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THE UNIVERSITY OF MELBOURNE

Guidelines for using carrageenans to heat stabilise wine



AUSTRALIAN GRAPE & WINE AUTHORITY

Supplement to FINAL REPORT

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1. Background

Pectins and carrageenans have been used as replacements for isinglass (animal protein) to clarify beer. Research on the use of these polysaccharides as clarification and fining agents in winemaking and brewing was initiated by Carlton and United Brewing (CUB), a division of Foster's, in collaboration with the Cooperative Research Centre (CRC) for Biopolymers. The outcome of this research was patented in 2006 (Duan et al., patent No WO2008/148156). An ideal 'pectin formulation' was developed for beer in which specific pectin properties 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).

Studies conducted by Treasury Wine Estates (TWE) and the Australian Wine Research Institute (AWRI) (Marangon et al., 2012; 2013) have shown that some carrageenans can be used to heat stabilise wine at lower rates than bentonite. They also found that such carrageenans can be added at the juice, ferment or wine stage and result in a heat-stable wine. However, the study also found that if added in excess and not removed by filtration, certain types of carrageenan can appear in the wine itself and cause a haze.

Further work conducted by TWE and The University of Melbourne (Stockdale, 2016) included screening a broad range of commercially available pectin and carrageenans to determine which were the best type(s) of polysaccharides to heat stabilise white wine. None of pectins tested resulted in a heat-stable wine; however, the study did find that in order to heat stabilise white wine without negative side effects such as poor filtration or inadequate heat stabilisation, certain types of carrageenan could be used. These were identified as kappa-carrageenans in either the sodium (Na^+) or potassium (K^+) salt form, or a blend of kappa- and iota-carrageenans that can contain up to 10% iota-carrageenan. Lambda-carrageenan, or 'pure' (approximately 100 %) iota-carrageenan does not heat stabilise wine.

The use of polysaccharides for the purpose of heat stabilising wine will perform the technical function of a processing aid. This document aims to provide some guidance on using carrageenans as an alternative to bentonite, based on data generated from pilot-scale winemaking trials and bench-scale trials (Marangon et al, 2012; 2013; Stockdale, 2016). At the time of writing these guidelines, carrageenans are not an approved wine processing aid under Food Standards Australia New Zealand (FSANZ) Food Standards Code – Standard 4.5.1. An application seeking approval of carrageenans and pectins in winemaking was submitted in July 2016.

2. A brief comparison between bentonite and carrageenan

Both bentonite and carrageenan can be used to heat stabilise wine. Wine heat instability is caused by proteins that are derived from the grape, and have been identified as grape pathogenesis-related proteins; specifically the thaumatin-like proteins and chitinases (Marangon et al., 2011). These proteins are protease resistant, outlast the fermentation process and remain in the wine. They can form undesirable hazes post-bottling, particularly if the wine becomes heated during transport or storage and hence, need to be removed.

Bentonite is an absorbent aluminium phyllosilicate clay formed from volcanic ash, and is very effective at removing heat-unstable wine proteins (Zoecklein, 1988; Muhlack et al 2016). The main types of bentonites used in winemaking are derived from dominant salts: potassium (K^+), sodium (Na^+) or calcium (Ca^{2+}). Bentonite clay also consists of plates of silicon, magnesium and aluminium. The hydration of these plates provides a relatively large negatively charged surface area. Heat-unstable wine proteins have a slight positive charge at wine pH and hence, bind electrostatically to the negatively charged bentonite plates. Bentonite is also an excellent settling agent and settles quite readily under gravity to the bottom of the tanks along with the removed proteins.

The commercial use of bentonite in wine was first reported more than 70 years ago (Majewski et al., 2011) and has become engrained in white winemaking practices ever since. Bentonite use in red wine is limited because of its ability to reduce colour by absorption of anthocyanins. A disadvantage of using bentonite is that it appears that some aroma and flavour molecules are also removed by the attractive force of the bentonite plates, causing an inadvertent loss of flavour character in the wine. Moreover, studies have shown that bentonite can remove ethyl esters and terpenes at levels that can impact wine sensory properties (Sanborn et al., 2010, Lira et al., 2015).

Carrageenans are polysaccharides extracted from various species of edible red seaweeds, or Rhodophyta, including *Gigiartina*, *Chondrus crispus, Eucheuma and Hypena* (Nayar et al., 2014). Carrageenans are hydrophilic, linear chains of sulfated galactans (Campo et al., 2011) and are used in the food industry for their gelling properties. In wine, their functional properties including the ability to heat stabilise wine depends on their type (kappa-, iota-, lambda-) and dominant salts, usually sodium (Na^+) or potassium (K^+). The heat stabilising mechanism of carrageenans in juice or wine is yet to be elucidated but it has been hypothesised to be through the formation of an electrostatic complex between the negatively charged sulfate groups and positively charged wine proteins. The formation of these aggregated double helices in the high concentration of potassium in wine provides the mechanism for network formation of the carrageenan chains to form gels that precipitate the wine haze proteins, along with the aggregated carrageenans through sedimentation.

