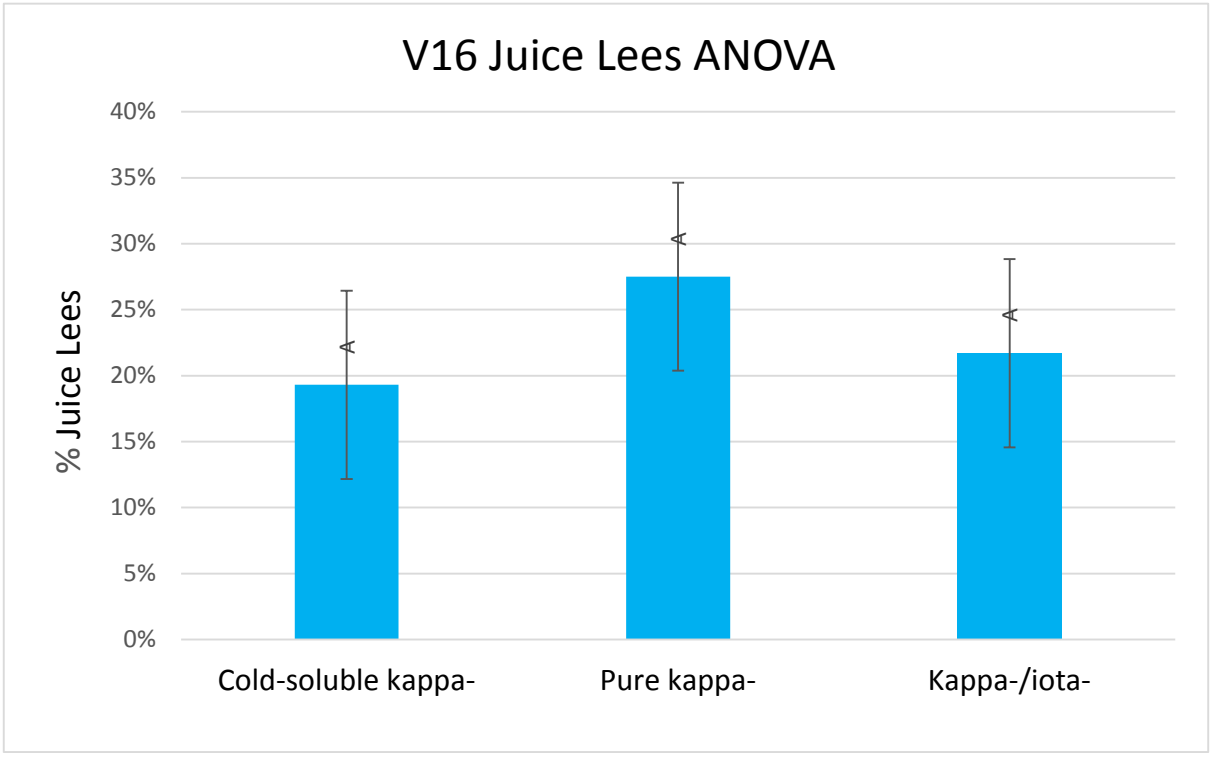
The mass of each vessel was taken and recorded before and after each racking, in order to enable the calculation of the wine recovery (*Figure 6.5*). Wine recovery data from the V15 trials has been excluded, as the juice used in this winemaking trial was not enzyme treated. Pectinase enzyme promotes the cold settling of juice and therefore provides more consistent lees settling. That said, it is difficult to extrapolate the wine recovery results from the V16 trials as a basis for commercial scale production and as such, the wine recovery data should be considered as a guide only.

Figure 6.5. The mass of recovered juice or wine was recorded after each vessel movement.



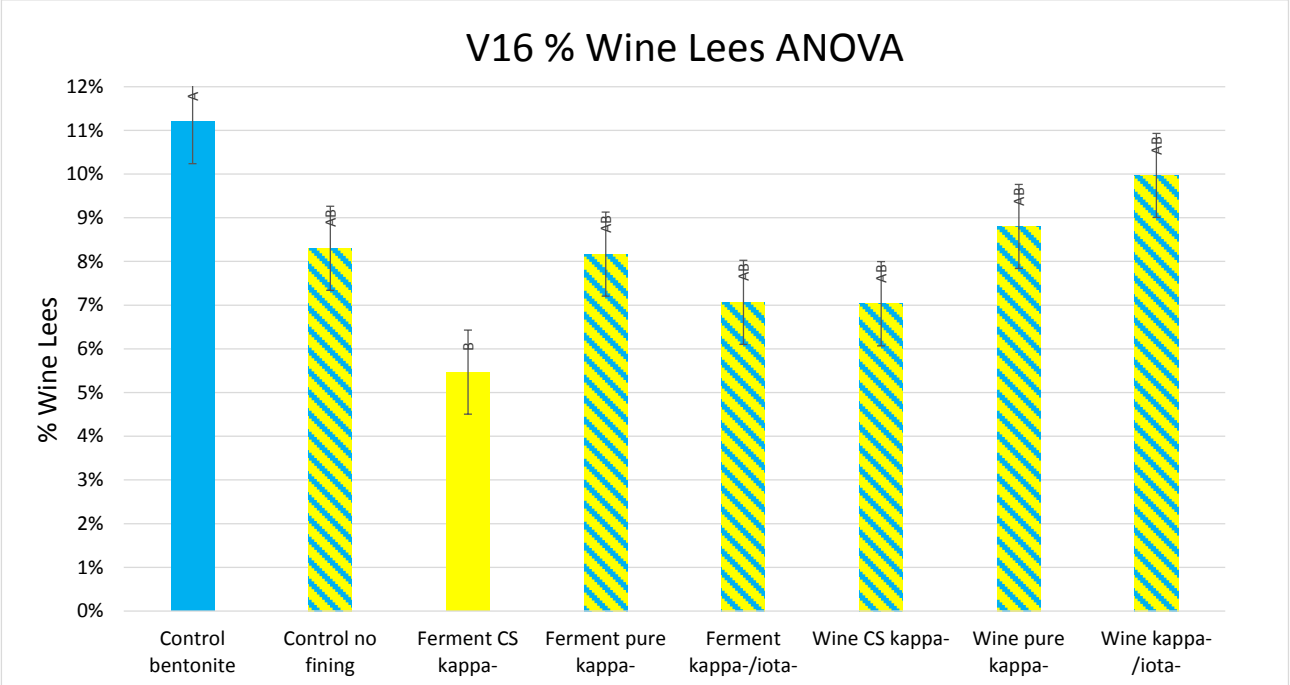
Figure 6.6 illustrates that there was no statistical difference between the recoveries of the juice lees. The percentage juice lees generated was typically higher than that observed in commercial scale cold settling of juice, which is usually less than 10% v/v. It is not known whether the higher level of juice lees occurred due to the carrageenan lees or the smaller scale of the pilot winery.

Figure 6.6. The amount of juice lees % w/w generated from cold settling in a 50L vessel during V16.



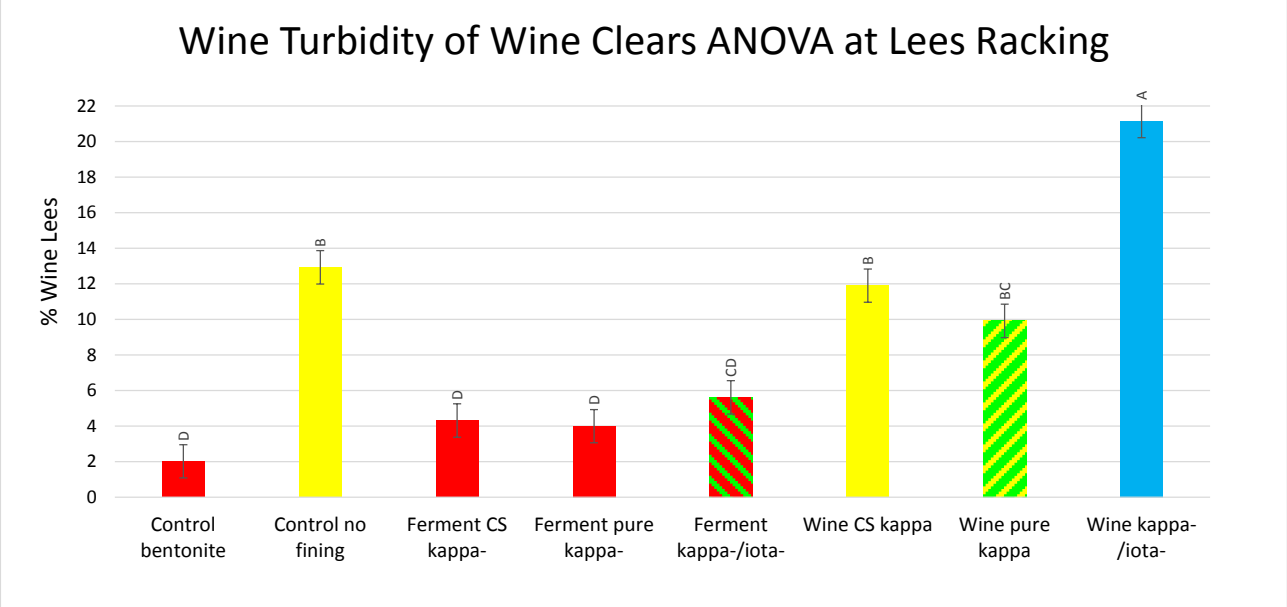
The amount of wine lees, following the racking of the vessels after 21 days of storage at 0°C, is shown in *Figure 6.7*. The ANOVA analysis indicates that the % of wine lees generated from the carrageenan treatments is statistically similar to that achieved using bentonite, with the exception of the cold soluble kappa-, which, when added at the ferment stage, generates statistically less lees.

Figure 6.7. The amount of wine lees % w/w generated following 21 days of storage at 0°C in a 50L vessel during V16.



To demonstrate whether the wines had settled to the same level of clarity, the turbidity of the clears was measured (*Figure 6.8*). As bentonite is an excellent and common settling agent commonly used in wine, this would set the ideal standard for settling of lees. The control (no fining agent) and wine addition treatments did not settle as well as the bentonite wine. However, the kappa- carrageenan, when added at the ferment stage, did settle to the same clarity of clears as the bentonite control.

Figure 6.8. Turbidity of wine clears after racking off gross lees.



Further data are required to better understand wine recovery when heat stabilising wine with carrageenan. The sedimentation test (Section 7.4) indicated that carrageenan would be more efficiently utilised alongside stages of the winemaking process that generate solid lees. The density of carrageenan gels in clarified wine resulted in flotation (*Figure 7.1*), indicating that the presence of lees may facilitate sedimentation.

6.7. Filtration

In certain circumstances, carrageenan additions to wines result in wines that are difficult to filter (Marangon, et al., 2013). To investigate this, samples were taken during bottling for filterability testing on all treatments and controls in V15 and V16. (Appendix 13.7, Appendix 13.8).

The V15 Sauvignon Blanc wines in which carrageenans were added, at either the juice or ferment stage, were difficult to filter (*Figure 6.8*, Appendix 13.7). The V16 Sauvignon Blanc wines — regardless of fining agent or the stage in which they were added to the juice, ferment or wines — passed filterability testing and were not difficult to filter (Appendix 13.8). Two variables were altered between the V15 and V16 trials, which may have affected the filterability of the wine. These were the addition of pectolytic enzyme in V16 (and not V15) and the utilisation of cross-flow rather than a depth cartridge to filter the wines pre-bottling.

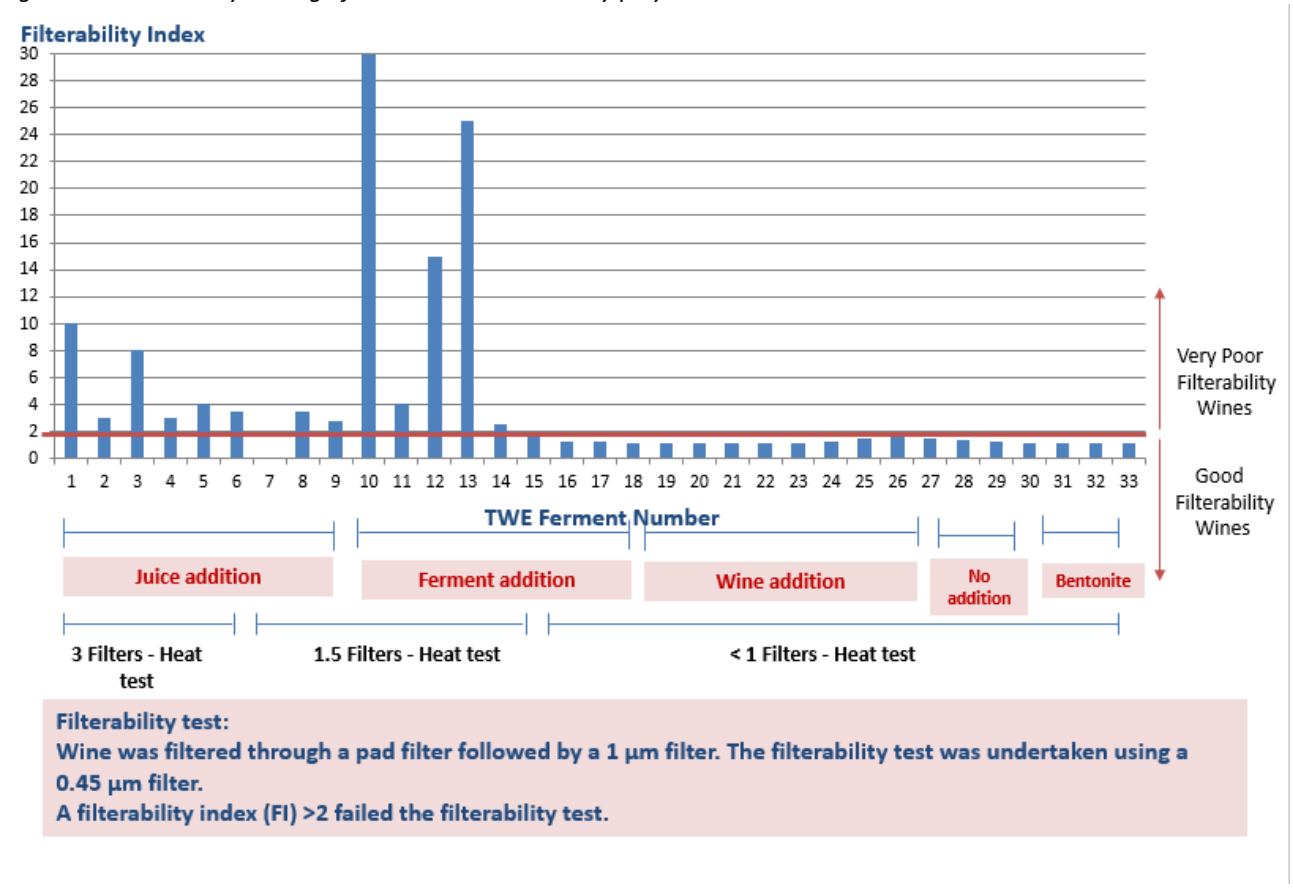
The addition of pectolytic enzyme in juice allows easy filtration of the juice to perform the heat test, in order to determine the required addition rate. Pectin negative juice also allows the initial turbidity in a heat test to be less than 1 NTU, a property that is very difficult to achieve in pectin positive juice.

Choosing not to add pectolytic enzyme in white winemaking is an option often used by winemakers to achieve a particular wine style. Pectin positive juice in bench testing is harder to filter and does not settle as well as pectin negative juice. It appears that in the presence of carrageenan, these properties are accentuated.

The poor settling properties of the V15 juice, without addition of pectolytic enzyme, is further illustrated in Appendix 13.1 and Appendix 13.3. The wine turbidity of the V15 wines fined with polysaccharides had an average turbidity of 166.5 NTU, compared to a turbidity of 8.5 NTU for the V16 polysaccharide treated wines (TWE 2, V16 excluded). The control wines (bentonite and no fining agent) for V15 had an average turbidity of 25.1 NTU, compared to a turbidity of 7.5 NTU for the V16 control wines (Appendix 13.3; also see *Figure 6.8*, wine turbidity of clears).

