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Carrageenans as heat stabilisers of white wine

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Abstract

Background and Aims: Carrageenan addition has been previously shown to remove proteins from wine and to heat-stabilise wines, however, many types of carrageenans are now available with potentially different protein-adsorbing properties. This study investigated a range of commercially-available carrageenans added at several stages of winemaking for efficacy of protein removal, heat stability and impact on wine sensory properties.

Methods and Results: In preliminary screening trials, 11 types of carrageenan were added to Chardonnay wine and the heat stability of the wine measured with a heat test. Three of the carrageenans successfully heat-stabilised the wines and were included in large-scale winemaking trials: sodium-rich *kappa* (*kN*), potassium-rich *kappa* (*kK*), and *kappa/iota* (90:10, *ki*) carrageenan. Each carrageenan was added at three stages of winemaking, to juice, during fermentation and to wine. All carrageenans produced heat-stable wine regardless of time of addition. Addition of *kN* during fermentation also improved wine recovery compared to bentonite addition, the positive Control, although sodium concentration also significantly increased in the wine. Addition of *kK*-carrageenan to either wine or clarified juice was the most effective treatment for producing heat-stable wine with minimal impact on the sensory profile, wine lees, turbidity and concentration of metal ions compared to that of untreated Control wines.

Conclusions: *Kappa*- and *kappa/iota*-carrageenans can be effective at heat stabilising white wines without negative impact on sensory properties, although the filterability and concentration of metal ions of the wines can vary with carrageenan structure and time of addition.

Significance of the Study: *Kappa*-carrageenan, a renewable fining agent, is effective in heat stabilising wines and maybe become a useful alternative to bentonite.

Keywords: *bentonite, heat stability, kappa-carrageenan, kappa-/iota-carrageenan, lambda-carrageenan, white wine protein haze*

Introduction

Grape pathogenesis-related proteins (PRPs), including thaumatin-like proteins (TLPs) and chitinases, are the major soluble proteins found in grape juice and are responsible for the formation of hazes in white wine (Tattersall et al. 1997, Vincenzi et al. 2011). These haze-forming proteins survive the winemaking process but during transportation and storage, when wine can be exposed to unfavourable environmental conditions, they can undergo aggregation resulting in the appearance of a haze (Waters et al. 2005, Vincenzi et al. 2011, Van Sluyter et al. 2015). These hazes are commercially unacceptable and therefore the haze-forming proteins must be removed before bottling. The current method of protein stabilisation in commercial white winemaking is by adding bentonite, a cheap, absorbent clay that is an efficient fining agent that binds grape proteins and removes them through sedimentation (Muhlack et al. 2006, 2016). The use of bentonite, however, has some negative aspects, such as loss of wine volume, incompatibility with filtration equipment, and sustainable waste disposal challenges (Majewski et al. 2011). Furthermore, the use of bentonite, particularly at high dose, can remove phenolic substances, flavour and aroma compounds and therefore cause an inadvertent loss of flavour in the wine (Lambri et al. 2013, Dordoni et al. 2015, Vincenzi et al. 2015). An alternative, cost-effective, fining agent would therefore be desirable to the wine industry.

Protein stabilisation using polysaccharides, such as carrageenans, is well established in the milk and brewing industries (Duan et al. 2006). Previous reports of the addition of carrageenans to white wine and grape juice for haze stabilisation have shown that they can be effective when added either during wine processing or to the finished wine (Cabello-Pasini et

al. 2005, Marangon et al. 2012, 2013). Carrageenans are naturally occurring polysaccharides extracted from red seaweeds of the *Rhophyta* species (Necas and Bartosikoya 2013) and are therefore a potential renewable heat-stabilising agent. They are also a permitted additive to wine in Australia, New Zealand, Europe and the USA (Food Standards of Australia and New Zealand 2017). These polysaccharides are composed of a galactan backbone of alternating 1,3-linked- β -D-galactopyranosyl (Gal) and 3,6-anhydro-D-galactose (AnGal) residues with a molecular mass between 200–800 kDa and can contain sulfate ester (SO_3^-) groups. There are three commercially-available structural types of carrageenans, namely *kappa* (k -, one SO_3^- -residue per disaccharide), *iota* (i -, two SO_3^- -residues per disaccharide) and *lambda* (λ -, three SO_3^- -residues per disaccharide). These sulfate ester polysaccharides are widely used in industrial applications in which *kappa*-carrageenans (G4S-DA repeat) and *iota*-carrageenans (G4S-DA2S repeat) are employed for their gelling properties and *lambda*-carrageenan (G2S-D2S6S), a non-gelling carrageenan, as a thickening agent. Strong interactions between the sulfate ester groups of neighbouring helices, mediated by potassium (K^+) and calcium (Ca^{2+}) ions, are responsible for stabilising the three-dimensional structures of *kappa*- and *iota*-carrageenans, respectively (Janaswamy and Chandrasekaran 2002). Carrageenans naturally occur as a K^+ salt, although they can be modified to the sodium (Na^+) salt through a patented ion exchange process (Campo et al. 2009, Trudsoe 2010). *Kappa*-carrageenan as the Na^+ salt is soluble in cold water and delays the aggregation of the helix structure thereby forming a hard gel.

Carrageenan has previously been shown to heat-stabilise white wines (Marangon et al. 2012, 2013), however, the impact of different carrageenan structures and times of addition on

winemaking processes and wine properties is unknown. In the current study, an initial screening was undertaken of several commercially-available carrageenans of varying structure types in a Chardonnay wine. From this initial screening process, the carrageenans that were identified as more effective at heat stabilising wines were selected for pilot winemaking trials. The impact of the addition to juice, wine or fermentation of different carrageenans, including *kappa*-carrageenans with either Na⁺, K⁺ ions or blended with *iota*-carrageenan, was explored in pilot-scale winemaking for heat stabilisation, fermentation time, wine recovery, clarity and filterability, concentration of metals and sensory profiles.

Materials and methods

Heat-stabilising and clarification agents

Carrageenans with different structures were obtained from CPKelco ApS (Lille Skensved, Denmark), Cargill Australia (Melbourne, Vic., Australia) and Herbstreith & Fox (Neuenbürg, Germany). Low-methyl citrus pectin (LM) was obtained from Herbstreith & Fox and bentonite (SIHA Active G, 0.5% sodium 0.8% calcium) was from E. Begerow, Langenlonshiem, Germany. Details of the carrageenan batches are shown in Table S1. Clarification agents tested prior to carrageenan addition included pectinase enzymes (polygalacturonase, VinoClear, Novozymes, Copenhagen, Denmark), a chitosan-based clarification agent [Qi-Up, comprising tartaric acid (47%), pea protein (33%), and chitosan (20%), Institut Oenologique de Champagne, Mardeuil, France], and a combined enzyme treatment [referred to as 'E2', 50:50 blend of endo- β -glucanase (Viscozyme) and cellulase (NS26215) enzymes (Novozymes, Copenhagen, Denmark)].

Stock solutions for protein removal

Stock solutions of carrageenans (10 g/L) were prepared for addition to either juice, ferment or wine to remove proteins. Stock solutions were prepared in the same media as the sample to be treated (juice, ferment or wine) and the volume required was removed from the vessel, mixed with the required dose of carrageenan, and returned to the original vessel to avoid dilution effects. Stock solutions were freshly prepared by slowly adding carrageenan (2 g) to either juice, ferment or wine (200 mL) while mixing using a mixing stirrer (ES Overhead Stirrer with stirring shaft with propeller, Velp Scientifica, Usmate, Italy) for at least 2 min until all material was in solution. Bentonite stock solutions (250 g/L) were prepared with water and included in the preliminary heat stabilisation trials and large-scale winemaking trials as positive Controls.