Potential benefits of using carrageenan as an alternative to bentonite are:

- No negative impact on sensory properties.
- Use of a fining agent derived from sustainable natural sources (seaweed).
- Improved winemaking process efficiency, as carrageenan can be used at a range of stages of the winemaking process such as addition to the juice, ferment or to the wine itself, and result in a heat-stable wine without negative quality impacts. It could therefore be combined with other process steps to reduce the number of tank movements and create a more continuous process flow.
- In initial studies, it appears that carrageenan can be dosed at lower levels than bentonite.
- The lees by-product is biodegradable.

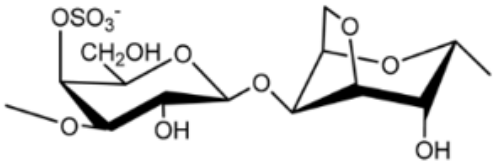
3. Types of carrageenan

3.1. General

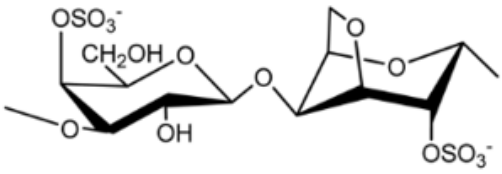
There are three types of commercially available carrageenan: kappa- (*κ*-), iota- (ι) and lambda- (*λ*), which are differentiated by the number and position of their sulfate ester substituents (Figure 3.0). These carrageenans are widely used in industrial applications with κ-carrageenan (kappa-) and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

The main ionisable cations found in carrageenans are sodium, potassium, calcium and magnesium. The type of cation salt impacts the functional properties of the carrageenan. 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-carrageenan are not soluble in cold water but the rheology of these carrageenans is also strongly influenced by the concentration and type of cations present in the product to which they are added, as well as the solution temperature and conditions of dispersion.

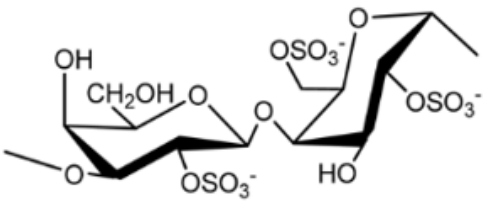
Figure 3.0. Schematic representation of the different structures of the three most commercially important carrageenans.



Kappa (κ-)-carrageenan (G4S-DA)



Iota (ι-)-carrageenan (G4S-DA2S)



Lambda (λ-)-carrageenan (G2S-D2S6S)

3.2. Most efficient types of carrageenan for heat stabilising wine

In a recent study (Wine Australia-funded project TWE 1301), 28 commercially available carrageenans were tested for their ability to heat stabilise the same 2014 Riverland Chardonnay wine in an attempt to identify which types of carrageenans were the most effective as possible alternatives to bentonite.

The study focused on commercially available blends of carrageenans provided by four different suppliers. It was by no means exhaustive of all of the different types of blend and permutations of carrageenan, but it provided a good baseline for establishing some guidelines on which types of carrageenan could replace bentonite for heat stabilising wine.

Certain types of carrageenan have been identified as being the most effective at heat stabilising wine, as summarised in Table 3.0. These are the kappa- carrageenans in either the sodium (Na^+) or (K^+) salt form, or a blend of kappa- and iota- (K+) carrageenan that can contain up to 10% iota-carrageenan. The presence of lambda-carrageenan will cause filtration problems in wine because it does not form sedimentation gels in the wine medium and therefore, cannot be effectively removed. Iota- carrageenan that the supplier claims is 'pure' i.e. contains close to 100 % iota-carrageenan, does not effectively heat stabilise wine.

Table 3.0. Types of kappa-carrageenans and kappa-/iota- carrageenans suitable to heat stabilise white wine.

Name	No. Batch Codes Tested*	Carrageenan	Description
Pure kappa- (K+) carrageenan	4	κ-carrageenan	K^+ dominant
Cold-soluble kappa (Na+) carrageenan	4	κ-carrageenan	Na^+ dominant
Kappa- (90%) iota- (10%) carrageenan[1]	2	ι-carrageenan	K^+ dominant.

**Batches of the same type of carrageenan were tested to be compositionally the same.*

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

4. Quality Control parameters for carrageenan used to heat stabilise wine

4.1. Polysaccharide specifications

Carrageenan products intended for use as a processing aid in winemaking should be supported with a Product Information Form (PIF) to ensure that they comply with the Australia New Zealand Food Standard Code. However, the current PIF documents requested from the suppliers did not contain all of the information required to determine whether the carrageenan blend would be efficient at heat stabilising wine. For example, it is often described in the PIF that the product is 100% carrageenan, which is not possible as samples will contain some salts and water; therefore, a dry weight calculation of the polysaccharide is recommended. It is also common for carrageenan samples to contain bulking agents such as sucrose or dextrose at levels up to 15% w/w. These bulking agents are not permitted additives for wine and should be avoided.