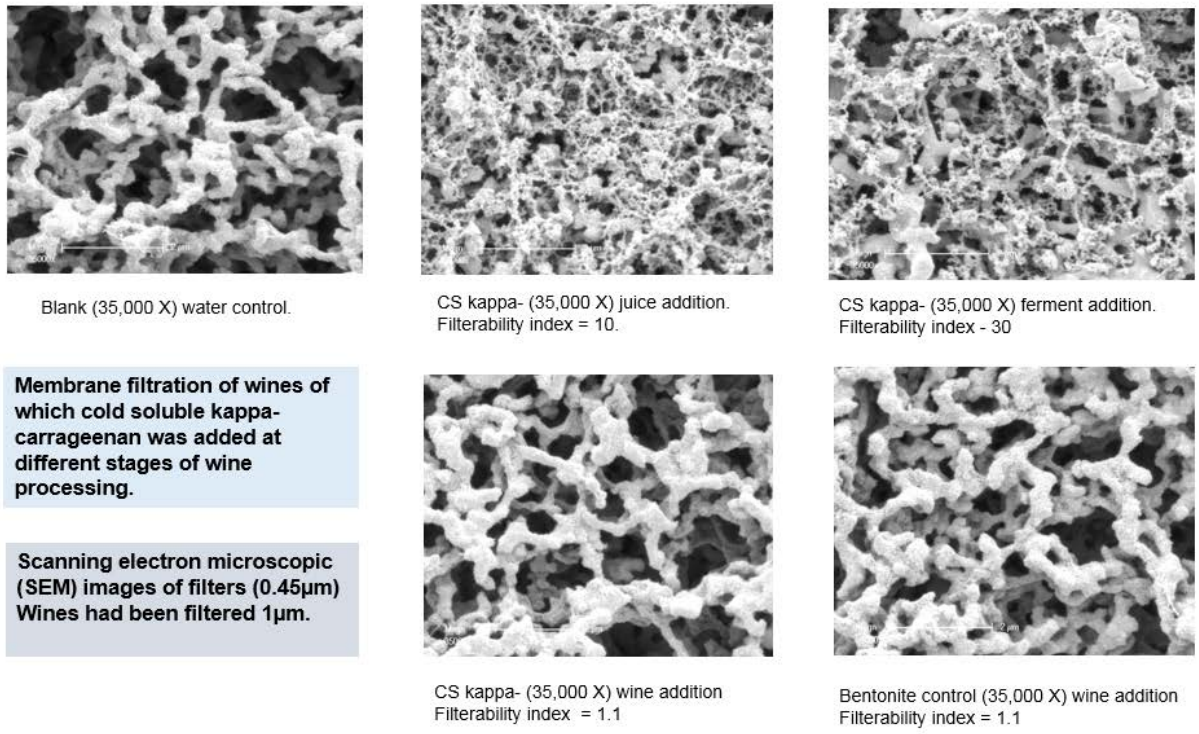
Figure 6.8 illustrates the difficulty of filtration in the V15 wines by measuring the filterability index for wines that were filtered with the 1µm depth cartridge but not the 0.45µm depth cartridge. During bottling of these wines, the cartridge filters (1µm and 0.45µm) both blocked and had to be replaced, resulting in a 48% increase in bottling cost. The difficulty observed in filtering the juice and ferment addition stage wine is commercially prohibitive.

Figure 6.8. Filterability testing of wines heat stabilised by polysaccharides and controls in V15.



The cold soluble kappa- carrageenan wines (V15) were further examined using a scanning electron microscope (SEM) (*Figure 6.9*). The 0.45µm membranes for the cold soluble kappa- carrageenan treated wines, at the juice and ferment stages, appear to have had strands of an unidentified polymer blocking the membrane pores. The pore-blocking strands were absent in the bentonite fined wine, as well as the wine fined with cold soluble kappa- carrageenan added at the wine stage.

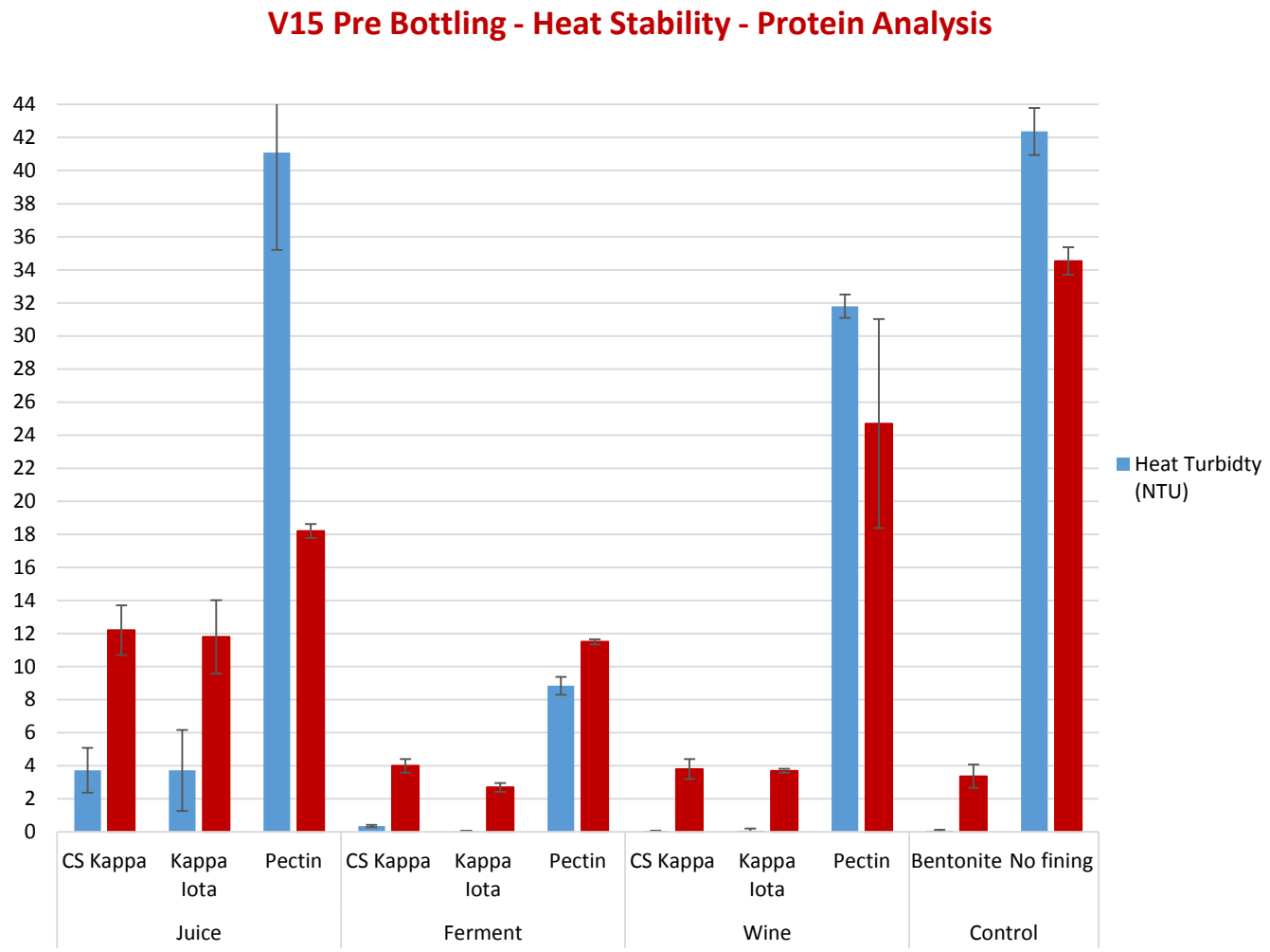
Figure 6.9. Scanning electron microscope images of 0.45µm nylon membranes following the filtration of wine that had been heat stabilised with cold soluble kappa- carrageenan and the bentonite control.



6.8. Heat stability of wines

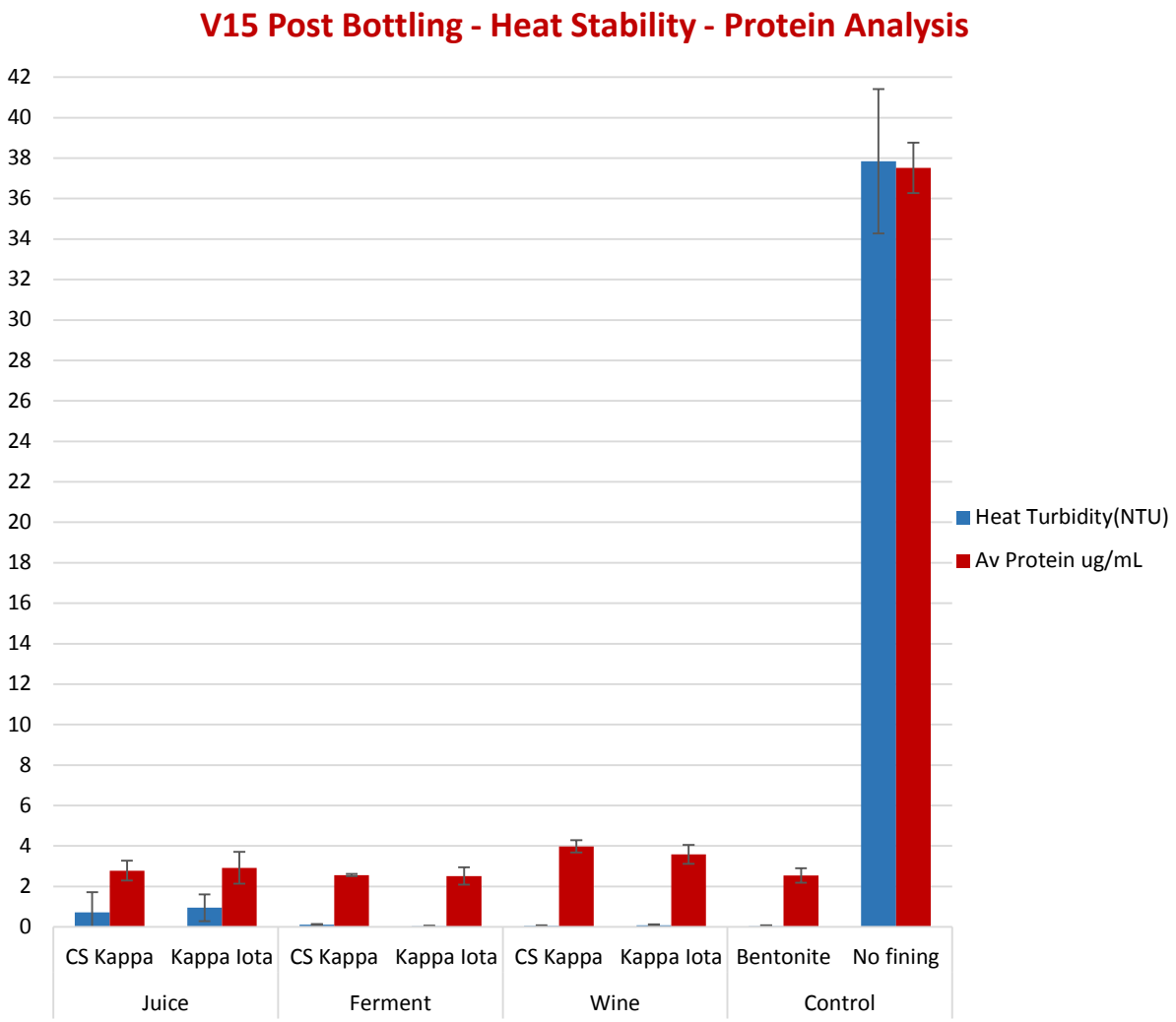
Heat test turbidity and protein concentration were measured before and after bottling for the V15 and V16 Adelaide Hills Sauvignon Blanc pilot winery trials (Appendix 13.1, Appendix 13.3). In V15, the pectin selected for use in the pilot-scale trial was unsuccessful at heat stabilising the Sauvignon Blanc wine. Prior to bottling, the wines derived from addition of carrageenan at the juice stage were also not consistently heat stable to less than 2 NTU (*Figure 6.10*).

Figure 6.10. Heat stability of the V15 Sauvignon Blanc wine post racking off cold stabilisation and pre-bottling. Average of three samples.



After bottling, all of the wines that had been fined with the cold soluble kappa- or the kappa-/iota-carrageenan had a heat stability of less than 2 NTU (*Figure 6.11*). For the juice addition samples, both the heat turbidity and the protein concentration decreased after bottling.

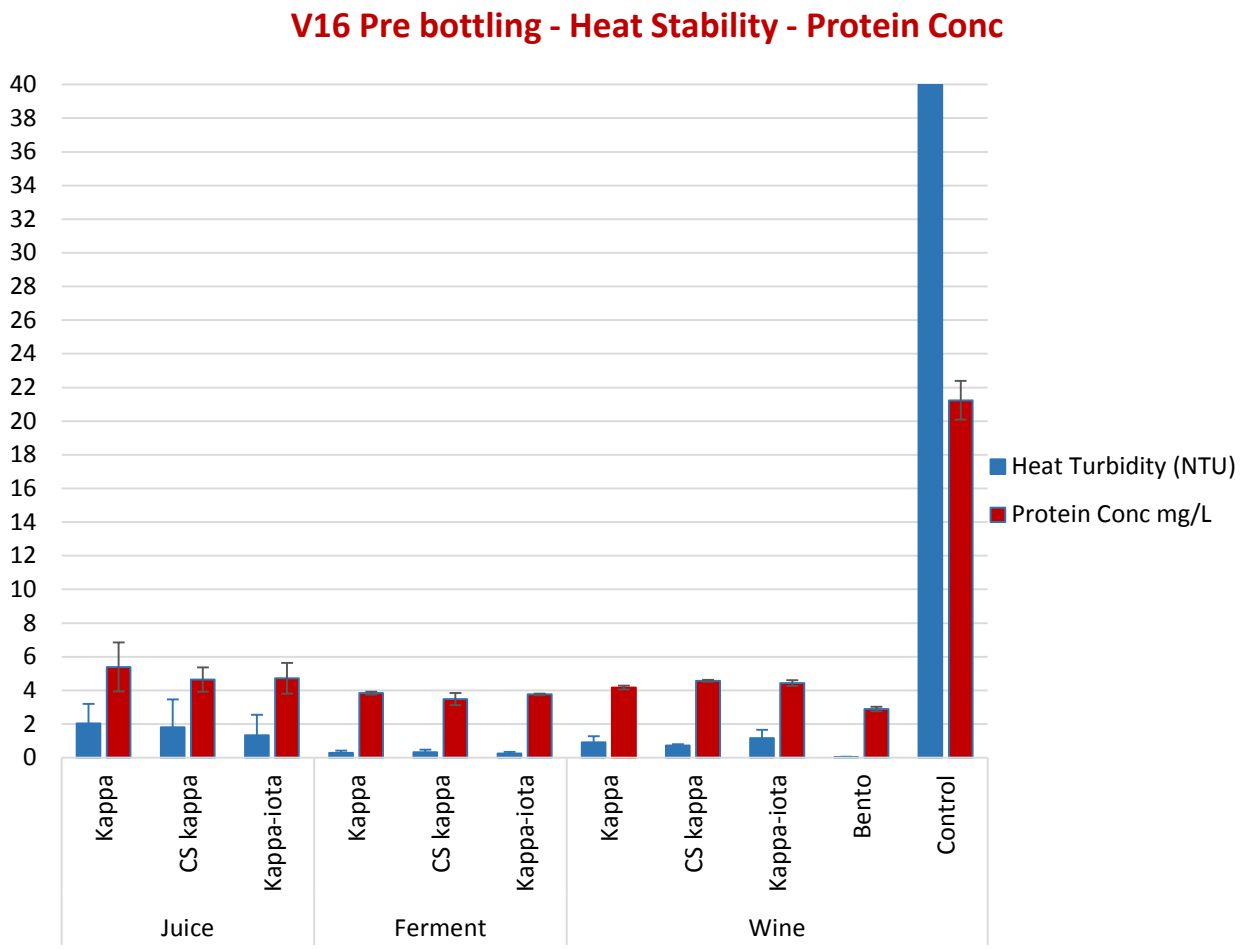
Figure 6.11. Heat stability of the V15 Sauvignon Blanc wine post -bottling. Average of three samples. Pectin data not shown.



A possible reason for the decrease in heat turbidity and protein concentration, post bottling, for the V15 wines, is that carrageenan collected on the depth filters (as described in *Figure 6.9*) formed a layer of fining agent that further removed heat unstable proteins. The post-bottling protein content for the LM pectin was not measured; however, the heat stability (NTU) had decreased between pre- and post- bottling (Appendix 13.1).

In V16, some of the wines that had been treated with carrageenan fining agent during the juice stage were not heat stable but had heat turbidity values close to heat stability (NTU <3.3) (Appendix 13.3). Wines with a heat test result of less than 3.0 NTU, post-bottling, may be commercially acceptable, at the winemaker's discretion. It is also common for heat turbidity to decrease slightly after post-bottling filtration, as was observed by the V15 wines. *Figure 6.12* describes the heat turbidity and protein concentration in the V16 wines that were tested following racking off cold stabilisation prior to bottling.

Figure 6.12. Heat stability of the V16 Sauvignon Blanc wine post racking off cold stabilisation and pre-bottling. Average of three samples. Average heat turbidity of no fining agent = 78 NTU.



The bottling process in V16 did not contribute to further protein removal as in V15 (*Figure 6.13*). Cross-flow filtration was used in V16 instead of the depth cartridge filtration. The tangential flow of cross-flow filtration may have distinctly separated the fining agent from the wine, thereby preventing additional protein from being removed. The slight increase in turbidity between pre-bottling and post-bottling, for the juice addition samples, may be due to variation in the turbidity reading. Where pre-cross flow wines do not have a heat test result of <2 NTU, if they are at least close to this value, it is recommended that additional fining agent (carrageenan or bentonite) be added such that heat stability is confirmed.

Figure 6.13. Heat stability of the V15 Sauvignon Blanc wine post-bottling. Average of three samples. Average heat turbidity of no fining agent = 92 NTU.