Carrageenan characterisation

Carrageenans were characterised for water- and wine-solubility using Viscosity Index measurements. Carrageenan solutions (1.5% w/v; 15 g/L) were prepared in both water and wine (Chardonnay) and the Viscosity Index of each solution was visually rated on a scale of 1–6, where 1 = low viscosity (liquid), 6 = high viscosity (solid). To determine total carbohydrate concentration, each carrageenan (200 mg) was dissolved in H₂O to which four volumes of absolute EtOH were added. After 18 h at 4°C, the solution was centrifuged, the pellet washed twice with EtOH, acetone and methanol, dried under vacuum and weighed. Carrageenan structures were elucidated using linkage composition analysis (Stevenson and Fumeaux 1991) and sulfate ester content (Craigie et al. 1984), as well as ¹H NMR spectroscopy (D₂O, 80°C, Bruker Avance III 600 MHz spectrometer with cryo-probe, Bruker Daltonics, Bremen, Germany) as a single-step analytical method to identify different types of

carrageenan (Figure S1 and Table S2). Carrageenans have been characterised extensively with ^1H -NMR and the diagnostic chemical shifts for the key structural units are published (Van de Velde et al. 2002). Molecular size distribution (data not shown) was determined using size-exclusion chromatography with an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA, USA) with a Shimadzu RID-10A refractive index detector (Shimadzu, Kyoto, Japan), GRAM 1000Å column (PSS Polymer, Mainz, Germany) at 80°C, and dimethylsulfoxide (DMSO) with 0.5% lithium bromide (LiBr) isocratic solvent system, 0.3 mL/min flowrate. Structures were found to be similar to the product specifications, as expected, and batches of the same type of carrageenan were compositionally the same regardless of sample code or production year.

Heat-stabilising trials

The efficacy of different carrageenans in producing heat-stabilised wines was assessed in preliminary heat-stabilising trials with a Riverland Chardonnay wine [Treasury Wine Estates (TWE), Nuriootpa, SA, Australia]. Carrageenan stock solution (10 g/L in wine) or bentonite stock solution (250 g/L in water) were added to wine samples (150 mL each in triplicate) to give a final concentration between 0.2–1.4 g/L in 0.2 g/L increments. Samples were then shaken to mix and kept overnight at room temperature before filtering at 0.45 µm (Ultipor N66, Pall Corporation, New York, NY, USA) and assessing the heat stability of each sample as previously described (McRae et al. 2018). The concentration of each heat-stabilising agent that produced a change in turbidity before and after heat treatment (80°C for 2 h, 20°C for 3 h) of < 2.0 NTU (Hach model 2100P nephelometer, Loveland, CO, USA) was considered the

recommended dose. Carrageenans that heat-stabilised the Chardonnay wine at a concentration less than 1.4 g/L were investigated further in large scale trials.

The concentration of heat-stabilising agent required to produce heat-stable wines for the large scale trials was also assessed using bench heat-stabilising trials. The dose for wine addition was determined as described above with addition of either carrageenan or bentonite at 0.2 g/L across the range of 0.2–1.4 g/L. The dose that gave $< 2.0 \Delta\text{NTU}$ before and after heating (80°C for 2 h, 20°C for 3 h) was selected. For juice addition, carrageenan was added in the same manner as with wine prior to heating, and the dose was determined as the carrageenan concentration that produced the lowest NTU after heating, to account for the interference of sugars and juice solids in the test. The addition rate to the fermentation was assumed to be the same as that for juice.

Preliminary clarification trials

Unclarified Sauvignon Blanc grape juice (2015 and 2016, Adelaide Hills, SA, Australia) was provided by TWE. For the clarification trials, juice was divided into batches for the three clarification treatments and two Controls (20 L each in triplicate) (Figure S2). For Treatment 1 (T1, chitosan treatment), juice was first settled (0°C, 24 h), carrageenan stock solution added (10 g/L *kappa*-*iota*- in 2 L grape juice to give 1 g/L in 20 L), further settled for 24 h and a chitosan-based clarification agent added (10 g/hL). Treated juice was then allowed to settle a further 24 h prior to racking. For Treatments 2 and 3 (T2 and T3), pectinase (3

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mL/hL) was added to juice and allowed to settle for 24 h prior to carrageenan addition. For T2 (E2 enzyme treatment), a 50:50 mixture of endo- β -glucanase and cellulase (E2 enzymes, 60 mg/L, 15°C) was added 24 h after carrageenan addition and allowed to settle for a further 24 h prior to racking. For Treatment 3 (T3, pectinase treatment), no further additions were made. The time between addition of carrageenan and enzyme was to minimise any potential interference of each treatment on the efficacy of the other. Pectinase was added prior to carrageenan to prevent interactions between juice solids and carrageenan. Chitosan and E2 were added after carrageenan to assess the efficacy of these treatments in aiding the removal of protein-rich carrageenan prior to filtration. Controls included juice with either carrageenan treatment only (C1) or juice with no treatment (C2). All treated and Control juices were then fermented (in triplicate) with *Saccharomyces cerevisiae* var. *bayanus* (Maurivin PDM, Mauri Yeast Australia, Sydney, NSW, Australia) at 15°C to dryness (residual sugar < 2.0 g/L). Potassium metabisulfite was added (to approximately 30 mg/L free SO₂) and wines were then settled (0°C, 24 h), cold stabilised (2 g/L potassium tartrate, 0°C, 21 days), filtered using crossflow filtration (0.4 μ m, Advanced Metallurgical Solutions, Lonsdale, SA, Australia) followed by 0.45 μ m cartridge filtration (Ultipor N66 Pall Corporation) and bottled in 750 mL green claret bottles. Samples were taken before and after crossflow filtration for the filtration analysis and after bottling for sensory and post-bottling analyses.

Large-scale heat-stabilising trials winemaking protocol

For the large-scale fining trials, Sauvignon Blanc grape juices were sourced from the Adelaide Hills region (South Australia) in 2015 (Wine 1) and 2016 (Wine 2), and stored at 0°C prior to use (up to 3 days). Wine 1 was produced from unclarified juice and Wine 2 was

produced from juice treated with pectinase (0.5 g/L, Ultrazym, Novozymes) in line with the outcome of the preliminary clarification trials described above. Juice (2000 L) was mixed to the same turbidity and transferred to 50 L fermentation vessels (48 kg juice in each vessel). Juice was cold-settled (0°C, 48 h), racked into clean vessels (turbidity <200 NTU, Hach nephelometer), and fermented to dryness (*S. cerevisiae*, PDM, 15°C) in triplicate. Diammonium phosphate (DAP, 200 mg/L) was added to each ferment after the initial 2°Be decrease.