Polysaccharide linkage analysis and ^{1}H-NMR of commercially available kappa-carrageenans have found that they contain 2.0 – 2.8% iota-carrageenan. This is unlikely to impact the performance of the carrageenan and is acceptable, since carrageenan is a plant extract and in nature, these polysaccharides do not occur as ‘pure’ types. In the same study, no evidence of the precursors of the kappa- or iota-carrageenans, which are the mu- and nu-carrageenans, was found, indicating that the reactions to form the active carrageenans were complete and that any precursors were removed.

To ensure integrity, additional information should be requested as part of the product specifications, including a Product Purity Certificate. The carrageenan type and cation concentration of the carrageenan blend is also an important specification because these influence the gelation properties of the carrageenan in the juice or wine. It is recommended that the wine industry adopts these specifications as part of its quality control parameters when requesting carrageenan from suppliers, as specified in Table 4.0.

Table 4.0. 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 method described in* Stockdale *et al 2016.*

[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.*

4.2. Impact of fining wine with carrageenan on wine chemical composition

Fining wine with carrageenan has been found to not impact % alcohol, pH, residual sugar, treatable acid (TA) or volatile acid VA (Marangon et al., 2012); however, using carrageenans can alter the cation composition of the wine (see also Table 5.0). Table 4.1 describes how bentonite and carrageenans impact the metal concentration of a V15 Adelaide Hills Sauvignon Blanc compared to the unfined control.

Metal content in wine is important due to wine import/export limits that are imposed by different countries, and for avoiding metal-complex instabilities. More recent studies have also showed that copper and iron contributes to wine oxidation (Danilewicz, 2007), therefore the AWRI recommends that residual copper and iron limits be kept to less than 0.5 mg/L. As shown in Table 4.1, the carrageenans did not significantly impact either iron or copper levels compared to the unfined control.

Table 4.1. Metal Analysis for Treatments TWE1 – TWE33 wines for a V16 Adelaide Hills Sauvignon Blanc wine.

Supplier Code	Polysaccharide	Addition Stage	K^+	Na^+	Ca^{2+}	Cu^{2+}	$Fe^{2/3+}$	Zn^{2+}	Mn^{2+}
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 rows means followed by a different lower case letter are significantly different (p≤0.05) according to the Turkey-Kramer test.

Zinc is an important yeast nutrient for building ethanol tolerance and promoting yeast membrane health. Only the treatment when cold soluble kappa- (Na^+) carrageenan is added during the ferment stage was a slight decrease in zinc levels observed. Chinese wine authorities are currently enforcing a 2 mg/L limit on the level of manganese content for wines imported into China. Table 4.1 illustrates that manganese levels are not altered when using carrageenans as fining agents, to a level that would be detrimental to export limits.

The cold soluble kappa- (Na^+) carrageenan in the 2016 Sauvignon Blanc elevated sodium levels to above the Swiss export limit of 60 ppm as the chloride salt. Calcium in the wine becomes elevated when kappa- (K+) carrageenan and kappa- iota- (K^+) carrageenan are added. Calcium levels above 70-80 mg/L are considered to elevate the risk of calcium tartrate instability for a sparkling base, as recommended by the AWRI, but are not considered a risk for a still Sauvignon Blanc wine.

4.3. Impact of fining wine with carrageenan on wine sensory properties

In recent studies, no taints were observed in any wines fined with commercially available carrageenans (Marangon et al., 2013; Stockdale, 2016). Descriptive sensory analysis was undertaken on a 2015 and 2016 Adelaide Hill Sauvignon Blanc fined with different treatments of carrageenan, as well as with bentonite and an unfined control (Stockdale, 2016).

In 2015, sensory descriptive analysis was completed on the cold soluble kappa- (Na+) and kappa-/iota- carrageenans tested in the V15 winemaking trials. The bentonite-fined Sauvignon Blanc for the V15 winemaking trials had lower intensities for most of the sensory attributes described including viscosity, flint, astringency, passionfruit, box hedge and sweaty/cheesy compared to the untreated control, and several of the carrageenan treatments. This is consistent with the literature (Sanborn et al., 2010; Lira et al., 2015) and the common winemaking belief that bentonite can strip wine of its flavour characteristics. The Sauvignon Blanc fined with carrageenan gave wines with higher intensities of tropical fruit-related attributes including: box hedge, sweaty and passionfruit compared to the same wine fined with bentonite. Carrageenan-fined wines were also higher in the viscosity attribute for the 2015 winemaking trials.

Wines fined with the pure kappa- (K+) carrageenan underwent descriptive sensory analysis for the V16 winemaking trials. For the 2016 Sauvignon Blanc wines, there was a significant difference in sensory properties because of the stage of the addition of the fining agent i.e. whether the pure kappa- carrageenan was added at the juice, ferment or wine stage. 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 had lower intensities of the fruity aromas. The bentonite wine rated lowest of all of the treatments in intensity of all fruity attributes and yellow colour intensity and was higher in green, vegetal and bitter attributes.

For both the 2015 and 2016 winemaking trials of an Adelaide Hills Sauvignon Blanc the bentonite treatment had the lowest intensity of fruity attributes.