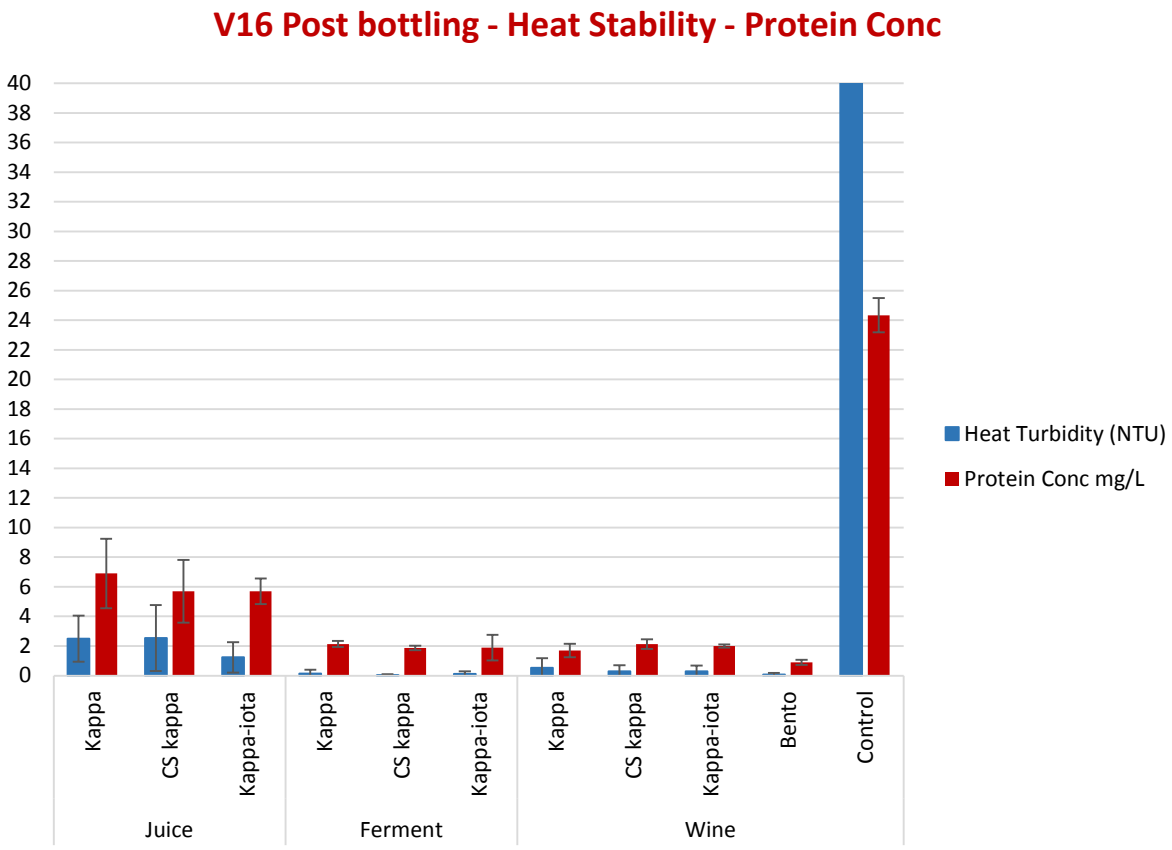
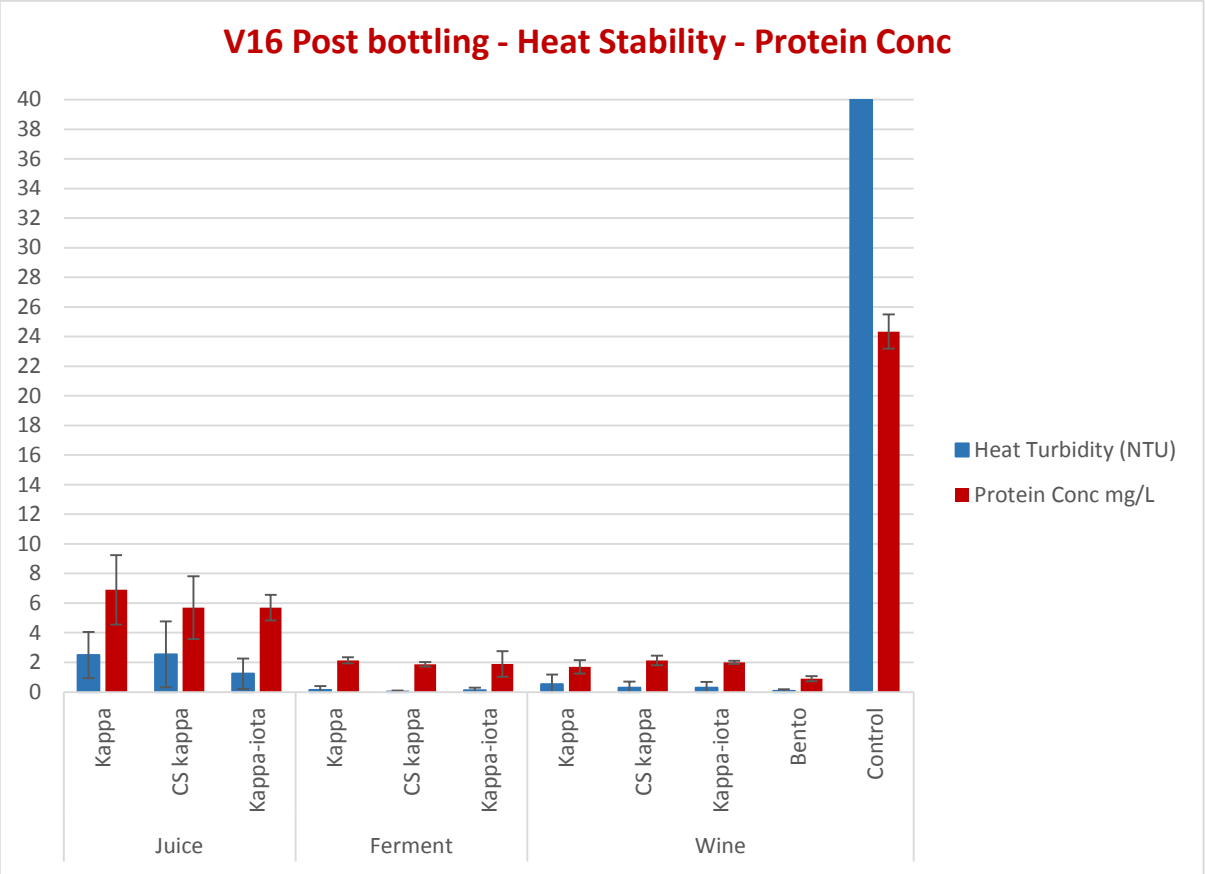


Figure 6.13. Heat stability of the V15 Sauvignon Blanc wine post racking off cold stabilisation and pre-bottling. Average of 3 samples. Average heat turbidity of no fining agent = 92 NTU.



6.9. Wine chemistry analysis

The use of carrageenans or pectin as fining agents did not impact on wine analysis parameters such as alcohol, pH, TA and VA (Appendix 13.2, Appendix 13.4).

Cations are known to play a part in the gelation of carrageenans and hence the V15 and V16 wines were analysed for metals, across the different treatments. The metals measured were potassium, sodium, calcium, copper, iron, zinc and manganese. Metal content in wine is important due to wine import/export limits imposed by different countries, as well as for avoiding metal-complex instabilities.

Potassium is the most abundant metal in wine (*Table 6.2*) and is a strong gelation agent for kappa-carrageenan. Sodium also plays a role in the solubility of different types of carrageenan in aqueous solutions (Kara, et al., 2006). There are some legal export restrictions around sodium levels for wine, for example, the current limit for sodium levels in Switzerland is 60 ppm as the chloride salt. Calcium levels above 70-80 mg/L in wine are considered to present a risk of calcium tartrate instability, particularly for sparkling base wines. The AWRI recommends that residual copper and iron limits be kept to less than 0.5 mg/L. Zinc is an important yeast nutrient for building ethanol tolerance and promoting yeast membrane health. Chinese authorities are currently enforcing a 2 mg/L limit on the level of manganese in wines imported to China.

Metal analyses for the V15 and V16 wines fined with pectin, carrageenans and bentonite, compared to an unfined control, are shown in Appendix 13.5 and Appendix 13.6. When using carrageenan as a fining agent the potassium, copper, iron, zinc or manganese levels are not altered to any degree that would be detrimental to wine quality, metal instability or existing export restrictions.

The V16 calcium levels in wine fined with the kappa- (K^+) and kappa-/iota- carrageenans were slightly elevated, reaching the range discussed in the AWRI recommendation as presenting a risk of calcium tartrate instability (70-80 mg/L), particularly if the product is a sparkling base wine (Appendix 13.6). However, these levels of calcium are not considered to be a risk for a Sauvignon Blanc table wine.

The cold soluble kappa- carrageenan in V15 and V16 had elevated sodium levels exceeding the Swiss export restrictions (*Figure 6.14*, *Figure 6.15*).

Figure 6.14. V15 sodium levels in wines fined with carrageenans, pectin and bentonite, compared to the unfined control.

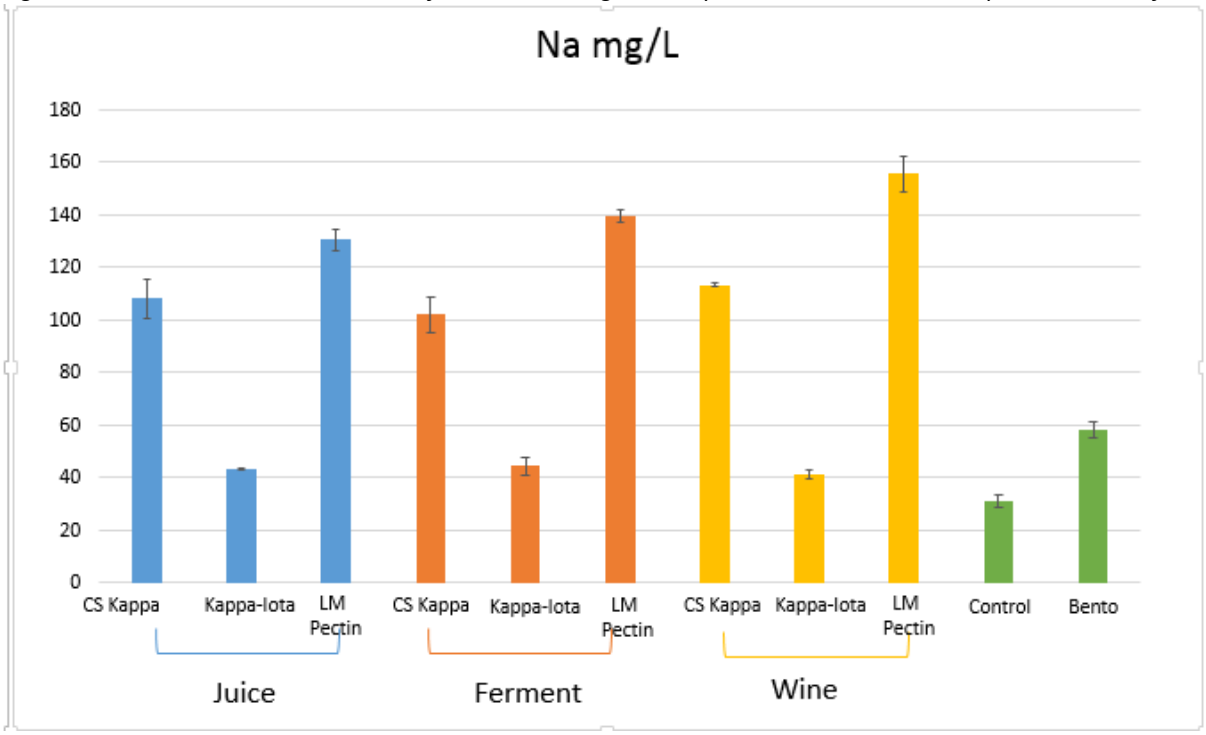
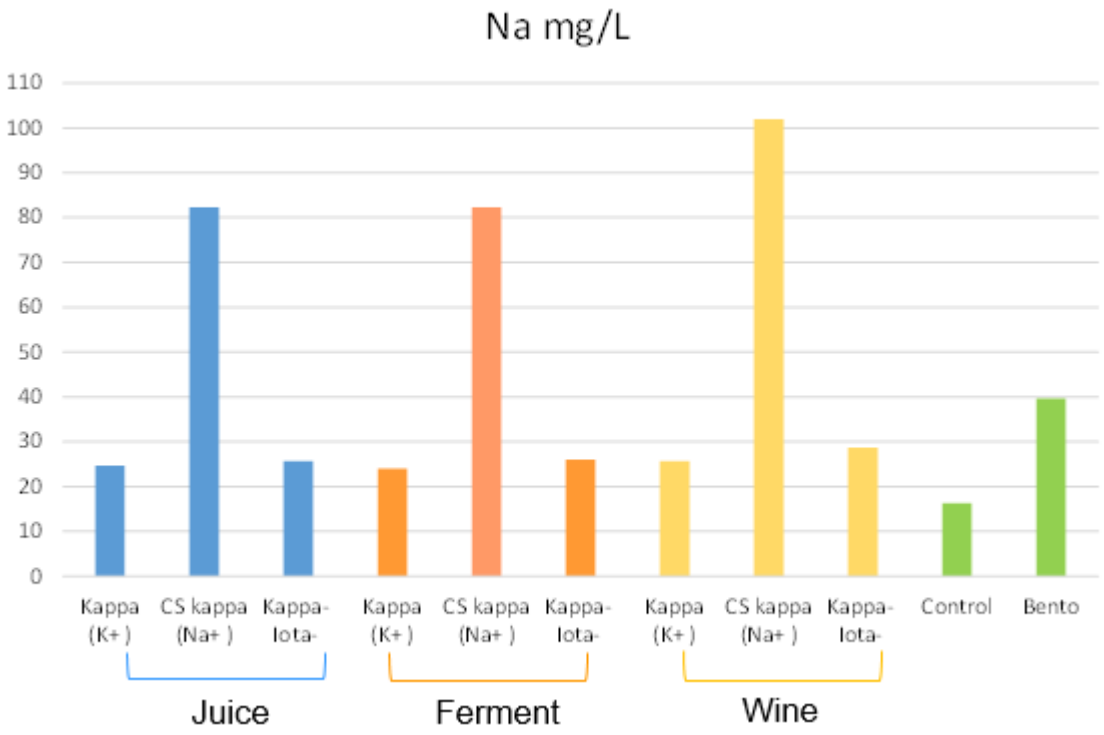


Figure 6.15. V16 sodium levels in wines fined with carrageenans and bentonite, compared to the unfined control.



6.10. Sensory analysis

Sensory descriptive analysis was completed on the cold soluble kappa- (Na+) and kappa-/iota- carrageenans tested in the V15 winemaking trials and on the pure kappa- (K+) carrageenan for the V16 winemaking trials.

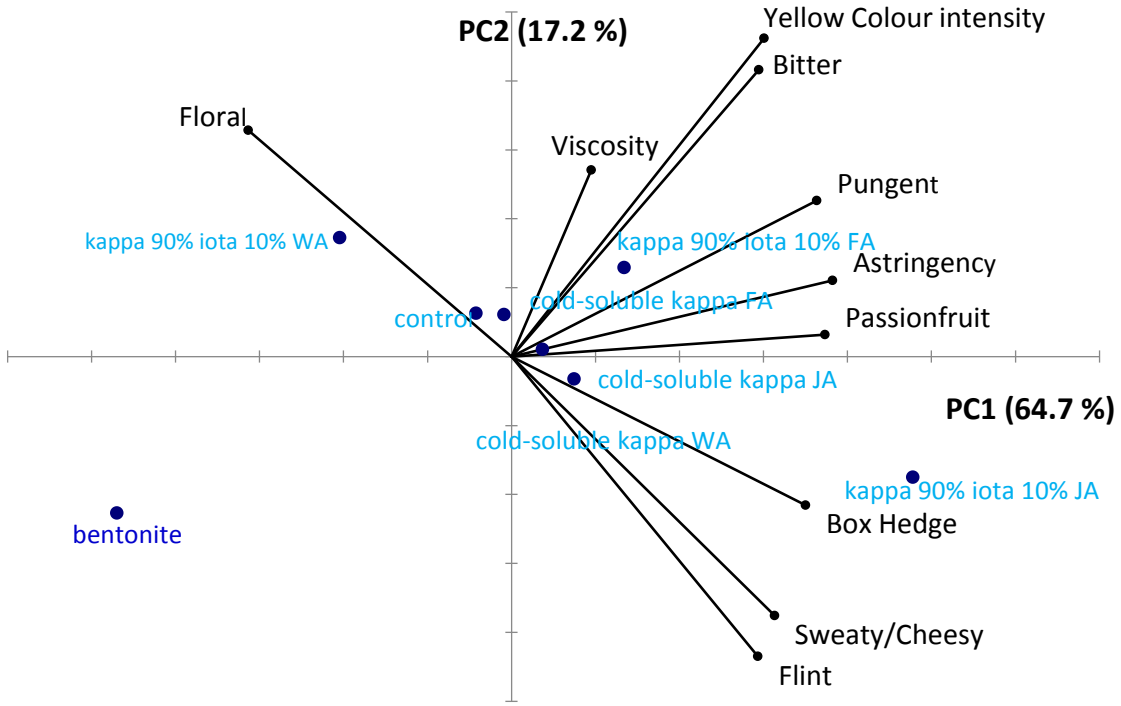
Bench screening tasting by winemakers at Wolf Blass Winery described the 2015 Adelaide Hills Sauvignon Blanc Control wine to be more complex and have higher tropical and stone fruit intensity than the 2016 Adelaide Hills Sauvignon Blanc, which had less aroma intensity and more floral and green characters than the wine produced from the 2015 winemaking trials.

For the 2015 wines, the analysis of variance ANOVA identified that eight sensory attributes differed significantly by treatment ($p < 0.05$). This significant difference in sensory properties was a result of the different fining agents applied and impacted aroma, palate and wine colour attributes.

