Claristar™ : a new ingredient for tartrate stabilisation

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Claristar™

A new ingredient for tartrate stabilisation

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Claristar™
tartaric stabilizer

Introduction

The preventative property of mannoproteins on tartrate crystallisation continues to raise considerable interest in the wine industry. However, at this time, little attention has been placed on the diversity of yeast mannoproteins and the quality of their effects in wine.Yeast mannoprotein is actually a large family of molecules and their specific composition and configuration confer their particular functionality and stability in wine. With more than a century of expertise in yeast technology and in the wine ingredients business, DSM has now identified the mannoprotein fraction which has the optimal functionality against tartrate crystallisation. This technology forms the base for a new wine ingredient, marketed under the brand name Claristar™.
In this time of increasing concern for our environment,Claristar™ provides a natural alternative to existing physical treatments like cold stabilisation or electrodialysis without any additional energy requirements, water consumption or effluent. Claristar™ is a liquid ingredient which can be directly added into the wine before bottling, thus maximising efficiency and minimising process change.
As for performance, the product has been extensively tested by the tartrate stabilisation experts at the Station Experimentale d'Oenologie INRA, Pech Rouge.
This article presents some of the research findings to date.

Claristar™ - The Natural Solution to Tartrate Stability

Various studies with wine aged on lees have shown that yeast, after alcoholic fermentation, release numbers of compounds such as mannoproteins, peptides, amino-acids and nucleic acids. Lubbers *et al.* showed that mannoproteins had a positive effect on tartrate stability. Further studies have confirmed that wine aged for several months on lees becomes relatively stable to tartaric precipitation and does not therefore require cold stabilisation (Ribéreau-Gayon *et al*., 2000 b). Following many years of dedicated research and development, DSM scientists and enologists have added to this knowledge and developed a new product to prevent tartaric precipitation in wines. This has involved developing a much greater understanding of the biochemistry of mannoproteins and their effects in wine. An equivalent challenge has been the development of an efficient process to extract the targeted mannoprotein fractions from the yeast.

In the recent past, mannoproteins used in enology were typically obtained by two different means:

- **A physical method** consisting of a heat treatment of yeast cell walls at very high temperatures (e.g. 120 ºC).
- **An enzymatic method** using ß-glucanases hydrolyzing yeast cell walls to release mannoproteins.

Both of these methods can give rise to unsatisfactory results. The mannoproteins isolated by such means have been found to be partially insoluble and/or to contain insoluble impurities. A post-treatment remedy would be to remove all insoluble material from the wine by filtration, taking the risk to remove functional mannoproteins as well. In addition to the above mentioned problems, the mannoprotein fraction obtained by these methods often has limited effectiveness against potassium bitartrate (KHT) crystallisation.
The technology developed by DSM now allows the isolation of the perfectly soluble mannoprotein fractions which are highly active in the prevention of KHT crystallisation. In this procedure, whole yeast cells are hydrolysed to yield a yeast extract and insoluble material.
Selected mannoproteins are then isolated from the yeast extract using ultrafiltration. Finally, the mannoprotein fraction is further purified to improve solubility and maximize functionality in wine. To preserve their unique properties,

DSM mannoproteins are kept in their natural liquid state which also makes the product very easy to use for the winemaker.

Tartrate Stability - Mechanisms and Existing Treatments

Potassium bitartrate is soluble under certain conditions of temperature and pressure. In wine, this salt is in an unstable state of super-saturation which can lead to crystal formation under certain conditions such as low temperatures. This phenomenon is known as tartaric precipitation or crystallisation.

Wine stability is influenced by a variety of factors including: tartaric acid content, concentration of potassium and calcium ions, pH, ethanol concentration, temperature and the presence of colloids.

Nowadays several treatments are used to prevent the precipitation of tartrate salts. The oldest, described by Scazzola E. (1956), consists of metatartaric acid addition into the unstable wine. This molecule prevents the growth of KHT crystals. However, its action is limited in time because of its gradual hydrolysis into free tartaric acid. This actually results in an increase of the super-saturation state which favours crystallisation of potassium tartrate (Carafa, 1958).

Cold stabilisation, another commonly used treatment, is characterised by long term (weeks) conservation of the wine at low temperature. Cold induces the formation of the potassium tartrate crystals. This technique can be accelerated by the addition of cream of tartar (solution of potassium tartrate micro crystals) which plays the role of crystallisation initiator. Once formed, the crystals grow and can then be removed by filtration.

A more recent method is based on electrodialysis technology. The wine is recycled between plates. The electric potential difference applied between these plates forces the migration of molecules through a selective membrane and thus removes ionic material from the wine (Saint Pierre *et al.*, 1995).