5. Addition of carrageenan to juice or wine

As with bentonite, adding carrageenan powder to a liquid to form a slurry or stock solution is a two-step process that involves:

1. Good dispersion of the powder into the liquid.
2. Mixing with sufficient energy and time to achieve hydration, or swelling.

5.1. Viscosity of carrageenan in wine

The viscosity of carrageenan in a wine solution is impacted by the type of carrageenan salt (Na^+ or K+) and the ionic composition of the wine. Table 5.0 describes the levels of key cations in wine. Potassium is the most abundant metal in wine and is also a strong gelation agent for kappa-carrageenan (Campo et al., 2009). Wine can also contain varying levels of sodium (Na^+) and calcium (Ca^{2+}). Cold-soluble (Na^+) carrageenan is known to be soluble in cold water hence, it was initially recommended by suppliers because they thought that it may be easier than kappa (K+) carrageenan to mix into the wine.

*Table 5.0. Composition of white wine. Juice will have higher levels of potassium and tartaric acid, as these compounds drop out 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

Table 5.1 outlines the viscosity measurements of different types of carrageenan in water and wine. The viscosity of the carrageenan slurries differs more in wine than in water, likely due to the complex ionic composition of wine. More understanding of the rheology of carrageenans in wine would help the development of better ways to add carrageenan to juice or wine.

Table 5.1. Viscosity data based on a 1 – 6 rating at 25 g/L solution where 1 = low viscosity, 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

Anecdotally, in pilot-scale winemaking trials, the cold-soluble kappa- (Na^+) carrageenan took longer to disperse and hydrate than kappa- (K^+) carrageenan. There was also limited evidence that the cold-soluble kappa-carrageenan settled better in the tank, possibly due to the formation of stronger gels. Such hypotheses are yet to be conclusively proven.

5.2. Wine processing stage to add carrageenan

Carrageenans can be added to the juice, ferment or wine stage and still result in a heat stable wine. However, outcomes from pilot winery trials indicated that adding carrageenan at the fermentation stage heat stabilises the wine more reliably than in unsettled juice, and also heat stabilises at a lower concentration of carrageenan than when the fining agent is added at the wine stage. There is also some evidence that adding at the fermentation stage could result in better compaction of the lees and improve wine recovery, although this hypothesis needs to be tested further (Stockdale, 2016).

It is recommended to add carrageenan during yeasting, when the juice has been warmed to 14 – 16°C, which 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 six hours to complete, doing a trial for each ferment would not be practical. Most likely the winery would have to determine for each vintage, a standard rate addition per variety and region ascertained through practice and time; for example 0.8 g/L for a cool-climate Chardonnay, 1.2 g/L for Sauvignon Blanc, etc. This could, however, not completely heat stabilise the wine if a more protein-rich batch went through and would require a ‘top up’ with a small addition of carrageenan post-fermentation. Adding carrageenans to unsettled juice could be beneficial, if the fining agent is added to perform the dual function of heat stabilisation and clarification. For example, the carrageenan could be added via an in-line injection system to a flotation tank, centrifuge or decanter.

Unlike bentonite, carrageenans do not have a good sedimentation rate or settling properties when added to juice or wine. Carrageenans should therefore be added at stages in the winemaking process prior to the generation of lees such as during juice clarification or before the separation of wine from yeast or tartrate lees by racking or centrifugation. Natural juice or wine lees would then facilitate sedimentation or separation of the carrageenan lees, along with the juice or wine solids.