Carrageenan stock solutions, *kappa*-carrageenan with K⁺ ions (*kK*), *kappa*-carrageenan with Na⁺ ions (*kN*), and *kappa*-(90%)/*iota*-(10%) with K⁺ and Na⁺ ions (*ki*), were prepared as described above and added to juice prior to cold-settling (Juice Addition), to ferment after a decrease of 2–3°Be (Ferment Addition) or to wine after fermentation and potassium metabisulfite addition (Wine Addition) (Figure S3). Doses for additions were determined using fining trials as described above for the preliminary heat-stabilisation trials. Bentonite (1.5 g/L final concentration) was added to wine for the positive Controls, wines without any additions were the negative Controls. After each addition, either the carrageenan or bentonite was mixed to ensure homogeneity and dry ice was added to the vessels during mixing to minimise oxygen exposure.

The lees volume was determined by measuring the mass of vessels before filling and after racking. Before 0.45 µm filtration, Wine 1 was pad-filtered (Z6 nominal pad filter, IMCD Australia, Mulgrave, Vic., Australia) and then passed through two cartridge filters (1.0 µm, absolute Pall Profile II, Pall, Cheltenham, Vic., Australia; and then (0.45 µm, absolute Ulitipor N66 Nylon) whereas Wine 2 was filtered using a titanium cross flow (0.4 µm,

nominal, Advanced Metallurgical Solutions) then filtered using a nylon cartridge filter (0.45 µm, absolute Ultipor N66 Nylon, Pall). Wines were bottled into clear punted claret bottles (750 mL, stock code 30143, O-I Glass, West Croydon, SA, Australia) and sealed with Saran-tin screw caps (standard matte black stelvin, Orora Closures, Dudley Park, SA, Australia). Samples were collected before the 0.45 µm filter for filterability analysis, before filtering and after bottling for protein analysis, and post-bottling for wine composition and sensory analyses.

Wine filterability and membrane analysis

The filterability of wines was measured using a filterability test as per Blue H₂O filtration protocol (BHTechnologies 2015). Wine samples were taken prior to absolute 0.45 µm filtration and post 1 µm cartridge or after nominal 0.4 µm crossflow-filtration. Wine (approximately 700 mL) was added to a stainless-steel chamber and filtered through a disc filter [0.45 µm polyethersulfone (PES) membrane, Millipore Express] under pressure (200 kPa) and collected in a measuring cylinder. For the preliminary clarification trials, the time taken to filter 200 mL, 400 mL and 600 mL of wine was recorded to calculate the filterability index (FI_V) (Equation 1), where $FI_V > 20$ was indicative of filtration issues. For the large-scale trials, the filterability index (FI_T) was calculated based on time, whereby the collected volume was measured at 30 s intervals up to 180 s (Equation 2), where $FI_T > 2.0$ is deemed a fail. Analyses were in triplicate.

$$FI_V = T_{400} - 2T_{200} \quad (1)$$

$$FI_T = \frac{V_{90} - V_{30}}{V_{180} - V_{120}} \quad (2)$$

Wines showing filterability issues in the filterability test were investigated further using scanning electron microscopy (SEM) of the post-test filter membrane (Wilson and Bacic 2012). Briefly, square pieces of the filters (approximately 1 cm) were fixed in 2.5% glutaraldehyde in phosphate buffered saline (PBS) for 2 h at room temperature. The filter pieces were rinsed in PBS (3 x 20 min), dehydrated by soaking (20 min) in solutions of increasing concentration of ethanol in water at 10, 30, 50, 70, and 90% (v/v) ethanol before soaking in 100% ethanol (3 x 1 h). The dehydrated filter samples were dried in a Balzers CPD030 critical point drier (BalTec, Pfäffikon, Switzerland) and then mounted flat onto aluminum SEM stubs using double sided carbon tape. The samples were then gold coated in a Dynavac Xenosput sputter coater (ProSciTech, Kirwan, Qld, Australia) and imaged in a Philips XL30 Field-emission scanning electron microscope (FEI Company, Hillsboro, OR, USA) at 2.0 kV. Digital images were captured at a magnification of 35 000x with a resolution of 1424 x 1024 pixels.

Wine and juice composition

Analysis of basic wine chemistry included alcohol concentration, pH, TA, volatile acidity (VA), glucose/fructose concentration, free and total SO₂ and metals (Iland 2012). Sugar concentration in grape juice was measured by refractometry (°Brix) with an Atago WM-7 digital handheld refractometer (Atago, Tokyo, Japan) and by densitometry (Baumé) with an Anton Paar DMA 35 densitometer (Anton Paar, Graz, Austria). Protein concentration of the juice and wine samples was determined using Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). Protein was quantified using Coomassie Brilliant Blue G-

250 dye (Bio-Rad Laboratories) with a calibration curve of bovine serum albumin as per manufacturer instructions.

Sensory analysis

For the preliminary clarification trials, sensory analysis was conducted using a balanced reference triangle test conducted in accordance with ISO 4120-1983(E) (Standards Association of Australia 1988) to determine any perceptible differences between wines produced using carrageenan treatment with or without pectinase addition. Samples were presented to panellists in 30 mL aliquots in three-digit-coded, covered ISO standard wine glasses at 22–24°C, in isolated booths under colour masking sodium lighting, with randomised presentation order across judges. Thirty-two experienced, screened and qualified assessors evaluated the wines for aroma and palate and selected one sample in the set that was different from the other two samples. Data were collected using Fizz 2.47B software (Biosystèmes, Couternon, France) and statistical significance was determined using a binomial model (Standards Association of Australia 1988).

For the large-scale carrageenan fining trials, descriptive sensory analysis (Siebert et al. 2018) was used to evaluate any differences in sensory attributes of Sauvignon Blanc wine treated with the *kN*, *ki* and *kK* carrageenans at three stages of winemaking. Appropriate descriptive terms were developed by panel assessors during three training sessions and used for rating in a practice session and in the formal sessions, which were conducted over 3 days (Table 1). In the formal sessions, panellists assessed 24 wines (eight treatments in triplicate)

twice using a modified Williams Latin Square incomplete random block design generated by Fizz 2.47B software. Aliquots of each wine (30 mL), were presented in random order in ISO standard wine glasses (coded and covered) at 22–24°C in isolated booths under daylight-type lighting. A rest of 90 s was enforced between samples and there was a minimum rest of 10 min between sets. Wines were assessed for appearance, aroma and palate. Each attribute was rated for intensity using an unstructured 15 cm line scale from 0 to 10, with ‘low’ and ‘high’ ratings given at 10% and 90%, respectively.

Statistical analysis

Sensory data were acquired and panel performance was assessed using Fizz Senstools (OP&P, Utrecht, The Netherlands) and PanelCheck (Nofima, Tromsø, Norway) as described previously (McRae et al. 2017). All judge responses were assessed for consistency using ANOVA of treatment, judge and ferment replicate results (Minitab, Sydney, NSW, Australia) and were found to be of required standard. Tukey’s honestly significant difference (HSD) value was calculated ($P < 0.05$) and principal component analysis (PCA) (XLSTAT, Addinsoft, Paris, France) was conducted using the correlation matrix of the mean values for the treatments averaged over ferment replicates, panellists and replicates.

The effects of adding different carrageenans during three winemaking stages compared to the untreated and bentonite Control wines were determined by one-way ANOVA, and the significance of the mean assessed by Tukey’s HSD test using GraphPad Prism statistics software (v6.04 GraphPad Software, La Jolla, CA, USA).