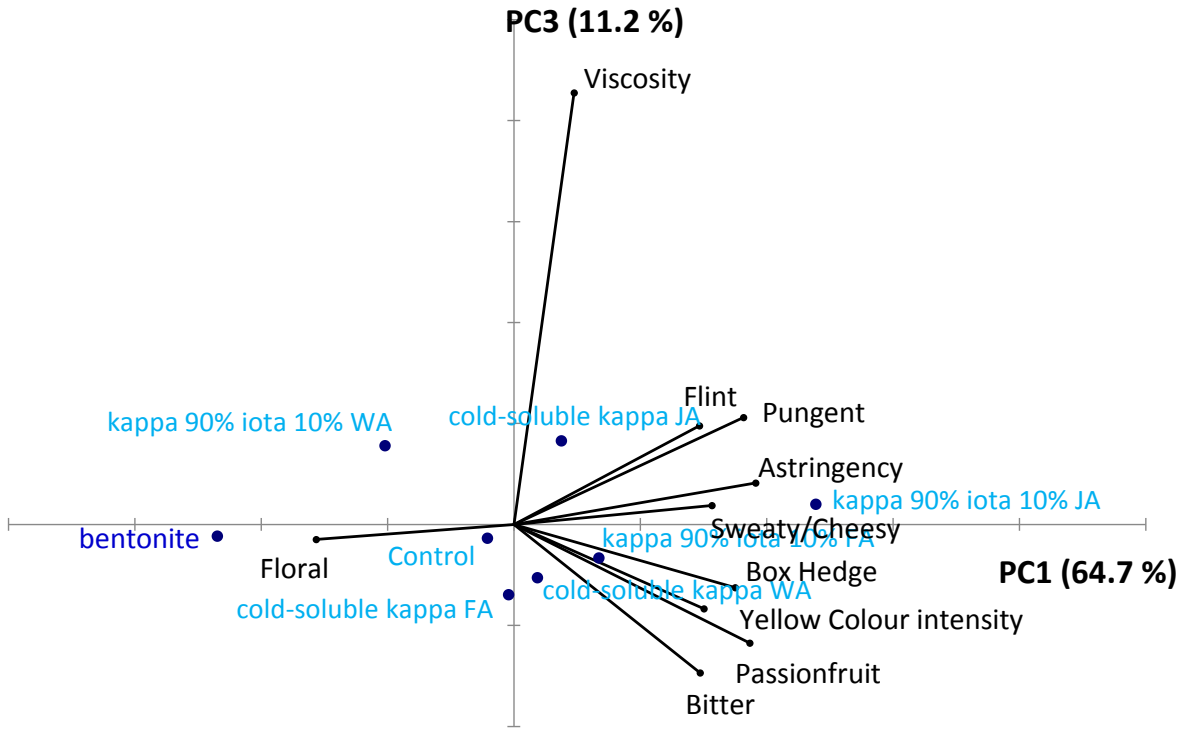
Following principal component analysis (PCA) of the significantly different sensory attributes for each treatment, the bentonite treatment gave a wine with a lower intensity of most sensory attributes compared to the untreated control. The cold soluble kappa- (Na+) and the kappa-/iota- carrageenan treatments gave wines with higher intensity of tropical fruit-related attributes, such as box hedge, sweaty and passionfruit, as well as higher viscosity. Figure 6.16 describes using PCA the significantly different attributes for each treatment. Figure 6.16(a) shows the scores and loadings for PC1 and PC2, which account for 81.9 % variation in the data set. Figure 6.16(b) accounts for a further 11.2 % of variation in the data set.

Figure 6.16. Scores and loadings bi-plot for Principal Component Analysis (PCA) of sensory attributes and samples: a) PC1 and PC2 and b) PC1 and PC3. JA = Juice addition; FA = Ferment addition; WA = Wine addition, PC=Principal component.

a)



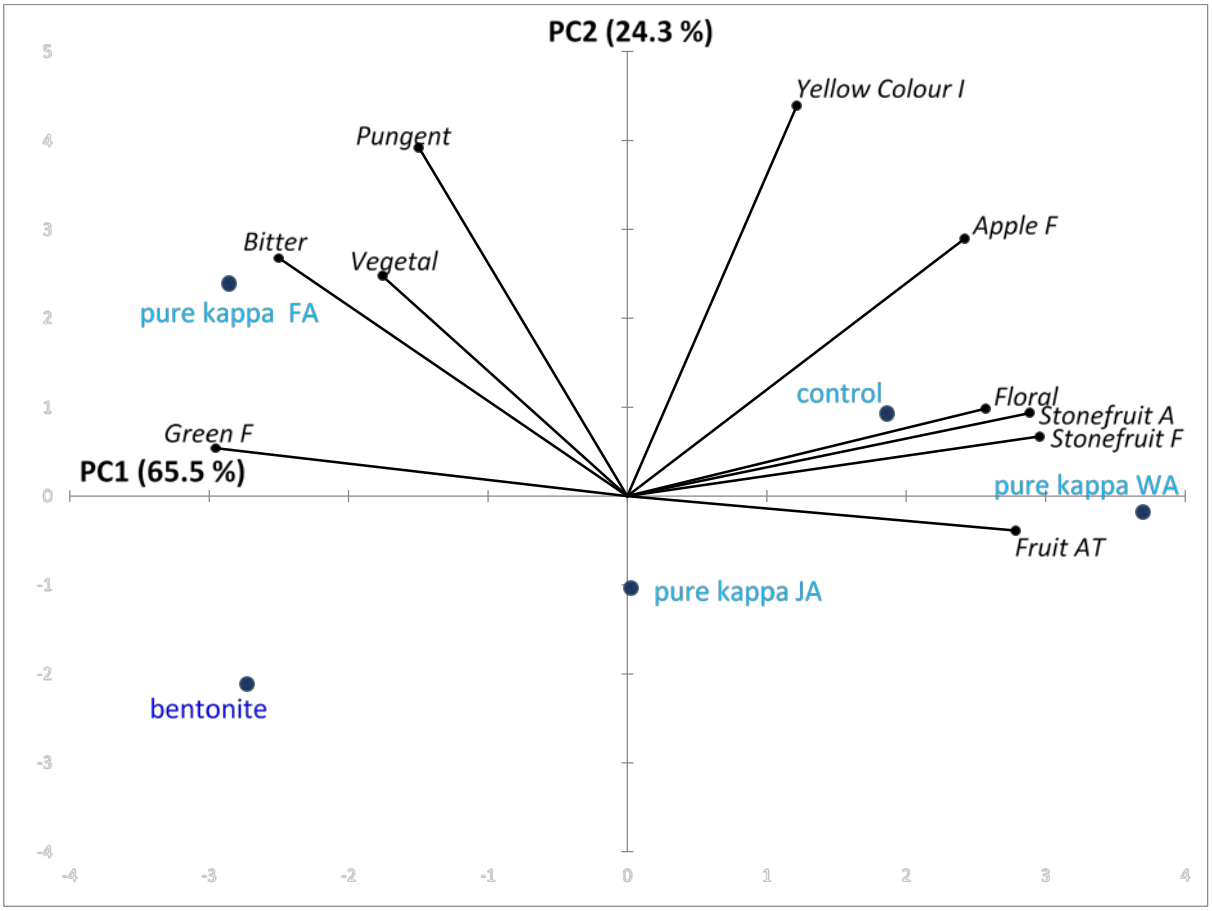
b)



These sensory results for the V15 winemaking trial, indicate that bentonite has fewer flavour attributes than the control (no fining agent). This outcome is consistent with the view that bentonite can strip the wine of certain flavour compounds — something that winemakers have long suspected to be the case. Wines from the 2015 vintage fined with carrageenan maintain desirable attributes for a Sauvignon Blanc wine relating to fruit and flavour complexity. Although wines fined with carrageenans are different from the control (no fining agent), these wines still maintain flavour complexity and intensity.

In 2016, only the pure kappa (K+) carrageenan was evaluated by descriptive sensory analysis. For the 2016 Sauvignon Blanc wines, there was a significant difference in sensory properties, dependant on the stage of the addition of the fining agent i.e. whether the carrageenan was added at the juice, ferment or wine stage. The analysis of variance (ANOVA) for the V16 pure kappa- carrageenan wines and controls identified that eight sensory attributes differed significantly by treatment ($p < 0.05$). Figure 6.17 describes using PCA the significantly different attributes for each treatment. Figure 6.17shows the scores and loadings for PC1 and PC2, which account for 89.8 % variation in the data set.

Figure 6.17. Scores and loadings bi-plot for Principal Component Analysis (PCA) of sensory attributes and samples: PC1 and PC2. JA = Juice addition; FA = Ferment addition; WA = Wine addition, PC=Principal component.



The wine addition treatment for the 2016 pure kappa- carrageenan had sensory attributes similar to the control (no fining agent), including apple, floral and stone fruit. The wine addition increased the intensity of the fruity attributes more than that for the control (no fining agent). The juice addition treatment had sensory attributes most similar to the control (no fining agent) indicating that addition of the pure kappa-carrageenan at this stage of the winemaking process had less impact than addition of the fining agent at other time points.

The ferment addition of the pure kappa- carrageenan accentuated the attributes bitter, vegetal and green, and lowered the intensity of the fruity aromas. The bentonite wine rated lowest in intensity for all fruity attributes and yellow colour intensity and was higher in green, vegetal and bitter attributes.

6.11. Cost benefit

Table 6.4 describes the costs of bentonite, pectin and carrageenan fining agents, as quoted in 2015 for a 20,000 kg order of each fining agent. Pectins are considerably more costly than carrageenan and do not result in a heat stable wine. Carrageenans can cost up to 20 times the price per kg of bentonite, despite often requiring slightly lower fining rates. The increased cost of the cold soluble kappa- carrageenan is due to the extra ion-exchange processing required to convert kappa- carrageenan from the potassium salt to the sodium salt.

Table 6.4. Cost per kg of fining agent quoted in 2015 for a 20,000 kg order.

Code	Fining Agent	Cost per kg	Fining rate[1,2] g/L	Cost per kg	Comments
Industry Standard					
	Bentonite NaCalit	$1.69	1.45	$2.45	
Carrageenans					
Ca-B-1R	Pure kappa (K^+)	$27.73	1.2	$33.28	
Ca-B-2	Kappa- (90%)/iota- (10%) blended	$27.73	1.0	$27.73	Hybrid-type kappa iota was quoted at $47.49 per kg
Ca-A-533	Cold soluble kappa (Na^+)	$48.00	1.1	$52.80	
Pectins					
P-A-373	Low methoxy DE (10%)	$35.00	1.9	$66.50	Did not heat stabilise wine. Final NTU = 14
P-A-374	Low methoxy DE (30%)	$30.00	1.9	$57.00	Did not heat stabilise wine. Final NTU = 11
P-D-910	Low methoxy DE (4%) (apple)	$53.98	1.8	$97.16	Did not heat stabilise wine. Final NTU = 14
P-D-902	Low methoxy DE (6%) (citrus)	$58.64	1.5	$87.96	Did not heat stabilise wine in V15 wine trial

[1]Based on average rate for fermentation addition (carrageenan) and wine addition (bentonite) for V15 and V16 Adelaide Hills Sauvignon Blanc. [2]Based on bench-testing of pectins in a 2014 heat unstable chardonnay wine.

Currently, fining wine with carrageenan is cost prohibitive, unless a wine recovery benefit over bentonite can be established. The wine recovery data for the V16 trial was inconclusive (Section 6.6) because racking wine is not a precise process and extrapolation from the pilot winery scale to the commercial scale would

require a greater number of vessel replications. If however, the wine recovery observed for the cold soluble kappa- carrageenan in Section 6.6 was repeatable and consistently achieved for the different types of carrageenan, then it is conceivable that carrageenan could be a cost effective fining agent, as a result of improved wine recovery relative to bentonite (*Table 6.5*).

Table 6.5. Wine cost = cost per L in which carrageenan can be used to be equivalent to using bentonite. Does not include cost of lees treatment/disposal/recovery from lees.

	% Wine lees	Volume (L) post racking from 1 kl	Wine cost CS kappa- (per L)	Wine cost pure kappa- (per L)	Wine cost kappa-/iota- (per L)
Control bentonite	**11.2%**	**888**			
Ferment carrageenan addition	**5.5%**	**945**	**0.8**	**0.54**	**0.44**

Understanding the compactability of carrageenan lees to improve wine recovery is a priority for future work, as carrageenans are unlikely to be cost effective unless a wine recovery saving can be identified (see also sedimentation studies of carrageenan in Section 7.4). Further sedimentation and compaction studies should be approached by observing the settling properties of carrageenan in conjunction with juice and wine lees, in order to mimic the production application.

It would also be useful as part of cost saving calculations if the amount of juice and wine absorbed as carrageenan lees were compared to that of bentonite. Barblet (2010) calculated that for every 1 kg of bentonite, 4.37 L of white wine was absorbed. The major costs of bentonite include the treatment of lees and the downgrading of wine that ensues (Majewski, et al., 2011). Wineries are currently moving away from using rotary drum vacuum (RDV) technologies, due to issues with product quality downgrades and waste. Carrageenan lees processing should be assessed with emerging technologies for processing lees, such as wide bore cross-flows, rotating ceramic cross-flows, centrifuges and decanters.

7. Polysaccharide compositional analysis

Polysaccharide analysis was applied to the carrageenans that were most effective at heat stabilising wine. Determining the most appropriate identification methods and characterising the most effective carrageenans for heat stabilising wine will allow the wine industry to ensure quality control of carrageenans in the heat stabilising process. It will also assist us in continuing to identify the most efficient carrageenans and to support future research in this area.

Key analytical parameters used for the identification and characterisation of carrageenans included percentage compositional analysis, sulfate analysis, molecular size distribution, characterisation of sedimentation and viscosity in wine, as well as ^{1}H-NMR spectrometry and polysaccharide linkage analysis. These parameters may be related to the solubility and gel properties of carrageenans in wine as well as their structural identification.

The degree of esterification, degree of amidation and galacturonic acid content were measured for five of the pectins from the screening process and were found to match the specifications of the manufacturer (Appendix 13.9, Appendix 13.10).

7.1. Percentage composition analysis

When purchasing commercial carrageenan products, the product information forms (PIF) will often describe the loss on drying or % moisture. It is important to ensure that the product is free of sugar or dextrose, which may be used as a bulking agent. The cation content should also be declared in the PIF, as cations can influence the functional properties of the carrageenan (Campo, et al., 2009).

The percentage compositional analysis is a good indication of the amount of long chained polysaccharides in the carrageenan sample, and is an important measure of product integrity. The remaining components include water, salts and sugars. *Table 7.0* describes the composition of the carrageenan samples, as determined by the method described in Section 4.2.1. These results indicate that the more efficient carrageenans that are capable of heat stabilising wine to less than 2 NTU turbidity have a total polysaccharide content of 95 % w/w or greater.

Table 7.0. The % composition of carrageenans tested for heat stabilising wine.

	Sample code (production year)	Carrageenan type	$CHO (%)
1.	Ca-A-367 (2014)	iota- / nu-	98%
2.	Ca-A-368 (2014)	lambda-	96%
3.	Ca-A-369 (2014)	kappa- (semi refined)	88%
4.	Ca-A-370 (2014)	iota- (semi refined)	89%
5.	Ca-A-371 (2014)	kappa-	96%
6.	Ca-A-372 (2014)	iota-/nu- (cold soluble)	97%
7.	Ca-A-CSW (2014)	kappa-, iota-, lambda- (sucrose 12%)	86%
8.	Ca-A-529 (2014)	lambda- (pure)	96%
9.	Ca-A-532 (2014)	kappa-, iota-, lambda- (cold soluble lambda-)	96%
10.	Ca-A-533 (2014)	kappa- (cold soluble)	98%
11.	Ca-A-978 (2015)	kappa-	97%
12.	Ca-B-1 (2014)	kappa- (dextrose 10%)	88%
13.	Ca-B-2 (2014)	kappa- (90%)/iota- (10%) blended	96%
14.	Ca-B-1R (2015)	kappa- (pure)	95%
15.	Ca-B-2R (2015)	kappa- (dextrose 10%)	88%
16.	Ca-B-3R (2015)	kappa- (90%)/iota- (10%) blended	98%

$ CHO= carbohydrate as total polysaccharide expressed as a % (w/w) and determined by the method described in *Section 4.2.1*.

7.2. Sulfate analysis

Sulfate groups are an important structural substituent of the galactan backbone of carrageenan, and are related to ionic interactions, as well as product solubility and rheology. Levels of sulfate were analysed in the kappa- and kappa-/iota- carrageenans (*Table 7.1*).

As kappa-, iota-, and lambda- carrageenans have one, two, or three ester groups, respectively, this would correspond stoichiometrically with sulfate contents of 20%, 33%, and 41 % (w/w), respectively, to their 'exact structures'. However, commercial kappa- carrageenan typically contains 22% w/w sulfate, while commercial iota- carrageenan contains 32% sulfate and commercial lambda- carrageenan contains 38% w/w. This indicates that carrageenan samples are not 'exact structures' and that small but real differences in quantities of sulfate esters for kappa-, iota-, and lambda- carrageenan samples occur, possibly due to small but significant variations in sulfate ester positions and different distributions along the galactan chain (De Ruiter, et al., 1997). Alternatively, the variation in % sulfate could be due to the presence of very tiny amounts of precursor material or low levels of another type of carrageenan structure

The sulfate content of the kappa- and kappa-/iota- carrageenans, determined by the barium chloride turbidimetric method, were 21.3% - 23.0% (w/w), which is consistent with sulfate levels typical of commercial carrageenans (Stevenson, 1991; Campo, 2009) (*Table 7.1*).