5.3. Determining the rate of carrageenan addition to the juice ferment or wine (bench scale)

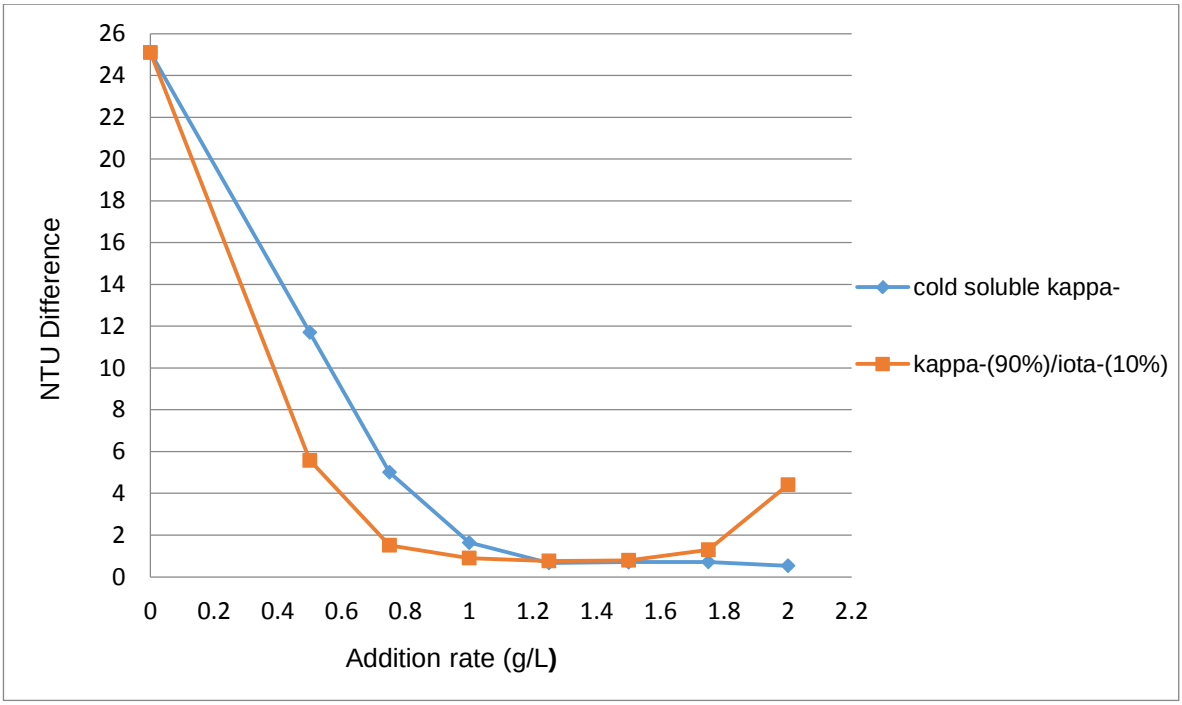
The rate of carrageenan required can be determined using a similar method to how the bentonite rate is determined in either juice or wine (Iland et al., 2004). It is important that juice samples are pectin-negative following treatment with pectolytic enzymes. For juice, this step is required because pectins will interfere with the juice's turbidity reading and cause filtration difficulties resulting in an invalid heat test. All samples should be filtered with a 0.45µm membrane and give an initial turbidity reading of less than 1 NTU prior to heat testing.

For bench lab trials, carrageenan stock solutions should be prepared at a specific concentration (1%; 10 g/L) in juice or wine, using a homogeniser to ensure a well-mixed solution Figure 5.1(a). Stock solutions are prepared by adding a small amount of the carrageenan powder to the juice or wine and allowing it to fully disperse for around 10 minutes, then adding the next small quantity of powder.

Fining trials to determine the amount of bentonite or polysaccharide required to achieve heat stability can be performed by adding an increasing dosage of fining agent to juice and wine from the stock solution and mixing well. Samples may be settled at room temperature for two hours, or overnight. Samples would then be filtered to 0.45um and submitted to heat test at 80°C for two hours and cooled to ambient temperature for two hours. Heat turbidity haze was measured by calculating the difference between heated (final) and unheated (initial) samples in nephelometric turbidity units (NTU) by means of a nephelometer. Samples with differences of <2 NTU were considered heat-stable.

Figure 5.0 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 addition rate to account for any variation in the method. Hence, in the fining trials described in Figure 5.0, the addition rate to the 2015 Adelaide Hills Sauvignon Blanc juice would be 1.0 g/L cold soluble kappa-carrageenan and 1.2 g/L kappa-/iota- carrageenan.

Figure 5.0. Determining the fining rate for V15 pilot-scale winemaking trials for Adelaide Hills Sauvignon Blanc juice.



5.4. Mixing and adding carrageenan (commercial scale)

A potential limitation of adding carrageenan to juice or wine is that due to gelation properties of the fining agent, a maximum stock solution concentration of 2% is possible before the carrageenan solution becomes saturated. This results in a rather large stock solution of around 12% of the total volume of the product. A typical bentonite slurry for a winery may be made up to a 20% v/v stock solution or slurry.

Systems typically used for adding bentonite to wine include a dedicated slurry tank or tub with an impeller mixing system extending approximately two thirds into the slurry vessel (Figure 5.1(b)). While wearing appropriate personal protective equipment (PPE), bentonite powder is slowly added to the mixing vessel (tub) until the bentonite is dispersed, then another quantity of bentonite powder is added, and so on. This system would also likely be adequate for preparing a carrageenan slurry, but it is also possible that the stock volumes prepared using existing winery mixing tubs would not be of sufficient size for the volume of slurry required for very large winery tanks.

Figure 5.1. a) Homogeniser used for bench trials of carrageenans; and b) Mixing tub for preparing bentonite slurry.



a)



b)

The methods for mixing and adding carrageenan outlined in sections 5.4.1 – 5.4.2 are untested for commercial use, as carrageenan is currently not a permitted additive in wine; therefore, there has not been an opportunity to run commercial-scale trials. It is recommended when developing methods for adding carrageenans to juice or wine that this is done in consultation with the carrageenan supplier, as they will have experience of the methods used to add carrageenan to products from other food industries.

5.4.1. In-line injection systems

Figure 5.2 illustrates an in-line injection pump system used with a decanter that clarifies juice lees. The Reynold's number at the inlet to the equipment following dosing of fining agent into the juice stream should be sufficient to ensure the juice changes from laminar to turbulent flow. For centrifuges and decanters, this may be achieved by placing an in-line static mixer on the outlet of feed pump to ensure that the fining reagent is in suspension and is fully dispersed. Contact time for carrageenan to react with the juice or wine is unknown and trials such as those by completed by Muhlack et al. (2006) may be required to determine the contact time for carrageenan to react within an in-line dosing system to heat stabilise a juice or wine.