Results and discussion

Preliminary heat stability trials

Carrageenans produced commercially have different composition, particularly relating to carbohydrate concentration and the concentration of associated metal ions, specifically K^+ , Na^+ or Ca^{2+} ions (Table 2). Of the selected carrageenans, *kappa*-carrageenans were most effective at improving heat stability by reducing haze formation after heating. Carrageenans incapable of heat stabilising wines contained either *lambda*- carrageenan or greater than 89% *iota*-carrageenan. These data indicated that only the predominantly *kappa*-containing carrageenans were capable of reducing heat haze through the potential to effectively either remove protein from wine or stabilise the protein in wine. A mixture of *kappa*- with *iota*-carrageenan for one batch was effective in heat stabilising the Chardonnay wine, whereas other blends and types of carrageenan were less effective in improving wine heat stability (Table 2). This demonstrated that the differences in the polysaccharide structures had a significant impact on the capacity to adsorb proteins from wine. *Kappa*-carrageenans have a single negatively charged sulfate ester group (SO_3^-) per disaccharide unit which forms a strong double helix structure as there is less repulsion between SO_3^- groups compared to that in *iota*- and *lambda*-carrageenans, which contain two and three SO_3^- groups per disaccharide unit, respectively. *Lambda*-carrageenans therefore do not form gels. *Iota*- carrageenans form gels in the presence of Ca^{2+} (Necas and Bartosikoya 2013, Alba and Kontogiorgos 2018). Potassium ions neutralise the charge between the SO_3^- groups in *kappa*-carrageenans, because the SO_3^- groups are located externally to the helix structure and this promotes either bundling or aggregation of the helical structure forming a three dimensional network (Campo et al. 2009). The aggregated helical structure can be removed from wine by either settling or centrifugation. The mechanism for heat stabilisation of juice/wine is likely to involve the

formation of an electrostatic complex between the negatively charged SO_3^- groups of neighbouring double helices in the *kappa*-carrageenan and positively charged wine proteins that entrap and precipitate the wine haze proteins through sedimentation (Kara et al. 2006, Campo et al. 2009). Based on these preliminary trials, three carrageenans were selected for larger-scale trials including natural *kappa*-carrageenan (*kK*), ion-exchanged Na^+ *kappa*-carrageenan (*kN*) and a *kappa*-/*iota*-carrageenan blend (*ki*) (Table S1).

Viscosity of carrageenans

The composition of each carrageenan determines its relative solubility in water and wine and may relate to the efficacy for removing proteins to heat stabilise wine. Carrageenans with a lower viscosity index can be readily mixed using standard winery equipment and may be more effectively dispersed. Higher viscosity carrageenans can also be processed with standard winery pumps or agitators and will facilitate protein removal by more efficient settling, but may be more difficult to initially disperse. Natural carrageenans contained more K^+ than Na^+ and were less soluble in water than wine. This is most likely to be due to the presence of other ions in wine that aid carrageenan solubility (Leske et al. 1997). The solubility of carrageenans depends on both their concentration of sulfate ester groups and the associated ionisable cations (Na^+ , K^+ , Mg^{2+} and Ca^{2+}), including the presence of other solutes in the media (Campo et al. 2009). The proportion of sulfate ester groups and equilibrium of cations and other solutes in solution determines the viscosity of the solution and the strength of the gel. Carrageenans that had been ion-exchanged with Na^+ , referred to as cold-stable carrageenans, had substantially lower K^+ concentration and greater Na^+ concentration compared to that of natural carrageenan. This led to greater solubility in water but reduced

solubility in wine, potentially due to the influence of the cation on the three-dimensional structure of carrageenans in liquid (Campo et al. 2009). In solutions such as wine, ionisable cations and soluble solutes either disturb the formation of the gel or, if formed, is not as stable. The higher viscosity in wine of ion-exchanged compared with natural carrageenan is likely to be the result of the complex matrix of wine suggesting that the ionic composition and other soluble solutes in wine/juice are likely play a role in affecting its structure (Kara et al. 2006). A comprehensive study of both types of *kappa*-carrageenan salts is needed to fully understand the differences in solubility.

Effect of juice clarification agents on wine filterability after carrageenan treatment

Carrageenan is a polysaccharide that can potentially hold juice solids in solution and adversely affect wine filterability. The relative filterability of wines produced after carrageenan addition to juice was assessed by juice clarification trials (Table 3). The addition of *ki* carrageenan to grape juice reduced the filterability of the resulting wine (C2) tenfold compared to that of the untreated Control wine (C1).

Enzyme addition to carrageenan-treated wine, including pectinase (T3) and the blend of endo- β -glucanase and cellulase (E2) enzymes in conjunction with pectinase (T2), significantly improved filtration compared to that of the Controls. Pectinase addition (T3) also improved the heat stability of the wine compared to the carrageenan-treated Control wine although the enzyme mixture (T2) reduced heat stability and increased haze. The cause of this haze is unclear and warrants further investigation. The use of a chitosan-based clarification agent (T1) improved wine heat stability compared to that of the Control wines although filterability was adversely affected. These results indicated that clarification of juice

with pectinase addition prior to carrageenan treatment will improve wine filterability. Sensory analysis using the triangle test indicated that there was a significant ($P < 0.05$) difference between wines produced using carrageenan treatment alone or carrageenan treatment with pectinase addition (data not shown). This difference, though not necessarily negative, was taken into consideration for the large-scale trials.

Influence of carrageenan structure and addition time on winemaking and filterability

Large-scale heat stability trials were conducted to assess the effect of carrageenans added to juice, during either fermentation or to wine, on winemaking processes and wine characteristics including filterability and heat stability. Treated wines were compared to an unfinned wine as a negative Control, and a bentonite-fined wine as a positive Control (Table 4). The required dose rate for addition during winemaking depended on the time of addition (Table 4). Generally, additions to juice or during fermentation were lower than those added to wine which may be due to difficulties in determining accurate heat test results in grape juices as the reverse has been noted for bentonite additions (Pocock et al. 2011). The amount of carrageenan required for the ferment additions was considered the same as for juice additions. Carrageenan addition increased the time of fermentation by approximately 1 day when added to juice and by around 2 days when added during fermentation compared to the untreated and bentonite Control wines (Table 4). A previous investigation reported that the presence of carrageenan could reduce the fermentation time (Marangon et al. 2012) although that was not observed in this case.

Wine lees were measured following the racking after cold stabilisation to determine the efficacy of carrageenan in wine recovery as compared with the bentonite and untreated

Control wines (Table 4). The addition of *kN* to the ferment generated around half the lees volume generated by bentonite. Wine recovery is one of the main reasons cited for finding an alternative to bentonite (Majewski et al. 2011). Improved wine recovery relative to bentonite is necessary for the adoption of carrageenans, which are more expensive fining agents compared to bentonite. These results indicated that *kN* carrageenan addition during fermentation can provide better wine recovery than bentonite for wines produced from clarified juice. Other carrageenan additions produced a similar volume of lees as the bentonite and untreated Control wines. Unlike bentonite, carrageenans tend to float rather than settle when added to clear wine (Figure S4), however, mixing carrageenan to juice or during fermentation will cause the carrageenan to settle for efficient racking and filtration. Therefore carrageenan is best added at stages in winemaking that already generate lees for more effective settling and removal. The potential for carrageenans to facilitate a dual role of a clarification agent in aiding flotation and heat stabilisation should also be explored. Wine turbidity, the measure of wine clarity prior to filtration, was generally not significantly altered by carrageenan addition to wine compared to that of the untreated Controls. The exception was *ki* addition, which increased wine turbidity but not to an extent that filterability was negatively influenced. Carrageenan addition during fermentation reduced wine turbidity to a similar extent as bentonite, indicating that the carrageenan lees had completely settled, and addition under these circumstances was as effective as bentonite in clarifying wines (Table 4). The addition of both K^+ and Na^+ *kappa*-carrageenan to wine produced similar turbidity to that of the untreated Control wine post-racking whereas addition of *ki*-carrageenan to wine

almost doubled the turbidity. This suggested that adding carrageenan during fermentation is a more effective strategy to reduce lees production and to improve wine clarity.