Table 7.1. Sulfate (% w/w) content of commercial kappa- and kappa-/iota- carrageenans used to heat stabilise white wine.

Sample code (production year)	Carrageenan Type	SO_3 (w/w %)
Ca-A-533 (2014)	kappa- (cold soluble)	21.28
Ca-B-2 (2014)	kappa- (90%)/iota- (10%) blended	22.79
Ca-B-1R (2015)	kappa- (pure)	22.5
Ca-A-980 (2015)	kappa- (cold soluble)	22.42
Ca-B-3R (2015)	kappa- (90%)/iota- (10%) blended	22.39
Ca-A-978 (2015)	kappa- (pure)	22.97

7.3. Molecular size distribution

Size-exclusion chromatography (SEC) is also known as gel permeation chromatography (GPC), and is used to measure the distributions of molecular sizes in order to make comparisons between the different carrageenans. SEC separates by hydrodynamic volume, (V_h), not by molecular weight. Carrageenans are single-chained polymers of similar conformation and are therefore described as linear. For linear polymers, there is a direct relationship between molecular weight (M) (and degree of polymerisation) and V_h. This is not the case for a branched polymer, as two polymer molecules with the same size can have different branching structures and thus different molecular weights (Vilapiano, et al., 2011). The size distributions for a pure kappa- carrageenan (Ca-A-978), cold soluble kappa- carrageenans (Ca-A-533 and Ca-A-980), and blended kappa-/iota- (Ca-B-2 and Ca-B-3R) carrageenans are shown in *Figure 7.0*. This describes the size of these carrageenans in terms of hydrodynamic volume *w* (log V_h), as a function of hydrodynamic radius R_h (related to V_h by $V_h = 4/3\ \pi\ R_h^3$). For linear polymers, how they fractionate is a product of their viscosity (weight-average viscosity) and molecular weight (number average molecular weight) (Gilbert, et al., 2011). There are no significant differences in molecular size distribution between the carrageenan samples, irrespective of the type, the supplier or the batch.

Figure 7.0 illustrates that the commercial carrageenans have similar hydrodynamic volumes.

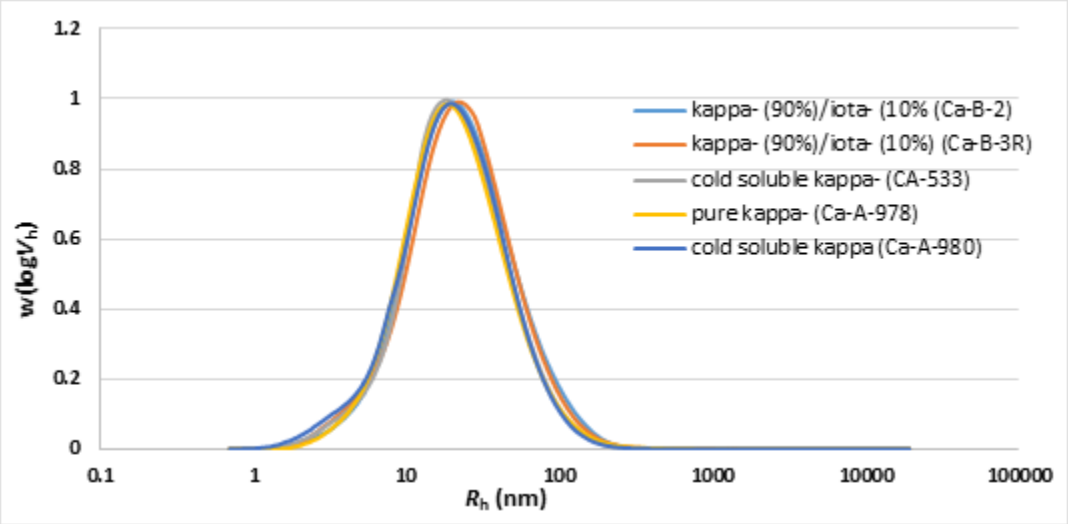


Figure 7.0. Molecular size distribution of commercially available kappa- and kappa-/iota- (blended) carrageenans.

7.4 Sedimentation

In a previous study on the ability of pectins to clarify beer, the clarification and sedimentation properties were found to be correlated with the degree of esterification (greater than 70%) and a specific molecular weight range (Duan, et al., 2008).

The same sedimentation test used in the beer study was repeated on a 2014 McLaren Vale Chardonnay wine that was clarified by centrifugation but not filtered (*Figure 7.1*). Unlike bentonite, which has excellent sedimentation properties, the carrageenans did not settle well but instead floated to the top of the measuring cylinder.

This suggests that carrageenans should be added at stages in the winemaking process that generate lees, such as during juice clarifying or before separation from yeast or tartrate lees. Natural juice or wine lees would facilitate sedimentation and removal of the carrageenan lees.

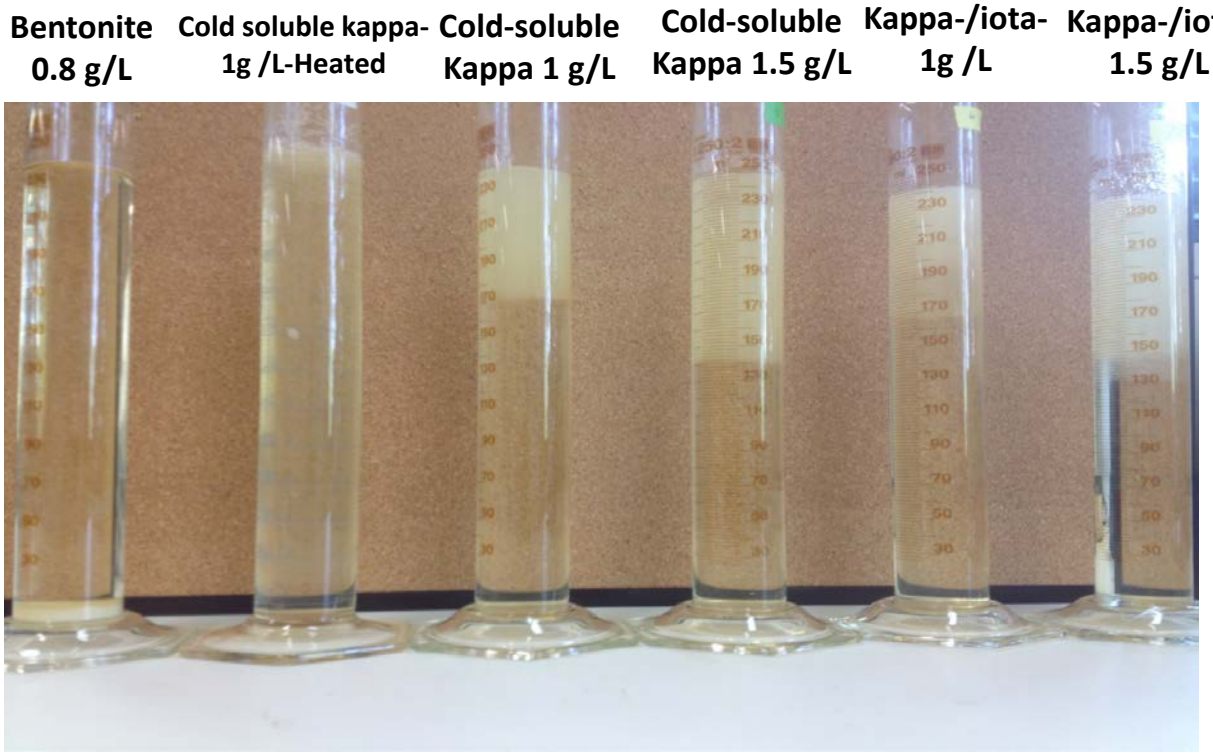


Figure 7.1. Sedimentation properties of kappa- (K^+), kappa- (Na^+), and kappa- (90%)/iota- (10%) blends of carrageenan using the sedimentation test on a 2014 McLaren Vale Chardonnay that was heat unstable but cold stable.

7.5 Viscosity measurements

The viscosity properties of carrageenans varied between water and wine (*Table 7.0*). This is significant because, although the viscosity of different types and blends of carrageenans in water is well understood by suppliers and described in the literature (Kara, et al., 2006), the viscosity of carrageenan in wine has not been previously characterised.

Cold soluble kappa- (K^+) carrageenan was described by suppliers and the literature (Kara, et al., 2006) as being soluble in cold water, unlike the potassium salt of the kappa- carrageenan, which quickly forms gels.

Table 7.0. Viscosity data is based on a 1-6 rating in a 25 g/L solution, where 1 = low viscosity and 6 = high viscosity for measurements conducted at room temperature.

Carrageenan	Viscosity in water	Viscosity in wine
Pure kappa- (K^+)	6	1
Cold soluble kappa- (Na^+)	4	3
Kappa- (90%)/iota- (10%) blended	6	1

However, in Table 7.2, cold soluble kappa- (Na^+) carrageenan is more viscous in wine than the corresponding (K^-) salt. This was also anecdotally observed in winemaking trials, as preparing the cold soluble kappa- carrageenan stock solution took longer to mix. The increased viscosity of cold soluble kappa-carrageenan in wine could make it more difficult to mix and disperse into the wine.

7.6. ^{1}H-NMR spectrometry

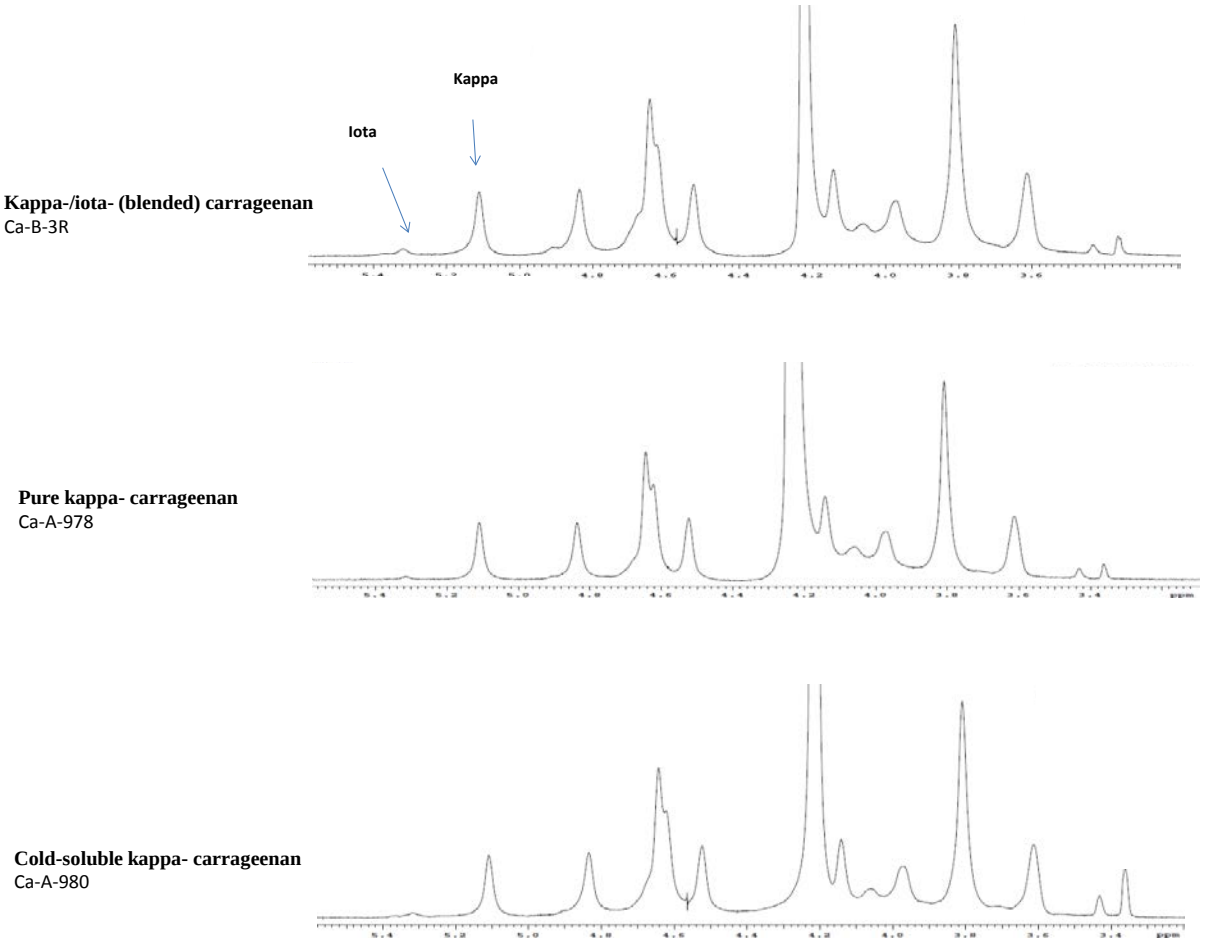
NMR allows for the identification of the types of carrageenans in a commercial blend of carrageenan fining agents. ^{1}H-NMR and ^{13}C-NMR have been reportedly used in identifying carrageenan types and possible contaminants (van de Velde, et al., 2002). However, in this study, only the ^{1}H-NMR was successful in producing clear spectra, due to difficulty in solubilising samples for ^{13}C-NMR.

Commercial carrageenan samples, including kappa- (K^+), cold soluble kappa- (Na^+), and kappa-/iota-carrageenans, were prepared for ^{1}H-NMR at a concentration of 1 % w/v and the spectra recorded at 80°C. The ^{1}H-NMR spectra of these carrageenans are presented in *Figure 7.2*, and the chemical shifts summarised in *Table 7.3*, assigned on the basis of literature values (van de Velde, et al., 2002).

Different carrageenan types in a sample are identified using ^{1}H-NMR by the presence of the α-anomeric protons in the regions from 5.1 to 5.7 ppm. The ^{1}H-NMR spectra of the kappa- carrageenan obtained from two different suppliers were typical of kappa- carrageenan, but a low intensity signal at 5.32 ppm was assigned to iota- carrabiose units, indicating that the commercial kappa- carrageenans contain some residual iota- carrageenans. This is not uncommon as 'pure' carrageenan classes are rare in nature.

Units typical of the biological precursor's mu- and nu- carrabiose units of kappa- and iota-carrageenans, respectively, were not detected. Absence of anomeric signals at 5.59 ppm indicated that none of the kappa-carrageenan consists of residual lambda- carrabiose units.

Figure 7.2. ^{1}H-NMR spectra of commercial carrageenans.



^{1}H-NMR is an effective single-step analytical method for identification of different types of carrageenans. Given that lambda- carrageenan is an undesirable additive in carrageenan samples intended for heat stabilising wine, ^{1}H-NMR — which can be used to detect its presence — is a valuable quality control tool.

Table 7.3. Proton (H^{1}) chemical shift for the main structural units of commercial carrageenans.

Carrageenan type	Unit	Sugar (Gal*p*)	Proton chemical shift (δppm)					
			H-1	H-2	H-3	H-4	H-5	H-6
κ-	G4S	3-linked	4.70	3.68	4.15	4.94	3.89	3.89
	DA	4-linked	5.17	4.22	4.61	4.73	4.68	4.30/4.16
ι-	G4S	3-linked	4.77	3.71	4.18	4.95	3.90	3.90
	DA2S	4-linked	5.35	4.77	4.86	4.78	4.78	4.53/4.21
λ-	D2S,6S	4-linked	5.59	-	-	-	-	-
μ-	D6S	4-linked	5.26	-	-	-	-	-
ν-	D2S,6S	4-linked	5.52	-	-	-	-	-

G-units = 3-linked β-D-galactopyranose; D-units= 4-linked α-D-galactopyranose; DA-units = 4-linked 3,6-anhydrogalactose.

7.8. Polysaccharide linkage analysis

Polysaccharide linkage analysis is a method by which the linkage patterns between individual monosaccharide units are determined and quantified by the molar ratio (Lau, et al., 1993). The linkage analysis of the commercially available carrageenans was determined by methylation and subsequent reductive hydrolysis (Stevenson, et al., 1991) (*Table 7.4*).

The key linkage patterns of the different types of carrageenans are the 3,4-linked Gal*p* and the 4-linked AnGal*p*, which are characteristically found in both the kappa- and iota- carrageenans. The 2,4-linked AnGal*p* was present in only minor quantities (<3%) and is a characteristic linkage type found only in iota-carrageenans. Absence of 2,3-Gal*p* and 2,4,6-Gal*p* linkages indicated that none of the kappa- carrageenans contain any lambda- carrabiose (G2S-D2S,6S) units diagnostic of lambda- carrageenans (*Figure 3.0*).

The low amounts of 2,4-AnGal*p* observed for the commercial kappa- (K^+) carrageenan (Ca-A-371), cold soluble kappa- (Na^+) carrageenan (Ca-A-533) and analytical grade pure kappa- carrageenan revealed that, although these kappa- carrageenans are described as 'pure' in the product information sheet, they do contain small amounts (2.2% - 2.4%) of iota- carrageenan. The commercial blended kappa- (90%)/iota- (10%) carrageenan (Ca-B-2) consists of 94.4 % kappa- and 5.6% iota- carrageenans when measured by linkage analysis. This differs from the product information sheets, which described the blend as 90% kappa- and 10% iota-; however, the method for determining these % types was not disclosed.

Table 7.4. Linkage composition of commercial carrageenan.