Mannoproteins - Definition and Properties

Mannoproteins are natural components of the yeast cell wall (Figure 1). The cell wall of *Saccharomyces cerevisiae* is composed of 90 % polysaccharides (glucans and mannans) but also contains proteins, lipids, phosphates, chitin and minerals. The architecture of the yeast cell wall has been reviewed extensively by Kapteyn *et al.* (1999) and by Lipke *et al.* (1998). Mannoproteins are released into the wine, firstly during alcoholic fermentation and then during yeast autolysis as wine is aged on the lees. Naturally prevalent in wine, mannoproteins represent the second largest family of wine polysaccharides (Vidal *et al.*, 2003).

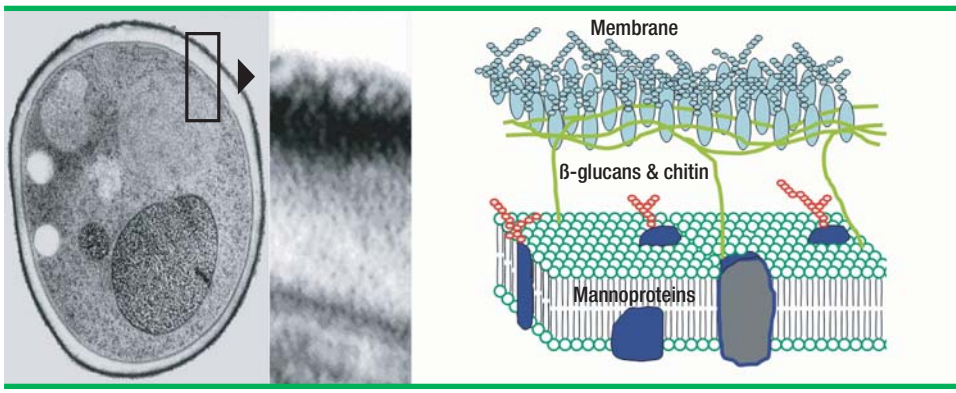

***Figure 1**:*
scanning electron microscopy view of a yeast cell and schematic representation of the yeast cell wall.

Mannoproteins are highly branched structures of mannose residues, linked by various glycosidic bonds and a polypeptide chain. Their molecular weight can reach as high as 800 kDa. Though all mannoproteins have a common structure (Figure 2), they do present a large diversity in their composition, type of glycosidic linkages and organisation. These differences are responsible for their functional properties and effects in wine, from mouthfeel improvement to induced tartaric stability.

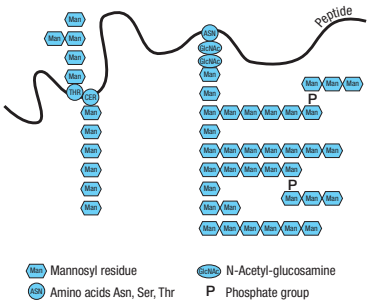

***Figure 2**:*
representation of the structural organization of Mannoproteins.

The protective colloidal effect of mannoproteins is well known in winemaking. Increased stability of wine when aged on lees has been observed for many years. Subsequently other properties have been described, such as mannoproteins stabilising protein haze (Ledoux *et al.*, 1992; Waters *et al.*, 1994) and more evidence on potassium tartrate stability (Lubbers *et al.*, 1993; Moine-Ledoux and Dubourdieu, 1997; Moine-Ledoux and Dubourdieu, 1999).

Claristar™
tartaric stabilizer

Mannoproteins appear to prevent the formation of crystals. It has been observed that a partial or complete elimination of protective colloids during the winemaking process generates a modification of the wine equilibrium leading to loss of tartaric stability. The mechanism of action is described to be based on a competitive inhibition, limiting crystal formation (Moutounet *et al*., 1999). It is generally agreed upon that mannoproteins do inhibit the nucleation (initial step of crystal formation) whilst their effect on the crystal-growth is less important. As a consequence, wine protection against tartaric instability can only be achieved, when there are no existing crystals in the wine (Moutounet *et al.* 1999).

Material and Methods

Validation trials described in this article have been performed in collaboration with the Station Experimentale d'Oenologie INRA Pech Rouge with wines from the 2004 and 2005 vintages.

Materials

Wine

■ *Blend of Grenache blanc and Viognier 2004 with a DIT of 18 % (Lot A) and 22.5 % (Lot B).*
■ *Sauvignon blanc 2005 with a DIT of 22 %.*

Mannoprotein

Claristar™ produced by DSM. This product can be used for the tartaric stabilisation of wine in compliance with the OIV International Oenological Codex and with European Council Regulation EC No 2165/2005 of 20-12-2005.