The impact on wine filterability of carrageenan addition at different stages of winemaking was also assessed prior to 0.45 μm filtration and bottling (Table 4). Carrageenan addition to juice or during fermentation reduced filterability in Wine 1 with filterability indices (FI_T) > 2, suggesting potential filtration issues. Carrageenan addition to wine, however, did not negatively impact filterability. The cause of the poor wine filterability after carrageenan addition was investigated further using SEM analysis of the 0.45 μm membranes (Figure 1). After filtering *kN* wines, membranes showed strands of polymeric material filling the regular membrane pores of the filters for *kN*-J and *kN*-F (Figure 1a,b, respectively). These strands were absent from *kN*-W wines and the bentonite-treated wines (Figure 1c,d, respectively). This suggested that the poor filterability may be due to an interaction between juice or ferment solids and the carrageenan polysaccharides which prevented adequate settling. A previous study also indicated that carrageenan addition to wines can reduce filterability (Marangon et al. 2013). To avoid further filterability problems, juice for Wine 2 was first treated with pectinase which was demonstrated in the preliminary clarification trials to be effective in preventing filterability issues. Pectinase promotes the cold settling of juice and therefore was likely to improve wine clarity and filterability (Sieiro et al. 2012). All wines treated with pectinase prior to carrageenan addition and subsequent cross-flow filtration (nominal 0.4 μm) passed the filterability test (absolute pore size 0.45 μm) (Table 4).

Influence of carrageenan structure and addition time on wine heat stability

The addition of each selected carrageenan to either juice, ferment or wine removed about 90% of the proteins present in the treated wines compared to that of the untreated Control wines (Figure 2). The proportion of protein removed from the wines was greater after filtration and bottling, suggesting that some of the protein-absorbed carrageenan is removed with filtration. Protein concentration in carrageenan-treated wines after filtering and bottling (Table 5) was marginally greater than the protein concentration in wines treated with bentonite. This reduction in protein contributed to the heat stability of most of the treated wines (Table 5). The only treated wines that were not heat stable were the addition of *kN*, *kK* and *ki* carrageenan to juice for Wine 2 with one or more replicates producing $>2.0 \Delta\text{NTU}$ in the heat test which was higher than the $\leq 2.0 \Delta\text{NTU}$ required to pass the test. This may relate to the difficulty in determining the heat stability of grape juice. After bottling, wines were stored under cellar conditions (16°C) and the heat stability of the wines was measured at 13 months for Wine 2 and 13 and 25 months for Wine 1 (Table 5). Wines with either carrageenan or bentonite treatment that were heat stable at bottling remained heat stable with storage, while the untreated Control wines were not stable. This indicated the long-term effectiveness of carrageenan treatment.

Influence of carrageenan structure and addition time on wine composition

Cations are known to play a part in the gelation of carrageenans and therefore the different metal ions present in juice and wine are likely to influence carrageenan rheology and viscosity (Leske et al. 1997, Campo et al. 2009). The concentration of metals in wine is also important due to limits for wine exports as well as influencing metal-complex instabilities.

The addition of carrageenans containing either Na^+ or K^+ ions was also likely to influence the concentration of these ions in each wine (Figure 3). Adding *kN*-carrageenan, which contained 55 mg/g sodium (Table 2), increased the Na^+ concentration of the wine fourfold compared to that of the Control wine (Figure 3a). The level of Na^+ present in the *kN* wines was greater than the 60 mg/L recommended maximum for export to Switzerland (de Loryn et al. 2014). Such increase in Na^+ concentration may prevent the commercial use of *kN* in export wines. The addition of K^+ -dominant carrageenan, *kK*, however, did not significantly increase the concentration of K^+ in the wines (Figure 3b). Potassium is the most abundant metal in wine and is a strong gelation agent for *kappa*-carrageenans (Leske et al. 1997, Campo et al. 2009). The concentration of calcium ions in Wine 2 with following addition of *ki* and *kK* carrageenan was significantly higher than that in the *kN*-treated wines (Figure 3c), although the concentration of calcium ions in the carrageenan product was low (Table 2). A Ca^{2+} concentration above 120 mg/L in wine may present a risk for Ca^{2+} tartrate instability (McKinnon et al. 1995). The concentration of copper, iron, manganese, zinc and magnesium (Table 6) was not altered by the addition of carrageenans at any winemaking stage to a level that would be detrimental to either wine quality or metal instability. Wine chemical analysis (Table 6) also indicated that the addition of carrageenan and the delay in fermentation did not significantly impact the basic wine parameters of alcohol, pH, TA and VA for each wine.

Influence of carrageenan structure and addition time on wine sensory profiles

For the large-scale trials, descriptive sensory analysis was conducted for Wine 1 and Wine 2 after 4 months bottle-ageing to determine the impact of carrageenan addition at different stages of winemaking on the sensory characteristics of the wine. For Wine 1, eight of the 27

sensory attributes (Table 1) differed significantly ($P < 0.05$) due to carrageenan type and addition time compared with a bentonite Control: yellow colour intensity, passionfruit, floral, box hedge, sweaty/cheesy, viscosity, astringency and bitterness (Figure 4). In addition, the attributes flint and pungent were found to be different at a low level of significance ($P \leq 0.10$). Carrageenan-treated wines and untreated Control wines rated higher in flavour and aroma intensity and yellow intensity than wines treated with bentonite (Figure 4a). The sensory characteristics of wines treated with *kN* carrageenan did not vary widely with addition time and were similar in sensory profiles to the untreated Control wines, whereas the time of addition influenced sensory profiles for wines treated with *ki* carrageenans (Figure 4b). For *ki* carrageenan, addition to juice resulted in wines with more box hedge character, whereas addition to wine produced more floral character. Ferment addition of *ki* resulted in more pungent and bitter wines. Bentonite addition to wine consistently showed a reduction in the flavour and aroma intensity of these wines. Wine 2 with *kK* carrageenan added at different stages of winemaking yielded ten significant attributes: yellow colour intensity, stone fruit aroma, floral, vegetal, pungent, stone fruit flavour, apple flavour, green flavour, bitter and fruit aftertaste (Figure 5). Addition of *kK* to wine produced more stone fruit aroma and flavour and were less bitter than the Control wines (Figure 5a). Bentonite-treated wines were less yellow and had a lower intensity of fruity aromas than the carrageenan-treated or untreated Control wines. Principle component analysis (PCA) also demonstrated substantial differences in wine sensory profiles with carrageenan addition time (Figure 5b). Addition of *kK* to Wine 2 during fermentation induced more bitter and green characters in the wine compared to addition to wine or juice. These results demonstrated that carrageenans added to

wine produce more fruity or floral characters than carrageenans added during fermentation or to juice.