Commercial sample	Carrageenan letter code	Deduced glyosidic linkages (mol %)				
		3,4-Gal*p*	**4-AnGal*p***	**2,4-AnGal*p***	**2,3-Gal*p***	**2,4,6-Gal*p***
Ca-A-371 (kappa-)	G4S-DA	58.5	38.4	1.1	ND	ND
Ca-A-533 (cold soluble kappa-)	G4S-DA	55.3	40.3	1.1	ND	ND
Ca-B-2 (kappa-/iota-)	G4S-DA G4S-DA2S	57.7	37.3	2.8	ND	ND
Ca-Sigma (kappa-)	G4S-DA	59.4	37.2	1.2	ND	ND

G-units = 1,3-linked-β- D-Galp; DA-unit = 3,6-anhydro-D-Galp; S = sulfate ester groups; κ-kappa; ι-iota; Ca- = Carrageenan; A- = CP Kelco; B- = Cargill; ND = Not detected.

The linkage results are consistent with the ^{1}H-NMR spectral data obtained for these same kappa- and cold soluble kappa- carrageenans. ^{1}H-NMR spectra also detected residual levels of iota- carrageenans in the commercial kappa- (K^+) or (Na^+) carrageenan samples, by the presence of low intensity signals observed at 5.3 ppm assigned to anomeric protons of an iota- carrabiose (DA2S-H1) unit.

8. Quality control parameters for carrageenans used to heat stabilise wine

8.1. Type of heat test

There are two types of standard heat tests used in Australian wineries. Pocock, et al. (1973) developed a test that heated wine for 6 hr at 80°C. In 2006, a similar test was validated as suitable for predicting a wine's ability to become heat unstable, post-bottling, but the heating time was reduced from 6 hr to 2 hr (Pocock, et al., 2006).

Table 8.0 illustrates that the carrageenans can be used for either the 2 hr heat test or the 6 hr heat test without significant differences in the heat turbidity reading.

Table 8.0. Carrageenan tested using the 2 hr and 6 hr heat test on a 2014 Riverland Chardonnay wine.

Treatment	**2014 sample code**	**Rate (g/L)**	**NTU (Initial)**	**NTU (2hr test)**	**NTU (6hr test)**
Control		0	1.6	34.9	35
Bentonite		0.5	0.5	1.3	1.3
Kappa- carrageenan	Ca-A-371	0.1	1.5	21.5	21.7
		0.3	0.4	6.1	6.2
		0.6	0.4	8.2	8.5
		1.0	0.6	6	6
Cold soluble kappa-carrageenan	Ca-A-533	0.1	0.5	16.5	16.5
		0.3	0.7	8.2	8.3
		0.6	0.6	7	7.1
		1	0.7	5.4	5.4
Kappa- (90%)/iota- (10%)	Ca-B-2	0.1	0.9	16.2	16.2
		0.3	1.3	6.3	6.4
		0.6	1.6	4.3	4.4
		1	2.7	5	5

8.2. Impact of carrageenan on cold stabilisation and impact of over-fining of polysaccharide on wine turbidity

To determine whether carrageenan caused a haze during cold stabilisation, a heat unstable but cold stable wine was selected and carrageenan was added in quantities up to double what is required to reach a heat turbidity of less than 2 NTU. Wines were then heat tested and cold stability tested. Wines were determined to be heat stable and cold stable via visual testing, regardless of the fining rate (*Table 8.1*).

Table 8.1. Carrageenan tested at double the rate of polysaccharide required to heat stabilise to turbidity <2 NTU and impact on cold stability¥. Testing was completed using a 2015 McLaren Vale Chardonnay that was heat unstable but cold stable.*

Treatment	2014 Sample Code	Rate (g/L)	NTU (Initial)	NTU (2hr Test)	Cold Stable
Control		0	1	29.5	Pass
Bentonite		0.8	0.6	1	Pass
Kappa- carrageenan	Ca-A-371	0.5	0.8	11.6	Pass
		1	0.7	4.3	Pass
		2	0.6	1.9	Pass
		3	0.7	2.7	Pass
Cold soluble kappa- carrageenan	Ca-A-533	0.5	0.8	8.2	Pass
		1	0.7	4	Pass
		2	0.7	1.9	NT
		3	0.8	0.8	Pass
Kappa- (90%)/iota- (10%)	Ca-B-2	0.5	0.5	9.5	Pass
		1	0.8	4.5	Pass
		2	0.9	3.2	Pass
		3	1.1	2.7	Pass

**Cold stability was tested at -4°C for 72 hours. ¥ Wine was heat stable at 1.5 g/L of carrageenan.*

The cold stabilisation test of the wine after over-addition of fining agent was performed in order to ascertain whether any residual fining agent remains in the finished wine and if there is any subsequent sediment formed after cold storage at -4^{o}C for 72 hr. The cold storage testing demonstrated that the over-addition of carrageenans does not affect the cold stability as no sedimentation was observed after cold storage. Collective observation of over-addition and the cold stability tests suggested that the carrageenans did not pass through the filters and contribute to haze formation during storage. In contrast, Marangon et al. 2013 reported that the addition of increasing doses of cold soluble lambda-carrageenans developed haze during the heat test possibly due to some residual carrageenan that had passed through the filters (0.45µm). A possible explanation for this result is that the lambda- carrageenan due to its non-gelation properties did not settle and precipitate in the wine to facilitate its removal thus being at risk of remaining in the wine and passing through filtration.

8.3. Product information specifications

Carrageenan product intended for use as a processing aid in winemaking should be supported with a product information form (PIF) to ensure that it complies with the Australia New Zealand Food Standard Code. Additional information that should be requested along with the product specifications includes a Product Purity Certificate, in order to ensure integrity. The product specifications identified as important characteristics for heat stability are listed in *Table 8.2*.

Table 8.2. Product specifications required for use of carrageenan to heat stabilise wine.

Component	**Specification and Description**
% w/w polysaccharide composition analysis	To determine the amount of polysaccharide component. For winemaking purposes, this should be free of buffering sugars such as sucrose and dextrose. % polysaccharide composition should be greater than 95% w/w[1].
% carrageenan type i.e. % of kappa-, iota-, lambda-, mu-, nu- or theta- carrageenans	90% - 100% kappa- carrageenan[2] Up to 10% iota- carrageenan[3] For heat stabilising wine, no lambda- carrageenan should be present. Pure iota- carrageenans cannot heat stabilise wine.
Cation concentration	Concentration of K, Na, Ca, or Mg ions in mg/L. The cations present will influence product gelation properties, along with the ionic content of the wine.

[1] *As measured by the 'Total Polysaccharide Analysis' described in Section 4.2.1.*

[2] *Based on current results only, as testing more than 10%—but less than 'pure'—iota blends has not yet been completed.*

[3] *The commercial blended kappa- (90%)/iota- (10%) carrageenan described in this report consisted of 94 % kappa- and 6 % iota- carrageenans by polysaccharide linkage analysis.*

9. Proposed mechanism for heat stabilisation of white wine using carrageenans

As grape-derived proteins are positively charged at wine pH, the mechanism for heat stabilising juice and wine using carrageenans is likely to revolve around the interaction between the negatively charged sulfate substitutions and the positively charged protein (*Figure 9.0*).

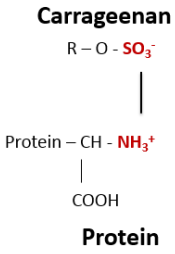


Figure 9.0. Interaction between positively charged protein and carrageenan.

Kappa- carrageenans and, to some extent, iota-type carrageenans form double helix or 'spiral noodle' type gels of which the sulfate groups are orientated toward the external part (*Figure 9.1*, Stage B). Kappa-carrageenans have the lowest negative charge per disaccharide unit, and therefore form the strongest helix, as there is less repulsion between sulfate ester groups. Lambda- carrageenans, which are more negatively charged per disaccharide unit than both kappa- and iota-, do not form helical structures due to the repulsion between the sulfate groups (Wu, et al., 2012). Consequently, in the food industry, lambda-carrageenan is used as a thickening agent, whereas kappa- and iota-carrageenans are used as gelling and stabilising agents.

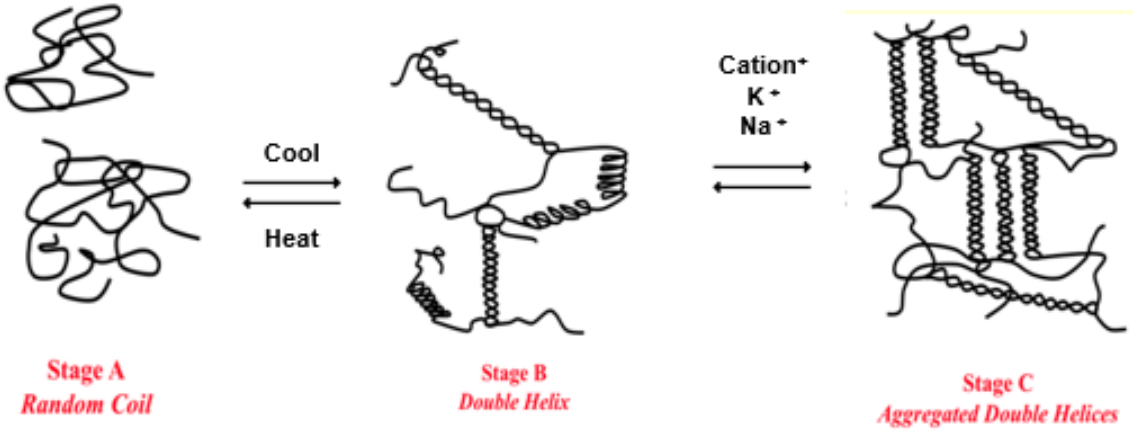


Figure 9.1. Double helix formation and cation dependent aggregation of kappa- and iota- carrageenan.

Following the formation of double helices, the kappa- and iota- carrageenan can form aggregates (*Figure 9.0*, Stage C). This process is mediated by strong interactions between the sulfate groups of neighbouring

helices and metal ions. Potassium (K^+), of which there is an abundance in wine, is considered more effective than other ions at inducing gelation (Campo, et al., 2009). Sodium (Na^+) promotes slower aggregations in water, and at low temperatures, which is why the Na^+ salts of carrageenans are referred to as cold soluble.

The mechanism for heat stabilisation of either juice or wine could be through the formation of an electrostatic complex between the negatively charged sulfate groups of neighbouring double helices in the kappa- or iota- carrageenan and positively charged wine proteins. The formation of the aggregated double helices in the high potassium environment of wine provides the gelation mechanism to precipitate the wine haze proteins along with the aggregated carrageenans through sedimentation.

10. Conclusions

Following a comprehensive and systematic screening of a range of commercially available pectin, carboxymethylcellulose and carrageenan type polysaccharides (*Table 5.0*, *Table 5.1*, *Table 5.2*), two types of carrageenan polysaccharide —the kappa- and a specific blend of kappa-/iota-[2] type carrageenans — were found to consistently heat stabilise white wines. The kappa- (Na^+) salt and the kappa- (K^+) salt were both able to heat stabilise wine although their viscosity in wine was different. Pure iota- and pure lambda-carrageenan types did not consistently heat stabilise wine and may cause filtration difficulties. In most cases, slightly lower quantities of carrageenan were required to heat stabilise the same wine compared to bentonite.

The cold soluble kappa- (Na^+) carrageenan gave high viscosity solutions in wine, which may make adding and mixing this type of carrageenan into the wine or juice more difficult. However, there was some evidence that this type of carrageenan compacts better in wine, which could lead to better wine recovery. Both a kappa-/iota- blend and the potassium salt kappa- (K^+) carrageenan were very effective at heat stabilising white wine. A possible mechanism responsible for the very good performance of kappa-/iota-blended carrageenan, particularly in the V15 winemaking trials (*Table 6.4*), may be that the increased amounts of iota- carrageenans could increase the solubility of the carrageenan and also increase the binding capacity to protein, due to the increased number of active SO_4^- group sites.

Unlike bentonite, carrageenans can be added at the juice or ferment stage of the winemaking process, as well as to the wine, post-fermentation, and still result in a heat stable wine without negatively impacting sensory qualities. Adding carrageenan to unclarified juice may be less reliable as, in V16, the juice addition stage wines were close but not quite heat stable, although the juice addition wines were heat stable in V15. There could be processing benefits through heat stabilising at stages in the winemaking process where separation from solids occurs, such as during racking or centrifugation. A potential benefit is that fewer tank movements are required, which would improve process flow.

Current evidence suggests that carrageenan must be used in conjunction with pectolytic enzyme to avoid commercially unviable filtration difficulties. The V16 pilot winery trial demonstrated that a wine derived from pectin negative juice and fined with carrageenan can be filtered using cross-flow technology.

In descriptive sensory evaluations of the wines made from the V15 pilot winemaking trials, using the cold soluble kappa- (Na+) and kappa-/iota- carrageenans had a positive impact on the sensory attributes on the Adelaide Hills Sauvignon Blanc wine and even improved the sensory properties of the wine compared to fining the same wine with bentonite. The bentonite wines had lower intensity of most sensory attributes compared to the untreated control. This suggested that using bentonite stripped the wine of certain flavour compounds. Several of the carrageenan treatments gave wines of higher intensity of tropical fruit-related attributes (such as box hedge, sweaty and passionfruit), as well as a higher viscosity mouthfeel attribute. In V16 descriptive analysis of the pure kappa- (K+) carrageenan wines compared to the bentonite and control (no fining agents), the bentonite fined wine likewise had the lowest intensity of fruity attributes.

Although the sensory aspects of carrageenans were favourable, the wines were more costly to make than the traditional process. Currently, bentonite costs approximately $1.50 - $2 per kg, whereas carrageenans can range in cost from $27 - $48 per kg.

[2] The commercial blended kappa- (90%)/iota- (10%) carrageenan consisted of 94 % kappa- and 6 % iota- carrageenans by polysaccharide linkage analysis.

11. Recommendations

If a winery wishes to use carrageenan to heat stabilise white wine, the outcomes from this project recommend using either a pure (K^+) kappa- carrageenan, a cold soluble (Na^+) kappa- carrageenan, or a kappa- (90%)/iota- (10%) blend[3]. A key benefit of using carrageenans to heat stabilise wine lies in the sensorial improvements relative to bentonite.

The sensorial differences between wines fined with carrageenan, bentonite or no fining agent could be explored in more detail by quantifying aroma molecule depletion or enhancement for various varietal wines treated with these different fining agents and a control (no fining agent). A comparative study of Gas Chromatography (GC) Mass Spectrometry (MS) profiles of volatile aroma compounds for bentonite- and carrageenan-treated wine could provide the details of desirable aroma or odour-active compounds that can be retained or removed after fining treatment and thereby contribute to the sensory profile of the various types of wines. The 2015 Adelaide Hills Sauvignon Blanc had distinctive tropical fruit and passionfruit aromas that were associated with the carrageenan-treated wines. It could therefore be of interest to quantify poly-functional thiol compounds that are associated with these flavour attributes to assess whether different fining treatments enabled the release of these compounds from their precursors in a sufficient concentration to affect wine sensory properties.

In the V15 pilot winemaking trial, the kappa-/iota- blend of carrageenan appeared to perform slightly better than the kappa- carrageenan, and anecdotally dissolved a little easier in the Adelaide Hills Sauvignon Blanc juice or wine. It is possible that the presence of some amount of iota-, and therefore an increased number of SO_4^- groups, also increases the solubility and binding sites, resulting in more efficient heat stability. Future research could determine whether a 'sweet spot' exists for the % amount of iota- in a kappa-/iota- blend that optimises the amount of carrageenan required to heat stabilise wine.

Although carrageenan can be added at the juice, ferment or wine stage, and still result in a heat stable wine, outcomes from the pilot winery trials indicated that adding carrageenan at the ferment stage more reliably heat stabilises the wine than juice stage carrageenan addition. Adding carrageenan at the juice or ferment stage of the winemaking process also heat stabilises at a lower rate than addition at the wine stage (*Table 6.4*). Adding carrageenan at the fermentation stage could result in better compaction of the lees and improve wine recovery. The author of this paper suggests adding carrageenan during yeasting or the early stages of fermentation when the juice has been warmed to 14 - 16°C. This temperature will promote both fermentation and dispersion of the carrageenan. Given that bench trials to determine the amount of carrageenan to add to a particular ferment take at least 6 hr to complete, conducting a bench trial for each ferment would not be practical. Most likely, the winery would have to determine during each vintage a standard rate addition per variety and region, ascertained through practice and time. For example, 0.8 g/L for cool climate Chardonnay, 1.2 g/L for Sauvignon Blanc, etc. This could, however, fall short of completely heat stabilising the wine if a more protein-rich batch went through, which would hence require a 'top up' or a small addition of carrageenan post-fermentation to heat stabilise (Stockdale, 2016).

The in-line injection of carrageenans during juice flotation or juice centrifugation should also be considered. It would be interesting to evaluate whether carrageenans can undertake a dual role of heat stabilisation as well as promoting clarification. The application of in-line injection of carrageenan in flotation or centrifugation would be interesting to determine, once commercial trials are permitted.

[1] The commercial blended kappa- (90%)/iota- (10%) carrageenan consisted of 94 % kappa- and 6 % iota- carrageenans by polysaccharide linkage analysis.