Methods

The wine was divided into two 10 L lots for the Grenache blanc / Viognier blend and 35 hl for the Sauvignon.

The first lot was an untreated control and the second was treated with Claristar™ at the dosage of 20 g/hl expressed in dry matter. All wines were previously fined with bentonite at 30 g/hl and filtered on Kieselguhr white earth.

Methods for wine characterisation

The following analyses were performed on the control wine and after the addition of Claristar™

- *Potassium concentration (measured by photometry).*
- *Tartaric acid concentration (measured by HPLC Ionic Chomatography).*
- *Turbidity (measured by nephalometry).*
- *Conductivity (with a reference at 20 °C)*
- *pH.*
- *Vmax (Filtration flow rate at 1 Bar on a 25 mm diameter membrane and a cut of 0,65 µm). Reference supplied below.*
- *Coating index (Filtration flow rate at 2 Bar on a 25 mm diameter membrane and a cut of 0,65 µm). Reference supplied below.*
- *DIT: 4 g/l of standard KHT crystals were added to the wine at - 4 °C, with constant stirring at 500 rpm. (Model based on "contact" treatment used in wineries). Conductivity was monitored for 4 hours and data extrapolated until equilibrium. The instability was expressed as the conductivity decrease percentage.*

Most commercial wines have a DIT of 20-25 % at time of bottling.

Methods to measure wine stability

- *Cold stabilisation: the wine was stored at - 4 °C. The visual appearance of the wine was checked daily until crystals were visible (0 no crystal, *suspicion of crystals, **visible crystals). A commercial wine is generally considered to be stable when no crystals are formed after 6 days.*
- *ISTC 50: 50 mg KHT was dissolved into 100 ml of wine. The wine was cooled down to - 4 °C with constant stirring at 500 rpm and conductivity monitored. The results were expressed in time (min) needed to get a conductivity decrease. The test is comparable to the cold stabilisation test at - 4 °C but gives a faster response.*

A commercial wine is generally considered to be stable when no crystals are formed after 180 min for red and rosé wines or after 120 min for white

	Grenache - Viognier 2004 Lot A		Grenache - Viognier 2004 Lot B		Sauvignon 2005	
	Before	After	Before	After	Before	After
Alcoholic degree	13.08	13.06	12.59	12.61	-	-
pH	3.43	3.43	3.42	3.42	3.39	3.37
Turbidity (NTU)	0.8	0.9	2	3.6	0.5	0.7
Conductivity µS/cm 20 °C	1638	1645	1836	1843	1708	1725
Tartric acid (g/l)	-	-	-	-	1.85	1.7
K+ (mg/l)	-	-	-	-	753	768
V max	-	-	-	-	4872	1044
Coating index	-	-	-	-	8	36
DIT	18 %	18.6 %	22.5 %	20 %	22 %	20.4 %

***Table 1**: wine composition before and after treatment with Claristar™.*

Results and Discussion

Effect on wine composition

Alcoholic degree, pH, turbidity, conductivity, tartaric acid concentration, potassium concentration, Vmax, coating index and DIT were measured on three different wines before and after the addition of Claristar™. The results are presented in Tables 1 and 2.

■ **Claristar™ did not affect the wine composition nor the turbidity (Table 1). The addition of Claristar™ impacted Vmax and coating index within acceptable limits for commercial bottling processes.**

Effect on wine flavour profile

Claristar™ was added at a dose of 20 g/hl (d.m.) to Sauvignon and Chardonnay. Each wine was tasted and compared to the control.

■ **A triangular test (n = 42) concluded that no significant difference was detected in flavour profile for either wine (Risk ß 50 % of 0.1 % and <0.01 %).**

Effect on wine stability

Stability tests at - 4 °C and ISTC 50 were completed on both wines before (control) and after the addition of Claristar™ (T0 = just after wine treatment, T12 = after 12 months storage at 18 °C). The analyses after 12 months were only performed on the Sauvignon 2005.