Most flotation systems have a built-in injection system for fining agents or alternatively, specific tank flotation dosing pumps such as the Juclas Easy N_2 flotation pump. These systems could be used for adding carrageenan to unsettled juice. However, testing of carrageenans in these systems cannot occur until they are approved as permitted additives.

Figure 5.2. a) In-line pumping systems to inject fining agent into a juice-clarifying decanter (b)



a)



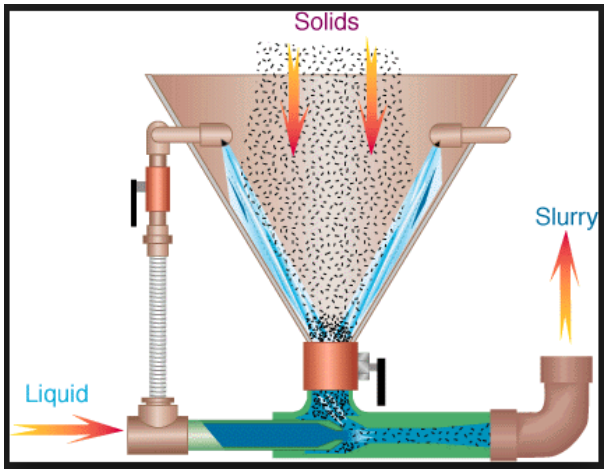
b)

5.4.2. Eductor systems

Eductors have been recommended by carrageenan suppliers as potential systems for preparing and dispersing stock solutions of carrageenan into either juice or wine. Eductors are systems without moving parts, which are designed to blend solids with liquids to continuously produce solutions or well blended slurries.

In an eductor system (Figure 5.2), juice or wine flows through a Venturi, which draws the carrageenan powder from the funnel into either a juice or wine stream so that the carrageenan reaches a region of strong agitation. This process wets the carrageenan particles so that effective hydration can occur. The slurry could be fed into a further tank fitted with an agitator to continue mixing and facilitate full hydration. Commercially available eductor systems are provided by a number of engineering equipment suppliers.

Figure 5.2. Eductor system for mixing carrageenan powder with juice or wine.



5.5. Impact of carrageenan on time to complete fermentation

In 40L pilot-scale winemaking trials on Adelaide Hills Sauvignon Blanc, adding carrageenan to either juice or ferment increased the time taken to complete fermentation by an average of 1.6 days in V15, and 1.0 days in V16. The reason for this is not clear, however it is possible that the carrageenan polysaccharides bind to a nutrient that facilitates fermentation, thereby slightly slowing the process.

Table 6.3. Days to complete fermentation for V15 and V16 pilot winery trial 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

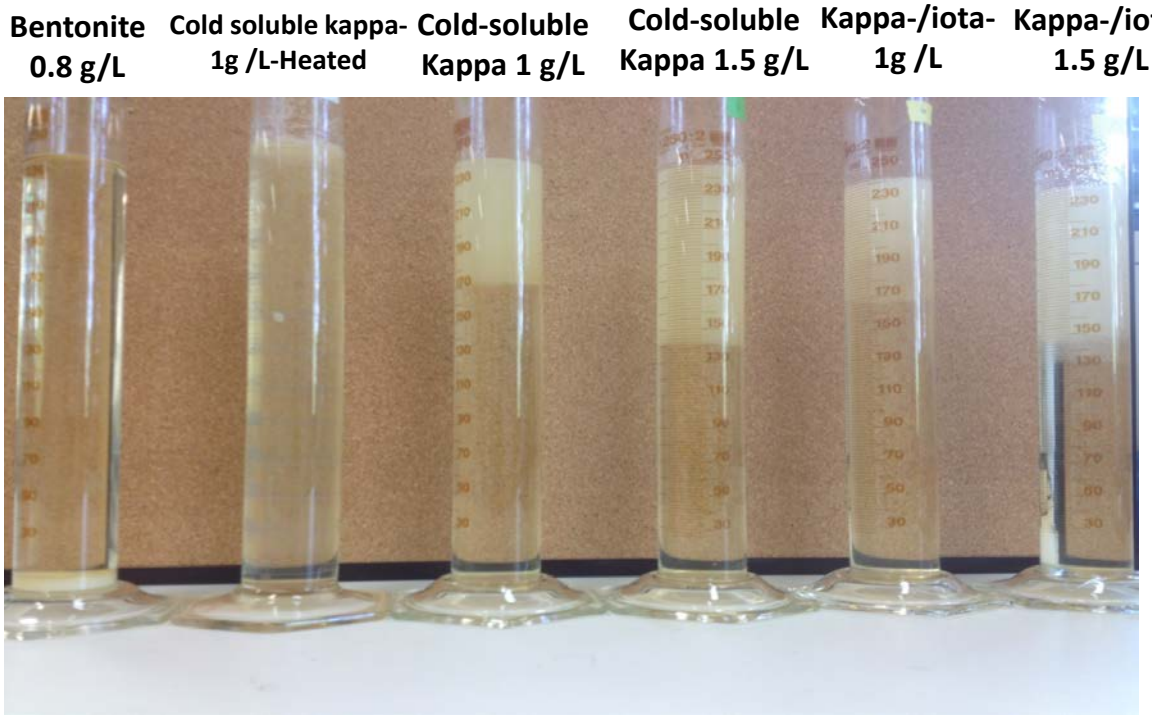
#Average of replicates.