Future research also needs to focus on how to effectively add the carrageenan to the juice or wine tank. As a result of gel formation, carrageenan can only be added in up to a 2% w/v solution. In the pilot-scale trials, this meant that 12% of the tank volume constituted the buffer volume used to mix carrageenan. This may not be practical in the very large 100 kL plus tanks occasionally used in commercial winemaking. Other physical methods for adding carrageenan may need to be explored, such as the use of an eductor system to promote mixing and dispersion of the carrageenan powder.

Another approach to improving the solubility of carrageenan could be to better understand the chemical factors that would aid carrageenan to dissolve and disperse in wine. The solubility of carrageenans depends upon their sulfate content (very hydrophilic) and the associated ionisable cations (Na^+, $K^{+,}$ Mg^{2+} and Ca^{2+}) in the wine. Both the sulfate content and its distribution on the polysaccharide backbone, together with the accompanying cations in the solution, determine the viscosity of the solution and the strength of the gel. Furthermore, the viscosity of carrageenans depends on concentration, temperature, presence of other solutes and the molecular weight of the carrageenan polymers. Kappa- carrageenans form harder gels in water compared to the gels they form in wine, suggesting that the ionic composition and other compounds in juice or wine may play a role in solubility. Comprehensive studies of cations and other compounds in juice and wine (or control model wine) that interact with the carrageenan fining agents, as well as the varying molecular weight of the carrageenan polymers are suggested in order to understand the conditions needed to improve carrageenan solubility. Such studies could develop an efficient delivery of 'active' carrageenans to bind to the targeted protein molecules.

A negative impact of fining with either bentonite or carrageenan is that the fining process produces lees solids that will require further treatment and disposal. Ideally, fining lees should be as compact as possible and absorb the lowest possible volume of juice or wine. Understanding the compactability of carrageenan lees to improve wine recovery is also a priority for future work, as carrageenans are unlikely to be cost effective unless a wine recovery saving can be identified. Further sedimentation and compaction studies should be approached by observing the settling properties of carrageenan in conjunction with juice and wine lees as the presence of lees, aids the settling and separation of the carrageenan. It would also be useful if the amount of juice and wine absorbed as carrageenan lees was compared to that of bentonite. One study, (Barblett, 2010) estimated that, for every 1 kg of bentonite used in winemaking, 4.6 L of wine is absorbed into bentonite lees.

Processing wine lees poses a challenge and a cost, as current methods to recover juice or wine result in downgrading of product due to oxidation and waste, as not all of the juice or wine can be recovered from the lees solids. A key challenge, therefore, in the adoption of carrageenan as a substitute for bentonite, is to reduce the amount of lees generated relative to bentonite.

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13. Appendices

Appendix 13.0. Oenological parameters and general composition of wine used in the heat-induced haze formation experiments. YAN = Yeast assimilable nitrogen; G = Glucose; F = Fructose ; *(Heat unstable / cold stable) wine; #Heat haze without fining agent.

Wine and region	**Chardonnay (Adelaide Hills)**	**Chardonnay (Riverland)**	**Chardonnay (McLaren Vale) ***	**Riesling (Barossa)**	**Chardonnay (Padthaway)**	**Pinot Gris (Langhorne Creek)**	**Sauvignon Blanc (Robe)**
Vintage	**2013**	**2014**	**2014**	**2015**	**2015**	**2015**	**2015**
Heat haze (NTU)#	41.5	34.6	29.5	38.8	27.4	24.7	40.0
Alcohol (% v/v)	12.75	13.65	12.92	13.43	13.41	13.56	13.15
Baume	-	-	0	0	0	0.1	0.1
G+F (g/L)	0.4	0.5	0.11	3.28	0	0.4	0.26
pH	3.44	3.25	3.35	3.15	3.42	3.29	3.31
Total acidity (g/L)	5.3	6.98	5.66	7.73	7.84	6.42	6.04
Volatile acidity (g/L)	0.09	0.24	0.18	0.41	0.15	0.2	0.2
YAN	-	192					
Free SO_2 (mg/L)	68	18	27	20	0	34	1
Total SO_2 (mg/L)	151	92	107	112	45	76	71
Bentonite fining (g/L)	1.2	0.8	0.8	1.2	0.8	0.6	1.4
Total protein (µg/ml)	31.5	31.9	-	27.2	24.1	21.4	27.4

Appendix 13.1. Turbidity, heat test, and protein data for treatment TWE1 - TWE33 wines for the V15 Adelaide Hills Sauvignon Blanc wines.

Treatment code	Supplier code	Polysaccharide	Rep	Fining agent conc	Pre-bottling NTU	Pre-bottling heat test	Post-bottling heat test	Pre-bottling protein conc	Post-bottle protein conc
Polysaccharide juice additions									
TWE1	CA-533	Cold soluble kappa- (Na^+) carrageenan	1	1.2	245	4.05	0.23	10.7	2.9
TWE2			2	1.2	192	2.25	0.04	13.7	3.2
TWE3			3	1.2	195	4.9	1.86	12.2	2.24
TWE4	CB-2	Kappa-(90%)/iota-(10%) carrageenan	1	1.2 (1.0)	148	2.76	0.19	10.5	2.23
TWE5			2	1.2 (1.0)	160	1.9	1.43	10.6	2.76
TWE6			3	1.2 (1.0)	165	6.5	1.22	14.4	3.77
TWE7	PD-902	Low methoxy citrus pectin	1	1.5	88	34.3	20.83	17.9	-
TWE8			2	1.5	132	44.4	28.24	18.7	-
TWE9			3	1.5	119	44.6	35.3	18.1	-
Polysaccharide juice additions									
TWE10	CA-533	Cold soluble kappa- (Na^+) carrageenan	1	1.2	80.3	0.3	0.08	4.3	2.6
TWE11			2	1.2	72.2	0.32	0.14	3.5	2.5
TWE12			3	1.2	80.1	0.44	0.11	4.1	2.58
TWE13	CB-2	Kappa-(90%)/iota-(10%) carrageenan	1	1.2 (1.0)	44.6	0.04	0.07	2.4	2.08
TWE14			2	1.2 (1.0)	49.6	0.05	0.02	2.9	2.53
TWE15			3	1.2 (1.0)	69.1	0.06	0.01	2.8	2.94
TWE16	PD-902	Low methoxy citrus pectin	1	1.5	36.4	8.32	5.45	11.7	-
TWE17			2	1.5	35.2	9.39	5.01	11.5	-
TWE18			3	1.5	22.9	8.81	4.53	11.4	-
Polysaccharide wine additions									
TWE19	CA-533	Cold soluble kappa- (Na^+) carrageenan	1	1.4	12.3	0.09	0.07	4.7	4.1
TWE20			2	1.4	16.0	0.04	0.06	3.7	4.2
TWE21			3	1.4	8.58	0.03	0	3.6	3.64
TWE22	CB-2	Kappa-(90%)/iota-(10%) carrageenan	1	1.0	3.33	0.22	0.07	3.6	4.08
TWE23			2	1.0	29.9	0.01	0.03	3.8	3.52
TWE24			3	1.0	7.32	0.01	0.13	3.6	3.15
TWE25	PD-902	Low methoxy citrus pectin	1	1.75	42.0	32.6	29.84	32	-
TWE26			2	1.75	57.3	31.5	28.59	21.2	-
TWE27			3	1.75	49.6	31.3	26.85	20.9	-
Controls									
TWE28	No fining agent		1	0	58.3	58.3	44.0	40.94	35.5
TWE29			2	0	83.6	83.6	41.4	38.64	34.1
TWE30			3	0	63.4	63.4	41.7	33.94	34
TWE31	Bentonite		1	1.4	19.3	19.3	0.03	0	3.5
TWE32			2	1.4	24.6	24.6	0.11	0.02	2.6
TWE33			3	1.4	97.9	97.9	0.13	0.08	4

Appendix 13.2. Wine analytical chemistry analysis for treatments TWE1 - TWE33 wines for the V15 Adelaide Hills Sauvignon Blanc wines.

Treatment code	Supplier code	Polysaccharide	Rep	Alc	Gluc / fruc	pH	FSO2	TSO2	TA	VA
Polysaccharide juice additions										
TWE1	CA-533	Cold soluble kappa- (Na^+) carrageenan	1	13.2	0.4	3.25	43	132	6.8	0.25
TWE2			2	13.1	0.4	3.25	42	128	6.7	0.25
TWE3			3	12.4	0.3	3.26	45	133	7.3	<0.25
TWE4	CB-2	Kappa-(90%)/iota- (10%) carrageenan	1	13.3	0.5	3.25	43	130	7.2	0.25
TWE5			2	13.3	0.5	3.25	41	131	6.8	<0.25
TWE6			3	12.8	0.4	3.27	43	131	6.5	0.26
TWE7	PD-902	Low methoxy citrus pectin	1	13.2	0.6	3.28	44	133	6.9	0.26
TWE8			2	13.2	0.5	3.3	45	131	6.9	0.25
TWE9			3	13.2	0.5	3.26	47	132	7.2	0.25
Polysaccharide ferment additions										
TWE10	CA-533	Cold soluble kappa- (Na^+) carrageenan	1	13	0.5	3.26	41	126	7	<0.25
TWE11			2	13.1	0.5	3.27	39	130	7.1	<0.25
TWE12			3	12.8	0.5	3.24	38	129	7.1	<0.25
TWE13	CB-2	Kappa-(90%)/iota- (10%) carrageenan	1	13.1	0.3	3.24	38	128	7.1	<0.25
TWE14			2	13.1	0.4	3.24	37	126	7.1	0.25
TWE15			3	13.1	0.5	3.27	37	127	7.1	<0.25
TWE16	PD-902	Low methoxy citrus pectin	1	12.6	0.4	3.27	39	131	7	<0.25
TWE17			2	13.1	0.5	3.27	38	127	7.1	<0.25
TWE18			3	13.1	0.4	3.28	41	129	7.1	<0.25
Polysaccharide wine additions										
TWE19	CA-533	Cold soluble kappa- (Na^+) carrageenan	1	13.1	0.4	3.27	38	125	6.9	<0.25
TWE20			2	13.1	0.4	3.27	39	128	6.9	0.27
TWE21			3	13.1	0.5	3.25	39	128	7	0.27
TWE22	CB-2	Kappa-(90%)/iota- (10%) carrageenan	1	13.1	0.5	3.25	35	123	6.9	0.27
TWE23			2	13	0.5	3.25	44	131	6.7	0.28
TWE24			3	13	0.4	3.25	40	126	6.8	0.27
TWE25	PD-902	Low methoxy citrus pectin	1	13.1	0.5	3.3	40	129	6.9	0.3
TWE26			2	13.1	0.5	3.32	40	128	6.9	0.3
TWE27			3	13	0.5	3.31	39	125	7	0.28
Controls										
TWE28	No fining agent		1	13	0.5	3.27	39	128	7	0.28.
TWE29			2	13.1	0.5	3.27	40	126	7.2	0.31
TWE30			3	13.1	0.4	3.27	41	129	7.2	0.31
TWE31	Bentonite		1	12.8	0.3	3.29	40	124	6.7	0.25
TWE32			2	12.8	0.3	3.28	40	124	6.7	0.28
TWE33			3	12.8	0.4	3.29	38	125	6.8	0.28

Appendix 13.3. Turbidity, Heat Test and Protein data for Treatment TWE1 – TWE33 wines for the V16 Adelaide Hills Sauvignon Blanc wines.

Treatment code	Supplier code	Polysaccharide	Rep	Fining agent conc	Pre-bottling NTU	Pre-bottling heat test	Post-bottling heat test	Pre-bottling protein conc	Post-bottle protein conc
Polysaccharide juice additions									
TWE1	C-A-978	Kappa (K^+)	1	1.2	4.00	2.84	3.57	7.04	8
TWE2			2	1.2	392	0.71	0.71	4.31	4.2
TWE3			3	1.2	10.1	2.57	3.20	4.83	8.5
TWE4	C-A-980	Kappa (Na^+)	1	1	2.31	0.04	0.00	3.89	1.8
TWE5			2	1	4.80	2.08	3.52	4.72	7.8
TWE6			3	1	1.80	3.31	4.12	5.34	7.5
TWE7	C-B-3R	Kappa-(90%)/iota-(10%)	1	1	15.1	0.10	0.21	3.68	4
TWE8			2	1	4.31	2.53	2.27	5.14	5.5
TWE9			3	1	3.72	1.38	1.24	4.39	4
Polysaccharide ferment additions									
TWE10	C-A-978	Kappa (K^+)	1	1.2	4.25	0.26	0.0	3.82	2.3
TWE11			2	1.2	4.85	0.18	0.43	3.93	1.9
TWE12			3	1.2	2.88	0.44	0.0	3.36	2
TWE13	C-A-980	Kappa (Na^+)	1	1	3.99	0.18	0.10	3.9	1.7
TWE14			2	1	3.46	0.30	0.0	3.2	1.9
TWE15			3	1	5.49	0.49	0.0	3.71	1.8
TWE16	C-B-3R	Kappa-(90%)/iota-(10%)	1	1	4.60	0.32	0.09	3.78	1.9
TWE17			2	1	4.33	0.31	0.30	3.8	2
TWE18			3	1	7.91	0.15	0.0	4.24	1.8
Polysaccharide wine additions									
TWE19	C-A-978	Kappa (K^+)	1	1.4	10.6	1.25	0.15	4.31	2.1
TWE20			2	1.4	9.16	0.96	1.29	4.73	2
TWE21			3	1.4	10.0	0.55	0.14	4.67	1.9
TWE22	C-A-980	Kappa (Na^+)	1	1.4	10.5	0.76	0.77	4.79	2.5
TWE23			2	1.4	15.0	0.77	0.13	4.23	1.9
TWE24			3	1.4	10.2	0.63	0.0	4.52	2
TWE25	C-B-3R	Kappa-(90%)/iota-(10%)	1	1.4	18.9	0.64	0.72	4.54	2.1
TWE26			2	1.4	23.8	1.26	0.0	21.25	23
TWE27			3	1.4	20.8	1.60	0.17	21.12	25
Controls									
TWE28	No fining agent		1	0	12.6	75.94	75.7	2.99	1
TWE29			2	0	12.1	76.80	94.9	2.95	0.7
TWE30			3	0	14.1	81.40	104.6	2.75	1
TWE31	Bentonite		1	1.5	2.23	0.04	0.20	7.04	8
TWE32			2	1.5	1.57	0.00	0.0	4.31	4.2
TWE33			3	1.5	2.25	0.06	0.03	4.83	8.5

Appendix 13.4. Wine analytical chemistry analysis for treatments TWE1 - TWE33 wines for the V16 Adelaide Hills Sauvignon Blanc wines.

Treatment code	Supplier code	Polysaccharide	Rep	Alc	Gluc / fruc	pH	FSO2	TSO2	TA	VA
Carrageenan juice additions										
TWE1	C-A-978	Kappa (K^+)	1	13.5	1	3.1	40	106	6.3	0.39
TWE2			2	13.5	1	3.09	45	113	6.2	0.31
TWE3			3	13.5	0.4	3.11	42	117	6.5	0.34
TWE4	C-A-980	Kappa (Na^+)	1	13.5	1.2	3.09	43	111	6.3	0.28
TWE5			2	13.4	1.6	3.11	33	104	6.4	0.33
TWE6			3	13.5	1.6	3.1	33	102	6.4	0.35
TWE7	C-B-3R	Kappa- (90%)/iota-(10%)	1	13.5	1.2	3.1	34	100	6.3	0.27
TWE8			2	13.5	1.4	3.1	42	113	6.3	0.31
TWE9			3	13.5	1.3	3.11	45	116	6.2	0.3
Carrageenan ferment additions										
TWE10	C-A-978	Kappa (K^+)	1	13.5	0.8	3.13	42	120	6.3	0.34
TWE11			2	13.5	0.7	3.12	41	120	6.4	0.34
TWE12			3	13.5	0.9	3.11	44	124	6.4	0.34
TWE13	C-A-980	Kappa (Na^+)	1	13.5	1.1	3.1	46	127	6.5	0.31
TWE14			2	13.5	1.1	3.09	46	125	6.5	0.31
TWE15			3	13.5	0.9	3.1	45	124	6.5	0.31
TWE16	C-B-3R	Kappa- (90%)/iota-(10%)	1	13.5	1	3.11	42	119	6.5	0.31
TWE17			2	13.5	0.9	3.11	44	120	6.5	0.32
TWE18			3	13.5	0.9	3.11	46	124	6.6	0.32
Carrageenan wine additions										
TWE19	C-A-978	Kappa (K^+)	1	13.5	1.5	3.14	38	118	6.2	0.34
TWE20			2	13.5	1.5	3.13	39	119	6.2	0.35
TWE21			3	13.4	1.6	3.14	38	119	6.3	0.34
TWE22	C-A-980	Kappa (Na^+)	1	13.4	1.5	3.12	39	119	6.5	0.33
TWE23			2	13.5	1.4	3.11	37	114	6.4	0.34
TWE24			3	13.4	1.5	3.13	40	120	6.5	0.34
TWE25	C-B-3R	Kappa- (90%)/iota-(10%)	1	13.5	1.4	3.13	37	113	6.4	0.31
TWE26			2	13.5	1.7	3.14	37	116	6.4	0.34
TWE27			3	13.5	1.4	3.13	38	114	6.4	0.34
Control										
TWE28	No fining agent		1	13.4	1.5	3.11	36	113	6.4	0.37
TWE29			2	13.5	1.6	3.11	39	122	6.5	0.38
TWE30			3	13.4	1.4	3.11	39	119	6.5	0.37
TWE31	Bentonite		1	13.3	1.4	3.11	35	116	6.3	0.34
TWE32			2	13.3	1.4	3.11	37	117	6.3	0.33
TWE33			3	13.3	1.5	3.11	37	117	6.3	0.33

Appendix 13.5. Metal analysis for treatments TWE1 - TWE33 wines for the V15 Adelaide Hills Sauvignon Blanc wines.