Conclusions

Kappa-carrageenans have demonstrated a capability to produce heat-stable wine without increasing wine lees or wine turbidity. *Kappa*-carrageenan with K^+ ions added to wine was the most effective treatment for producing heat-stable wine with minimal impact on sensory profiles, wine lees, turbidity, filterability and concentration of metal ions compared to untreated Control wines. Sensory analysis also indicated that wines heat-stabilised with carrageenans had more intense flavour and aroma profiles than those heat-stabilised with bentonite. Juice clarification with pectinase substantially improved the filterability of all wines treated with carrageenan. The potential for carrageenans to facilitate a dual role as a clarification agent in aiding flotation and as a heat-stabilising agent should be further explored given the flotation properties of carrageenans. The results from this study demonstrated that *kappa*-carrageenan, a renewable fining agent, can be used to heat stabilise wines and may become a useful alternative to bentonite.

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Figure captions

Figure 1. Scanning electron micrographs (35 000x) of filter membranes after filtering Wine 1 containing *kappa*-carrageenan (*kN*) added at different winemaking stages. (a) juice addition [*kN*-J, filterability index (FI_T) = 7 ± 4]; (b) ferment addition (*kN*-F, $FI_T = 16 \pm 13$); (c) wine addition (*kN*-W, $FI_T = 1 \pm 0$); and (d) bentonite Control ($FI_T = 1 \pm 0$).

Figure 2. Proportion of protein removed by carrageenan treatment pre-bottling (■) (and post-bottling (■) of (a) Wine 1; and (b) Wine 2 when added at different stages of winemaking compared to the untreated Control wines. Results are shown as the average of triplicate ferments $\pm$ one SD. Dash line represents 90% protein removal. Bent, bentonite; *kN*-J, *kappa*-carrageenan with sodium ion, juice addition ; *kN*-F, *kappa*-carrageenan with sodium ion, ferment addition; *kN*-W, *kappa*-carrageenan with sodium ion, wine addition; *ki*-J, *kappa*-*iota* carrageenan blended juice addition; *ki*-F, *kappa*-*iota* carrageenan blended ferment addition; *ki*-W, *kappa*-*iota* carrageenan blended wine addition; *kK*-J, *kappa*-carrageenan with potassium ion, juice addition; *kK*-F, *kappa*-carrageenan with potassium ion, ferment addition; *kK*-W, *kappa*-carrageenan with potassium ion, wine addition;

Figure 3. Concentration of (a) sodium ions, (b) potassium ions and (c) calcium ions in Wine 1 and Wine 2 after addition of heat-stabilising agents including Control (■), bentonite (■), *kappa*-carrageenan with sodium ions (*kN*) (■), *kappa*-*iota* carrageenan blend (*ki*) (■) and

kappa-carrageenan with potassium ions (*kK*) (■). Results are shown as the average of different treatment times $\pm$ one SD.

Figure 4. Sensory analysis for Wine 1 presented as (a) Spider plot and (b) Scores and loadings bi-plot for principal component analysis of sensory attributes that varied significantly with carrageenan type or addition time for heat-stabilised wines and untreated Control wines. Spell out treatment abbreviations, i.e. kiK FA

Figure 5. Sensory analysis for Wine 2 presented as (a) Spider plot and (b) Scores and loadings bi-plot for principal component analysis of sensory attributes that varied significantly with carrageenan type or addition time for heat-stabilised wines and untreated Control wines.

Supporting data

Table S1. Properties of the selected carrageenan samples for large-scale trials.

Table S2. Proton (^1H) NMR chemical shifts for the main structural units of commercial carrageenans.

Figure S1. Schematic representation of the preliminary clarification trials showing type of clarification agent and addition stages for each treatment.

Figure S2. Schematic representation of the pilot scale winemaking process and points for addition of fining agents as described in the materials and methods section.

Figure S3. ^1H NMR spectra of (a) κ -*l*- (blended) carrageenan and (b) κ -carrageenan.

Figure S4. Wines treated with heat stabilising agents after 96 h settling at 4°C. Each solution was mixed with a homogeniser for 2 min before transferring to a glass measuring cylinder.

(a) 0.8 g/L bentonite; (b) 1.0 g/L *kappa*-carrageenan; (c) 1.5 g/L *kappa*-carrageenan.

Figure 1. Scanning electron micrographs (35,000x) of filter membranes after filtering Wine 1 containing kappa-carrageenan (kN) added at different winemaking stages. a) juice addition (kN-J, FI = 7 ± 4); b) ferment addition (kN-F, FI = 16 ± 13); c) wine addition (kN-W, FI = 1 ± 0); d) bentonite control (FI = 1 ± 0).

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AJGW_12411_Figure 5_TL edit (002).tif

Supporting information

Table S1. Properties of the selected carrageenan samples for large-scale trials.

Code	Name	Structures	Metal ion	Batch No.	Year
<i>kN</i>	<i>Kappa</i>	<i>Kappa</i> -carrageenan	Na ⁺	A-533, A-980	2014, 2015
<i>ki</i>	<i>Kappa</i> / <i>iota</i> blended [†]	90% <i>Kappa</i> , 10% <i>iota</i> [‡]	Na ⁺ /K ⁺	B-2, B-3	2014, 2015
<i>kK</i>	<i>Kappa</i>	<i>Kappa</i> -carrageenan	K ⁺	A-978	2015

[†]*Kappa*- and *iota*- carrageenans can occur in the same carrageenan chain (hybrid) or as a blend (blended) of different *kappa*- and *iota*- carrageenans; [‡]94 % *kappa*- and 6 % *iota*- carrageenans by polysaccharide linkage analysis.

Table S2. Proton (^1H) NMR chemical shifts for the main structural units of commercial carrageenans.

Carrageenan type	Unit	Sugar (Galp)	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	-	-	-	-	-

DA-unit, 3,6-anhydro- α -D-Galp; G, 1,3-linked- β - D-Galp; κ -, *kappa*; ι -, *iota*; λ -, *lambda*; μ -, *mu*; ν -, *nu*; *p*, pyranose; S, sulfate ester groups.

Tables

Table 1. Sensory descriptive analysis attributes, definitions and standards.

Attribute	Definition/synonyms	Standard
Appearance		
Yellow colour intensity	Intensity of the yellow colour	
Aroma		
Overall fruit intensity aroma	Intensity of the fruit aromas in the sample	
Passionfruit	Intensity of the aroma of passionfruit	1 tsp Passionfruit pulp (John West)
Pineapple	Intensity of the aroma of pineapple	4 x 2 cm Cubes fresh pineapple, canned pineapple juice (1 tsp, Golden Circle)
Stone fruit	Intensity of the aroma of stone fruits: peach, apricot both fresh and dried, loquat	1 Can of Goulburn Valley apricots and peaches
Lemon	Intensity of the aroma of lemon	1 x 2 cm Piece of lemon, lemon rind
Confection	Intensity of the aroma of confection: banana lolly, musk	1 x Banana lolly and 1 x musk lolly
Floral	Intensity of the aroma of flowers: violets and blossoms	100 mg/L Linalool (40 µL), 200 mg/L 2-phenyl ethanol (25 µL)
Green	Intensity of the aroma of green grass, green leaves, stalks, green capsicum, green beans	Freshly picked grass and half a chopped fresh green bean
Box hedge	Intensity of the aroma of box hedge	Fresh box leaves
Flint	Intensity of the aroma of flint, wet stones, metals, toast	20 µL of 1 mg/L Benzyl mercaptan
Sweaty/Cheesy	Intensity of the aroma of sweat, cheese, blue cheese, cheddar cheese, body odour, sour milk, raw meat	100 µL Mix of hexanoic acid and isovaleric acid