The time to complete fermentation could be important, as during vintage it is useful to having a faster turnaround of tanks between fermentations to facilitate tank utilisation. However, the difference in time to complete fermentation was considered small. The TWE 1301 project had an Industry Reference Group (IRG) and when consulted, the winemakers did not consider the slight increase in fermentation time to be a hindrance.

5.6. Recovery of carrageenan lees from juice or wine

Unlike bentonite, carrageenan is not a good settling agent. Figure 5.3 illustrates that when added to a clarified wine, carrageenan does not settle. Therefore, carrageenan should be added at stages in winemaking that already generate lees, so that it could be removed as part of existing or established lees clarification processes. These stages could include the addition of carrageenan to unclarified juice, prior to or during fermentation, or to wine containing gross fermentation lees, or prior to cold stabilisation, which generates tartrate lees.

Figure 5.3. Sedimentation properties of cold soluble kappa- $(Na)^+$, and kappa- (90%) /iota- (10%) blends of carrageenan using a sedimentation test in a 2014 McLaren Vale Chardonnay that was heat-unstable but cold-stable.

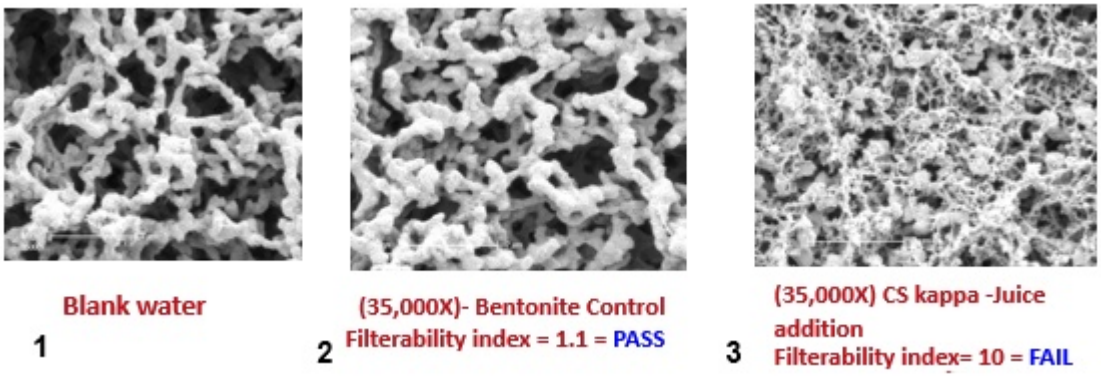


Clarification of carrageenan lees has not yet been tested on a commercial scale. Current methods of lees clarification in wineries include Rotary Drum Vacuum (RDV), centrifuges, decanter, rotating ceramic cross flows and wide-bore cross flows. It is recommended that the clarification of carrageenan lees should be tested with the established lees processing equipment.

6. Filtration and Bottling

Current evidence suggests that carrageenans should to be added in conjunction with pectolytic enzyme treatment when added at 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; however, if carrageenans are not removed by sedimentation they can block depth filters. Figure 6.0 shows Scanning Electron Microscope (SEM) images of nylon membrane filters, which have been used to filter wine fined with a cold-soluble (CS) kappa-carrageenan added at the juice stage, where it was not treated with pectolytic enzyme.

Figure 6.0. SEM images of 0.45um nylon membrane filters use to filter: 1. Water; 2. Wine fined with bentonite; 3. Wine fined with cold-soluble kappa-carrageenan at the juice stage, where the juice was not treated with pectolytic enzyme



In Figure 6.0, membrane strands of a polymer that is possibly carrageenan can been seen blocking the pores of the filter. It is therefore recommended that wine fined with carrageenans is well racked, centrifuged or filtered with a tangential cross-flow prior to fine depth filtration.

7. Shelf life

At the time of writing, a 12-month shelf life study had been completed on V15 wines fined with cold-soluble kappa- (Na^+) carrageenan, as well as kappa- (90%)/iota- (10%) blended carrageenans at the juice, ferment and wine stages. The resulting wines were still heat stable post-bottling.

8. Cost

Currently, bentonite costs approx. $1.50 - $2 per kg, whereas carrageenans can range in price from $27 - $48 per kg. The use of carrageenans will therefore not be cost-effective unless improved recovery of wine or juice from carrageenan lees relative to bentonite lees can be achieved, which would offset the higher materials cost.

We currently do not have solid data on lees recovery following carrageenan treatment to heat stabilise wine and this should be a component of any future work.