Supplier code	***Polysaccharide***	***Addition Stage***	***K***	***Na***	***Ca***	***Cu***	***Fe***	***Zn***	***Mn***
CA-533	*Cold soluble kappa- (Na^+) carrageenan*	*Juice*	507 bc	108 c	56 c	0.2 abc	1.8 ab	0.8 a	0.5 bc
CA-533	*Cold soluble kappa- (Na^+) carrageenan*	*Ferment*	482 c	102 c	62 bc	0.2 bcd	1.7 b	0.8 a	0.5 b
CA-533	*Cold soluble kappa- (Na^+) carrageenan*	Wine	479 c	113 c	60 bc	0.2 d	1.8 ab	0.8 a	0.5 b
CB-2	*Kappa- (90%)/iota- (10%) carrageenan*	*Juice*	515 bc	43 ef	62 bc	0.2 abcd	2.1 abcd	0.8 a	0.5 ab
CB-2	*Kappa- (90%)/iota- (10%) carrageenan*	*Ferment*	539 bc	44 e	63 b	0.2 cd	1.8 a	0.8 a	0.5 abc
CB-2	*Kappa- (90%)/iota- (10%) carrageenan*	*Wine*	516 bc	41 ef	61 bc	0.2 bcd	1.8 ab	0.8 a	0.5 ab
PD-902	*Low methoxy citrus pectin*	*Juice*	526 b	131 b	49 d	0.3 a	1.9 a	0.6 b	0.4 bc
PD-902	*Low methoxy citrus pectin*	*Ferment*	518 bc	140 b	46 de	0.2 bcd	1.4 bcd	0.4 b	0.4 c
PD-902	*Low methoxy citrus pectin*	*Wine*	538 b	156 a	39 e	0.2 abcd	1.3 abcd	0.5 bc	0.4 c
No fining agent		-	600 a	31 f	65 ab	0.3 ab	1.9 ab	0.9 a	*0.6* a
Bentonite		*Wine*	488 bc	58 d	70 a	0.2 ab	2.0 bcd	0.8 a	*0.6* a

Mean values are shown (n=3). Within each row, means followed by a different lower case letter are significantly different (p≤0.05), according to the Turkey-Kramer test.

Appendix 13.6. Metal analysis for treatments TWE1 - TWE33 wines for the V16 Adelaide Hills Sauvignon Blanc wines.

Supplier code	***Polysaccharide***	***Addition stage***	***K***	***Na***	***Ca***	***Cu***	***Fe***	***Zn***	***Mn***
C-A-978	*Kappa- (K^+) carrageenan*	*Juice*	421 ab	25 e	83 ab	0.0 b	0.3 ab	1.4 bc	1.3 a
C-A-978	*Kappa- (K^+) carrageenan*	*Ferment*	400 abc	24 e	78 abcd	0.0 b	0.2 ab	1.5 abc	1.0 a
C-A-978	*Kappa- (K^+) carrageenan*	*Wine*	440 a	26 e	74 cde	0.0 b	0.1 b	1.5 a	1.3 a
C-A-980	*Cold soluble kappa- (Na^+) carrageenan*	*Juice*	363 bc	82 b	69 def	0.1 a	0.5 ab	1.4 abc	1.3 a
C-A-980	*Cold soluble kappa- (Na^+) carrageenan*	*Ferment*	341 c	82 b	65 fg	0.0 ab	0.2 ab	1.3 c	1.2 a
C-A-980	*Cold soluble kappa- (Na^+) carrageenan*	*Wine*	367 bc	102 a	57 cde	0.0 ab	0.1 b	1.4 abc	1.2 a
C-B-3R	*Kappa- (90%)/iota- (10%) carrageenan*	*Juice*	375 bc	26 e	82 abc	0.1 ab	0.8 ab	1.3 abc	1.4 a
C-B-3R	*Kappa- (90%)/iota- (10%) carrageenan*	*Ferment*	418 ab	26 de	85 a	0.0 b	0.2 ab	1.5 abc	1.3 a
C-B-3R	*Kappa- (90%)/iota- (10%) carrageenan*	*Wine*	438 a	29 d	80 abc	0.0 b	0.2 ab	1.5 ab	1.2 a
No fining agent		-	393 abc	16 f	69 ef	0.0 b	0.1 b	1.5 ab	1.3 a
Bentonite		*Wine*	346 c	40 c	76 bcde	0.0 b	0.9 a	1.5 abc	1.4 a

Mean values are shown (n=3). Within each row, means followed by a different lower case letter are significantly different (p≤0.05), according to the Turkey-Kramer test.

Appendix 13.7. Filterability testing of TWE1 - TWE33 wines for the V15 Adelaide Hills Sauvignon Blanc wines.

Treatment code	Supplier code	Polysaccharide	Rep	FI	Result
TWE1	CA-533	Cold soluble kappa-(Na^+) carrageenan	1	10.0	Fail
TWE2			2	3.0	Fail
TWE3			3	8.0	Fail
TWE4	CB-2	Kappa- (90%)/iota-(10%) carrageenan	1	3.0	Fail
TWE5			2	8.0	Fail
TWE6			3	3.0	Fail
TWE7	PD-902	Low methoxy citrus pectin	1	4.0	Fail
TWE8			2	3.5	Fail
TWE9			3	0.9	Invalid
TWE10	CA-533	Cold soluble kappa-(Na^+) carrageenan	1	3.4	Fail
TWE11			2	2.8	Fail
TWE12			3	30.0	Fail
TWE13	CB-2	Kappa- (90%)/iota-(10%) carrageenan	1	4.0	Fail
TWE14			2	15.0	Fail
TWE15			3	25.0	Fail
TWE16	PD-902	Low methoxy citrus pectin	1	2.5	Fail
TWE17			2	1.0	Pass
TWE18			3	1.2	Pass
TWE19	CA-533	Cold soluble kappa-(Na^+) carrageenan	1	1.1	Pass
TWE20			2	1.1	Pass
TWE21			3	1.1	Pass
TWE22	CB-2	Kappa- (90%)/iota-(10%) carrageenan	1	1.1	Pass
TWE23			2	1.1	Pass
TWE24			3	1.2	Pass
TWE25	PD-902	Low methoxy citrus pectin	1	1.5	Pass
TWE26			2	1.6	Pass
TWE27			3	1.4	Pass
TWE28	No fining agent		1	1.3	Pass
TWE29			2	1.2	Pass
TWE30			3	1.1	Pass
TWE31	Bentonite		1	1.1	Pass
TWE32			2	1.1	Pass
TWE33			3	1.1	Pass

Wine was filtered through a pad filter, followed by a 1µm cartridge filter. The filterability test was undertaken on a 0.45µm filter. A filterability index (FI) >2 failed the filterability test.

Appendix 13.8. Filterability testing of TWE1 - TWE33 wines for the V15 Adelaide Hills Sauvignon Blanc wines.

Treatment code	Supplier code	Polysaccharide	Rep	FI	Result
TWE1	C-A-978	Kappa (K^+)	1	1.2	Pass
TWE2			2	1.1	Pass
TWE3			3	1.0	Pass
TWE4	C-A-980	Kappa (Na^+)	1	1.2	Pass
TWE5			2	1.2	Pass
TWE6			3	1.2	Pass
TWE7	C-B-3R	Kappa- (90%)/iota-(10%)	1	1.2	Pass
TWE8			2	1.2	Pass
TWE9			3	1.2	Pass
TWE10	C-A-978	Kappa (K^+)	1	1.0	Pass
TWE11			2	1.1	Pass
TWE12			3	1.2	Pass
TWE13	C-A-980	Kappa (Na^+)	1	1.3	Pass
TWE14			2	1.2	Pass
TWE15			3	1.1	Pass
TWE16	C-B-3R	Kappa- (90%)/iota-(10%)	1	1.1	Pass
TWE17			2	1.2	Pass
TWE18			3	1.1	Pass
TWE19	C-A-978	Kappa (K^+)	1	1.0	Pass
TWE20			2	1.0	Pass
TWE21			3	1.2	Pass
TWE22	C-A-980	Kappa (Na^+)	1	1.1	Pass
TWE23			2	1.2	Pass
TWE24			3	1.3	Pass
TWE25	C-B-3R	Kappa- (90%)/iota-(10%)	1	1.0	Pass
TWE26			2	1.1	Pass
TWE27			3	1.2	Pass
TWE28	No fining agent		1	1.2	Pass
TWE29			2	1.1	Pass
TWE30			3	1.4	Pass
TWE31	Bentonite		1	1.1	Pass
TWE32			2	1.3	Pass
TWE33			3	1.2	Pass

Wine was filtered through a titanium cross-flow filter (0.2 µm). The filterability test was undertaken on a 0.45µm filter. A filterability index (FI) >2 failed the filterability test.

Appendix 13.9. The composition (manufacturer supplied specification) and heat stability of pectin tested for 2014 Riverland Chardonnay wine.

	Code	Pectin type	GalA (%)	DE (%)	DA (%)	*Viscosity index	#Rate (g/L)	Initial haze NTU	Final haze NTU
1.	P-A-373	LM	87	10	-	2	1.70	0.5	14.7
2.	P-A-374	LM	95	30	-	5	1.65	0.5	11.0
3.	P-A-384	HM	94	70	-	4	1.75	1.0	23.6
4	P-D-701	LM, Apple	82	38	-	4	1.70	0.7	15.8
5	P-D-910	LM, Apple	75	4.1	-	2	1.65	0.6	14.0
6.	P-D-005	LM, Citrus, Amidated	88	40	10	3	1.75	0.7	26.9
7	P-D-025	LM, Citrus, Amidated	89	30	20	3	1.75	0.8	12.2
8.	P-D- 04	HM, Citrus	80	59	-	3	1.75	0.9	12.5
9.	P-D-902	LM, Citrus	78	6.1	-	2	1.75	0.6	6.2
10.	P-B-1	HM, Orange, Dextrose (15%)	82	68		3	1.6	7.6	17.4
11.	P-B-2	HM, Lime, Dextrose (15%)	85	70	-	3	1.65		27.0
12	P-B-1880CN				-	2	1.75	7.8	28.0
13	P-B-2880CN				-	5	1.75	4.0	19.5
14	P-S-9135	LM, Citrus	84	9.4	-	2	1.75	3.5	28.0
15.	P-S-1879	Sodium salt/poly pectate, Orange	85		-	2	1.75	0.7	12.3
16.	P-S-2157	LM, Apple	77	7	-	2	1.75	0.7	21.9

P- = Pectin; A- = CP Kelco; B- = Cargill; C- = IMCD; D- = Herbstreith & Fox; S- = Sigma; GalA = Galacturonic acid; DE = Degree of methyl esterification; DA = Degree of amidation; LM = Low methyl esterification (<50 DE); HM = High methyl esterification (>50 DE);

*Viscosity index data is based on a 1-6 rating of 1.5% solution (15g/L) in water, where 1 = low viscosity (liquid) and 6 = high viscosity (solid) at room temperature; #Rate= Highest addition rate;

$ = did not dissolve in water.

Appendix 13.10. Composition of pectins tested for heat stability of 2014 Riverland Chardonnay wine.

Batch code	Pectin	Source	Parameter	Manufacturer Supplied Specification	Experimentally Determined
TS-373	Low methoxy	Unknown	Degree of esterification (DE)	10%	11%
			Degree of amidation (DM)	-	-
			Galacturonic acid (GalA)	87%	83%
CU-025	Amidated	Citrus	Degree of esterification (DE)	30%	36%
			Degree of amidation	20%	Not tested
			Galacturonic acid (GalA)	89%	85.3%
CU-L-004/14	High methoxy	Citrus	Degree of esterification (DE)	59%	66%
			Degree of amidation (DM)	-	
			Galacturonic acid (GalA)	80%	81.7%
					-
CU-902	Low methoxy	Citrus	Degree of esterification (DE)	6.1%	10%
			Degree of amidation (DM)	-	-
			Galacturonic acid (GalA)	78%	69%
AU910	Low methoxy	Apple	Degree of esterification (DE)	4.1%	5%
			Degree of amidation (DM)	-	-
			Galacturonic acid (GalA)	75%	65.3%

Appendix 13.11. The composition and heat stability of carrageenan tested for of 2014 Riverland Chardonnay wine.

	Sample code (batch number production year)	Carrageenan type	$(%)	K^+ (mg/g)	Na^+ (mg/g)	Ca^{2+} (mg/g)	*Viscosity index in		#Rate (g/L)	Initial haze (NTU)	Final haze (NTU)
							Water	Wine			
1.	Ca-A-367 (2014)	ι- / ν-	98%	43	51	0.3	4		0.2	9.08	26.9
2.	Ca-A-368 (2014)	λ-	96%	18	50	8	5		0.2	14.0	50.6
3.	Ca-A-369 (2014)	κ- (Semi refined)	88%	85	11	5	2		0.2	0.6	50.6
4.	Ca-A-370 (2014)	ι- (Semi refined)	89%	112	9	5	2		0.2	5.0	41.2
5.	Ca-A-371 (2014)	κ-	96%	91	6	0.05	6	1	1.0	0.57	**2.77**
6.	Ca-A-372 (2014)	ι-/ν- (Cold soluble)	97%	6	80	<0.05	4		0.3	25.2	55.5
7.	Ca-A-CSW	κ-, ι-, λ-Sucrose-(12%)	86%				3		0.95	0.7	4.9
8.	Ca-A-529 (2014)	λ- (Pure)	96%	14	57	6	6		0.95	19.9	32.7
9.	Ca-A-532 (2014)	κ-, ι-, λ- (Cold soluble λ-)	96%	23	63	1.6	4		0.90	0.8	6.7
10.	Ca-A-533 (2014)	κ- (Cold soluble)	98%	1.1	55	0.9	4	3	1.0	0.6	**2.4**
11.	Ca-A-978 (2015)	κ-	97%	91	6		6	1	1.2	0.7	**2.7**
12.	Ca-A-979 (2015)	κ-, ι-, λ- (Cold soluble λ-)	nd	23	63	1.6		-	1.2	0.9	-
13.	Ca-A-980 (2015)	κ- (Cold soluble)	nd	1.1	55	0.9	4	3	1.2	.5	**3.5**
14.	Ca-B-1 (2014)	κ- Dextrose (10%)	88%	100			4		0.95	0.6	**3.5**
15.	Ca-B-2 (2014)	κ- (90%), ι- (10%)	96%				6	1	1.00	0.8	**2.2**
16.	Ca-B-1R (2014)	κ-(Pure) (Protex)	nd	100			6	1	0.95	0.86	**3.5**
17.	Ca-B-2R (2014)	κ-Dextrose(10%)(Protex)	nd				4	1	1.00	1.80	**3.9**
18.	Ca-B-1R (2015)	κ-(Pure)	95%	100			6	1	1.2	0.8	3.9
19.	Ca-B-2R (2015)	κ- Dextrose (10%)	88%				6	1	1.2	0.6	3.9
20.	Ca-B-3R (2015)	κ- (90%), ι- (10%)	98%				6	1	1.2	0.5	3.9
21.	Ca-C-359 (2014)	(Viscarin SA)	nd				6		1.00	9.0	35.0
22.	Ca-C-812 (2014)	(Gelcarin GP-Prod 303)	nd				4		1.0	1.06	8.31
23.	Ca-C-209 (2014)	(Viscarin GP- Prod 209)	nd				4		0.80	5.42	22.0
24.	Ca-S-3889	λ-	nd	49	49	6	3		0.7	10.7	37.3
25.	Ca-S-1263	κ-	nd	38	7	26	6		1.00	0.5	5.0
26.	Ca-S-4014	ι-	nd	26	16	33	6		1.00	14.4	37.3
27.	Ca-S-Blend	λ- (90%), ι- (10%)	nd				3		1.00	9.4	53.0
28.	Ca-S-Blend	κ-(90%), ι- (10%)	nd				6		1.00	0.6	5.0

κ-kappa; ι-iota; λ-lambda;μ-mu; ν-nu; Ca- = Carrageenan; A- = CP Kelco; B- = Cargill; C- = IMCD; D- = Herbstreith & Fox; S- = Sigma

$ CHO= carbohydrate as total polysaccharide expressed as a % (w/w) and determined by the method described in *Section 4.2.1*.

Cation content is commercial data obtained from suppliers.

*Viscosity index data is based on a 1-6 rating of 1.5% solution (15g/L), where 1 = low viscosity (liquid) and 6 = high viscosity (solid) at room temperature;

#Rate= Highest addition rate.

Appendix 13.12. The Industry Reference Group (IRG) for TWE 1301 Novel fining agents to heat stabilise wine.

	Representing
Vanessa Stockdale	Treasury Wine Estates
Peter Munro	Treasury Wine Estates
Gioia Small	Treasury Wine Estates
Iain Jones	Treasury Wine Estates
Sunil Ratanyake	University of Melbourne
Tony Basic	University of Melbourne
Heather Fraser	Yalumba
Jean McIntire	Pernod Ricard
Toby Barlow	St Hallet Wines
Adam Wadewitz	Shaw and Smith
Liz Waters	Wine Australia
Adrian Loschiavo	Wine Australia
Paul Smith	Australian Wine Research Institute
Marcus Herderich	Australian Wine Research Institute
Steven Guy	Wine Australia