Pungent	Intensity of the aroma and effect of alcohol	4 mL Ethanol
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Palate

Overall fruit intensity palate	Intensity of the fruit flavours in the sample
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Tropical	Intensity of the flavour of tropical fruits; pineapple, passionfruit, melon
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Stone fruit	Intensity of the flavour of stone fruits; peach, apricot, nectarine
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Lemon	Intensity of the flavour of lemon including aftertaste
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Green apple	Intensity of the flavour of green apple including aftertaste
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Green	Intensity of the flavour of green grass, green capsicum, stalks, herbal
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Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low, watery, thin mouth feel; high, thick mouth feel
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Oily	The perception of oiliness in the mouth
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Sweet	Intensity of sweet taste in the mouth including aftertaste
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Acid	Intensity of acid taste in the mouth including aftertaste
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Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation; low, warm; high, hot
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Astringency	The drying and mouth-puckering sensation in the mouth; low, coating teeth; medium, mouth coating and drying; high, puckering, lasting astringency
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Bitter	The intensity of bitter taste perceived in the mouth, or after expectoration
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Fruit after taste	The lingering tropical or stone fruit flavour perceived in the mouth after expectorating
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Table 2. Composition of different batches of carrageenans , including type, carbohydrate concentration and cation concentration (provided by suppliers), as well as the relative solubility in water and in wine and the heat stability of a Chardonnay wine after treatment with each carrageenan at the stated dose.

Type	Batch No. †	CHO (%)	K ⁺ (mg/g)	Na ⁺ (mg/g)	Ca ⁺ (mg/g)	Viscosity index		Dose (g/L)	Heat Δ NTU
						H ₂ O	Wine		
κ -	A978	97	91	6	ND	6	1	1.2	2.0 $\pm$ 0.1‡
κ -	A371	96	91	6	0.05	6	1	1.0	2.2 $\pm$ 0.2
κ -	B1R	95	100	ND	ND	6	1	1.2	2.5 $\pm$ 0.1
κ -	S1263	ND	26	16	33	6	ND	1.0	4.5 $\pm$ 0.1
κ - (CS)	A533	98	1.1	55	0.9	4	3	1.0	1.8 $\pm$ 0.2‡
κ - (CS)	A980	ND	1.1	55	0.9	4	3	1.2	3.1 $\pm$ 0.3
κ - (SR)	A369	88	85	11	5	2	ND	1.0	50.0 ^f
κ -/ ι - (90:10)	B2	96	100	ND	ND	6	1	1.0	1.9 $\pm$ 0.0‡
κ -/ ι - (90:10)	B3R	98	100	ND	ND	6	1	1.2	3.4 $\pm$ 0.4
κ -/ ι - (90:10)	SBe	ND	43	51	ND	6	ND	1.0	4.4 $\pm$ 0.65
κ -/ ι -/ λ -	A532	96	23	63	1.6	4	ND	1.0	5.8 $\pm$ 0.6
λ -	A529	96	14	57	ND	6	ND	1.0	12.8
λ -	S3889	-	38	7	ND	3	ND	1.0	26.6
λ -	A368	96	18	50	ND	5	ND	1.0	36.6
λ -/ ι - (90:10)	SBe	ND	ND	ND	ND	3	ND	1.0	43.6
ι -	S4014	ND	ND	ND	ND	6	ND	1.0	22.9
ι - (SR)	A370	89	112	9	ND	2	ND	1.0	36.2
ι -/ ν -	A367	98	43	51	ND	4	ND	1.0	17.8
ι -/ ν - (CS)	A372	97	6	80	ND	4	ND	1.0	30.3

Heat stability of the untreated Chardonnay wine was 34.6 Δ NTU; wines with < 2.0 Δ NTU were considered heat-stable. Results are shown as the mean of triplicate analyses $\pm$ one SD except where indicated. Values without SD indicate an initial NTU >2.0 and therefore the heat test was not repeated. †Batch numbers include the following supplier codes: A, CP

Kelco ApS; B, Cargill Australia; S, Sigma. ‡Heat stability results produced by the carrageenans that were selected for further trials. CHO, carbohydrate as total polysaccharide expressed as a % (w/w); CS, cold soluble; ι -, *iota*-carrageenan, λ -, *lambda*-carrageenan; κ -, *kappa*-carrageenan; ν -, *nu*-carrageenan; k -/ ι - (90:10), commercial blended κ -/ ι - (90:10) carrageenan consists of 94.4% κ - and 5.6% ι - carrageenans when measured by linkage analysis; ND, not determined; SR, semi-refined.

Table 3. Effect of grape juice additives for the preliminary clarification trials on wine filterability indices before and after cross-flow filtration, and wine heat stability.

Sample number	Additive	Carrageenan treatment (g/L)	Post-settling additive	FI pre-crossflow	FI post-crossflow	Haze (Δ NTU)
C1	-	-	-	64 $\pm$ 4	29 $\pm$ 1	53 $\pm$ 13
C2	-	1.0	-	711 $\pm$ 264	29 $\pm$ 1	1.0 $\pm$ 0.2
T1	-	1.0	Chitosan [†]	107 $\pm$ 15	35 $\pm$ 3	0.3 $\pm$ 0.4
T2	Pectinase	1.0	E2	41 $\pm$ 12	24 $\pm$ 9	8.4 $\pm$ 1.3
T3	Pectinase	1.0	-	36 $\pm$ 6	29 $\pm$ 7	0.3 $\pm$ 0.3

Results are given as the mean of six replicates $\pm$ one SD.[†]Chitosan-based clarification agent. C1, Control wine (no treatment); C2, wine treated with *kappa-/iota*-carrageenan; E2, blend of endo- β -glucanase and cellulase; FI, filterability index; T1, flotation agent addition; T2, E2 enzyme addition with pectinase; T3, pectinase addition; -, no addition.

Table 4. Effect of the addition during large scale winemaking trials of carrageenans and bentonite to either juice, during fermentation or to wine on fermentation time, quantity of wine lees, wine filterability and filterability index.