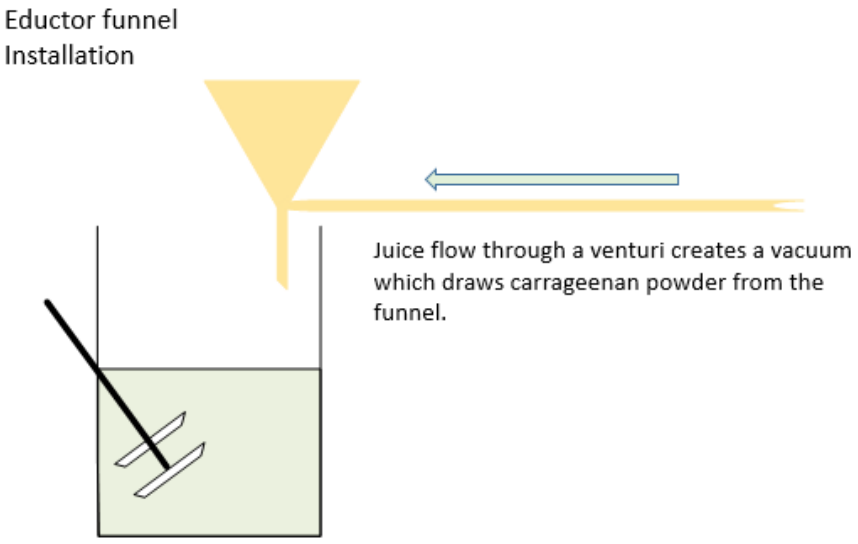
9. Proposed scenario for using carrageenans in commercial-scale production

Carrageenan can be added to the juice, ferment or wine stage and still result in a heat-unstable wine. A possible scenario for using carrageenan during white winemaking is described below, based on results from 40L pilot winery trials. However, if using carrageenans in a production facility becomes feasible and is eventually permitted under the Food Standards Code, other scenarios may well be developed.

Carrageenan could be added to clarified juice at the same time the yeast is added to the tank. Pilot winery trials have indicated that carrageenan added at the fermentation stage heat stabilises the wine more reliably than addition to unclarified juice and also heat stabilises at a lower rate of fining agent than when added at the wine addition stage. There was also some indication that adding carrageenan at the fermentation stage could result in better compaction of the lees and improve wine recovery.

Carrageenan could be added during yeasting using an eductor system (Figure 9.0) to disperse and promote hydration of the polysaccharide. This would occur in conjunction with yeast addition and the mixing of the tank, using the tank agitator for around 30 minutes. This is also when the juice has been warmed to 14 – 16°C, which would promote both fermentation and the reaction of the carrageenan. Alternatively, carrageenan could be added in the early stages once fermentation has started, provided the fermentation is not too active, as this may cause foaming issues.

Figure 9.0. Proposed system of eductor funnel addition of carrageenan to a yeasting tank.



Given that bench trials to determine the amount of carrageenan to add to a particular ferment may take at least six hours to complete, doing a bench trial for each ferment would not be practical. Most likely the winery would have to determine for each vintage, a standard rate addition per variety and region ascertained through bench trials, practice and time. For example, 0.8 g/L carrageenan for cool-climate Chardonnay, 1.2 g/L for Sauvignon Blanc etc.

A standard addition rate could however, not completely heat stabilise the wine if a more protein-rich batch went through. In such a case, a ‘top up’, or a small addition of carrageenan post-fermentation may be required to achieve heat stability. The wine should be heat tested prior to cold stabilisation, so that if an extra addition of carrageenan is required, it can be added and mixed prior to cold stabilisation and removed in conjunction with tartrate lees.

10. Conclusions

To date, the types of carrageenan polysaccharides that have been found to consistently heat stabilise white wines are the kappa-carrageenans and a specific a blend of kappa- (up to 90%) and iota- (up to 10 %) carrageenans. Either the sodium or the potassium salt of these carrageenans can be used. Compared to bentonite, in most cases, slightly less carrageenan was required to heat stabilise the same wine (Marangon et al., 2013; Stockdale, 2016).

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. The cold-soluble (Na^+) type kappa-carrageenan increased sodium levels in the wine three-fold compared to the control wine, which had no fining agent added. Wineries should monitor calcium levels when using carrageenans as the calcium level in the wine can increase slightly, but significantly.

Unlike bentonite, carrageenans can be added to 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 adverse sensory impact. This could result in processing benefits by 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. Carrageenan is not a good settling agent in wine and therefore, should be added at stages in winemaking that generate wine solids (lees) e.g. during juice clarification, fermentation or prior to cold stabilisation so that the carrageenan can be removed, along with the juice or wine lees. Current evidence suggests that carrageenan must be used in conjunction with pectolytic enzyme to avoid commercially unviable filtration difficulties.

Although the sensory aspects of carrageenans were favourable, particularly when compared to bentonite which can strip wines of aromas, the wines fined with carrageenan were more expensive to make than with the traditional process. Currently, bentonite costs approx. $1.50 - $2 per kilogram, whereas carrageenans can range in price from $27 - $48 per kilogram.

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