Wine and Treatment	Addition time [†]	Dose (g/L) [‡]	Length of fermentation (days)	Wine lees (%)	Wine turbidity (NTU) [§]	Filter type	Filterability index (FI _T)
Wine 1							
Control	None	0.0	6.0	ND	ND	PF ^c	1 ± 0
<i>kN</i> -J	Juice	1.2	7.0	ND	ND	PF	7 ± 4
<i>kN</i> -F	Ferment	1.2	8.0	ND	ND	PF	16 ± 13
<i>kN</i> -W	Wine	1.4	ND	ND	ND	PF	1 ± 0
<i>ki</i> -J	Juice	1.2	7.3	ND	ND	PF	3 ± 1
<i>ki</i> -F	Ferment	1.2	8.0	ND	ND	PF	10 ± 13
<i>ki</i> -W	Wine	1.0	ND	ND	ND	PF	1 ± 0
Bentonite	Wine	1.5	6.0	ND	ND	PF	1 ± 0
Wine 2							
Control-p	None	0.0	10.0	8.3a b	12.9b	CF ^d	1 ± 0
<i>kN</i> -Jp	Juice	1.0	10.6	ND	ND	CF	1 ± 0
<i>kN</i> -Fp	Ferment	1.0	11.6	5.5b	4.3d	CF	1 ± 0
<i>kN</i> -Wp	Wine	1.4	ND	7.0a b	11.9b	CF	1 ± 0
<i>ki</i> -Jp	Juice	1.0	10.0	ND	ND	CF	1 ± 0
<i>ki</i> -Fp	Ferment	1.0	11.0	7.1a b	5.6cd	CF	1 ± 0
<i>ki</i> -Wp	Wine	1.4	ND	10.0 ab	21.2a	CF	1 ± 0
<i>kK</i> -Jp	Juice	1.2	11.3	ND	ND	CF	1 ± 0
<i>kK</i> -Fp	Ferment	1.2	11.3	8.2a b	4.0d	CF	1 ± 0

κ K-Wp	Wine	1.4	ND	8.8a	9.9bc	CF	1 ± 0
				b			
Bentonite-p	Wine	1.5	10.0	11.2	2.0d	CF	1 ± 0
				a			

Values with different lowercase letters in the same column are statistically different. †Juice additions were made prior to cold-settling, ferment additions were made after a decrease of 2–3°Be and wine additions were made immediately after fermentation at the same time as SO₂ addition. Juice for Wine 2 was treated with pectinase (0.5 g/L) prior to fermentation. ‡Dose rates were determined using a heat test. §Measure of wine clarity post-racking of fining agent and gross lees prior to filtration. ¶Wines with a filterability index (FI)_T >2.0 have potential filterability issues. CF, crossflow filtration; F, fermentation; *ι*, *iota*-carrageenan; j, juice; κ -, *kappa*-carrageenan; κ K, potassium-rich κ ; κ N, sodium-rich κ ; ND, not determined; p, pectinase treated; PF, pad filtration; W, wine.

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Table 5. Impact of carrageenan addition during large scale winemaking trials on protein concentration and heat stability post-bottling.

Sample	Protein (mg/L)	Heat stability post-bottling (Δ NTU) [†]	Rating	Heat stability 13 months (Δ NTU) [†]	Heat stability 25 months (Δ NTU) [†]
Wine 1					
Control	37.5 a	37.8 $\pm$ 3.6	Fail	37.0 $\pm$ 7.7	48.0 $\pm$ 8.2
<i>k</i> N-J	2.7 b	0.7 $\pm$ 1.0	Pass	0.8 $\pm$ 0.4	0.8 $\pm$ 0.2
<i>k</i> N-F	2.5 b	0.1 $\pm$ 0.0	Pass	0.3 $\pm$ 0.1	0.1 $\pm$ 0.1
<i>k</i> N-W	3.9 b	0.0 $\pm$ 0.0	Pass	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1
<i>ki</i> -J	2.9 b	0.9 $\pm$ 0.7	Pass	0.5 $\pm$ 0.1	0.8 $\pm$ 0.7
<i>ki</i> -F	2.5 b	0.0 $\pm$ 0.0	Pass	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
<i>ki</i> -W	3.5 b	0.1 $\pm$ 0.1	Pass	0.2 $\pm$ 0.2	0.6 $\pm$ 0.2
Bentonite	2.5 b	0.0 $\pm$ 0.0	Pass	0.1 $\pm$ 0.0	0.6 $\pm$ 0.5
Wine 2					
Control-p	24.3 a	78.0 $\pm$ 2.9	Fail	89.9 $\pm$ 5.2	ND
<i>k</i> N-Jp	6.9 b	1.8 $\pm$ 1.7	Fail	3.9 $\pm$ 3.3	ND
<i>k</i> N-Fp	2.1 cd	0.3 $\pm$ 0.2	Pass	0.2 $\pm$ 0.1	ND
<i>k</i> N-Wp	1.7 d	0.7 $\pm$ 0.1	Pass	1.7 $\pm$ 1.9	ND
<i>ki</i> -Jp	4.5 bcd	1.3 $\pm$ 1.2	Fail	3.0 $\pm$ 2.1	ND
<i>ki</i> -Fp	1.9 cd	0.3 $\pm$ 0.1	Pass	0.4 $\pm$ 0.1	ND
<i>ki</i> -Wp	2.0 cd	1.2 $\pm$ 0.5	Pass	0.5 $\pm$ 0.2	ND
<i>k</i> K-Jp	5.7 bc	2.0 $\pm$ 1.2	Fail	4.8 $\pm$ 2.3	ND
<i>k</i> K-Fp	1.8 cd	0.3 $\pm$ 0.1	Pass	0.4 $\pm$ 0.2	ND
<i>k</i> K-Wp	2.1 cd	0.9 $\pm$ 0.4	Pass	0.7 $\pm$ 0.5	ND
Bentonite-p	0.9 d	0.0 $\pm$ 0.0	Pass	0.1 $\pm$ 0.0	ND

Results are expressed as an average of each treatment replicate $\pm$ one SD. For protein concentration, means with a different letter are significantly different ($P \leq 0.05$). [†]Heat stability measured as the difference in NTU of a wine sample before heating (80°C, 2 h) and after subsequent cooling (20°C, 3 h). F, fermentation ; *ι*, *iota*-carrageenan; *κ*-, *kappa*-carrageenan; *κ*K, potassium-rich *κ*; *κ*N, sodium-rich *κ*; J, juice ; ND, not determined for these samples; p, pectinase ; W, wine.

Table 6. Composition of Sauvignon Blanc wines.

	Wine 1	Wine 2
Alcohol (%)	13.0 $\pm$ 0.1	13.4 $\pm$ 0.1
TA (g/L)	6.9 $\pm$ 0.1	6.4 $\pm$ 0.1
Residual sugar (g/L)	0.4 $\pm$ 0.1	1.5 $\pm$ 0.0
pH	3.3 $\pm$ 0.0	3.1 $\pm$ 0.0
Free SO ₂ (mg/L)	34.7 $\pm$ 12.0	39.2 $\pm$ 2.0
Total SO ₂ (mg/L)	120.4 $\pm$ 16.4	116.3 $\pm$ 1.3
Copper (mg/L)	0.21 $\pm$ 0.03	0.02 $\pm$ 0.01
Iron (mg/L)	1.92 $\pm$ 0.10	0.17 $\pm$ 0.06 [†]
Manganese (mg/L)	0.54 $\pm$ 0.07	1.28 $\pm$ 0.07
Zinc (mg/L)	0.83 $\pm$ 0.05	1.44 $\pm$ 0.04
Magnesium (mg/L)	77.9 $\pm$ 6.9	ND

Results are shown as the mean of all treatments (untreated Control wines, bentonite-treated wines, and wines after carrageenan addition to juice, fermentation or wine) $\pm$ one SD, except where indicated. [†]Iron concentration for bentonite-treated wine was 0.86 mg/L and was excluded from the averaged value. ND, not determined for these samples.

Figure 2 Percent of protein removed by carrageenan treatment pre-bottling (blue) and post-bottling (red) when added at different stages of winemaking compared to the untreated control wines. a) Wine 1; b) Wine 2

AJGW_12411_Stockdale Figure 2 18-115 R4.tif

AJGW_12411_Stockdale Figure 3 18-115 R3.tif

AJGW_12411_Stockdale Rev Figure 4 18-115 R4.tif

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