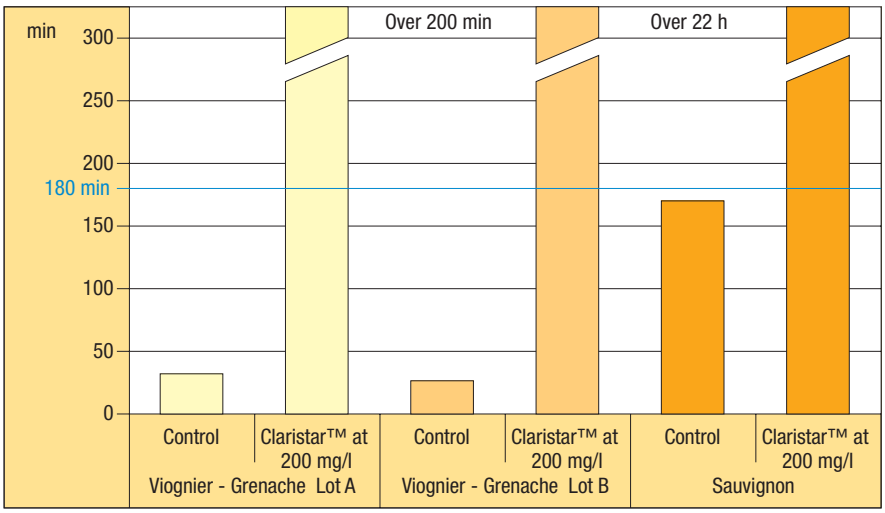

Figure 3:
ISTC 50 (experiments conducted at INRA). Time when conductivity decrease (min) as a function of wine type.

■ **Figure 3 shows the effect of Claristar™ on the ISTC 50 on Viognier / Grenache and Sauvignon. Results are expressed in time necessary to obtain a conductivity decrease. A wine is generally considered to be stable when the time for detectable drop in conductivity is greater than 180 min.**

■ **Figure 3 shows that all the untreated wines were unstable (25 min for the Viognier-Grenache and 160 min for the Sauvignon). All the wines treated with Claristar™ were stable (times exceeding 180 min).**

0: no crystal **: suspicion of cristal* ***: visible crystals*	Grenache - Viognier Lot A		Grenache - Viognier Lot B		Sauvignon	
	Control	Claristar™ at 200 mg/l	Control	Claristar™ at 200 mg/l	Control	Claristar™ at 200 mg/l
Day 1	*	0	*	0	0	0
Day 2	**	0	**	0	*	0
Day 3	-	-	-	-	**	0
Day 4	-	-	-	-	**	0
Day 5	**	0	**	0	**	0
Day 6	**	0	**	0	**	0
Day 7	**	0	**	0	**	0
Day 8	**	0	**	0	**	0
Day 9	**	0	**	0	**	0
Day 10	**	0	**	0	-	-
Day 16	-	-	-	-	-	-
Day 29	**	0	**	0	**	0
Day 33	**	0	**	*	-	-
Day 34	**	0	**	**	-	-
Day 36	**	0	**	**	-	-
Day 37	**	0	**	**	-	-

***Table 2**: effect of Claristar™ on the kinetics of crystal appearance.*

■ **Table 2 shows the effectiveness of Claristar™ when monitored with the cold stabilisation test at - 4 °C.**

■ **Table 2 shows that crystals appeared in the control wines within one or two days. However, when Claristar™ had been added, the appearance of crystals was delayed to more than 29 days at -4 °C. Therefore, the addition of Claristar™ improved the stability of these highly unstable wines.**

Effect on conductivity

Conductivity was measured over time for the Sauvignon 2005. Results are presented in Figure 4.

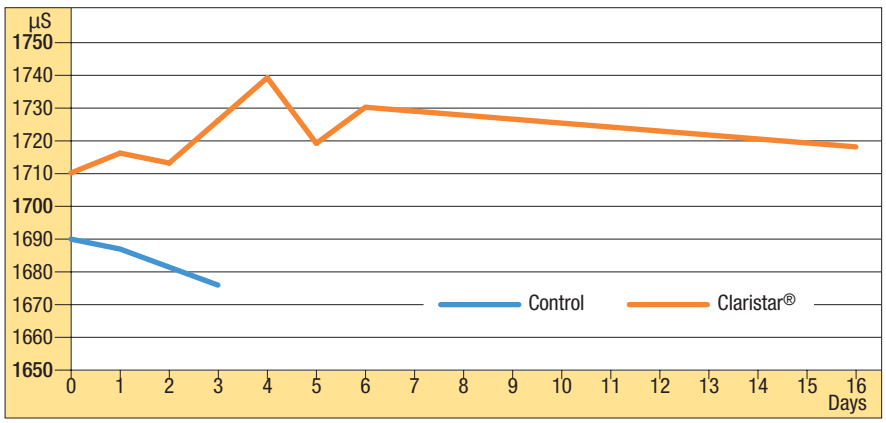

***Figure 4**:*
Conductivity as a function of time.

■ **Significant conductivity decrease was observed on day 3 in the untreated wine. No conductivity decrease was observed in the Claristar™ treated wine after 16 days at - 4 °C. This result is in line with the visual observations and confirms the protective effect of Claristar™.**

		Control		Claristar™ at 200 mg/hl	
		Conductivity	Visual	Conductivity	Visual
ISTC 50 min		110		>300	
Conductivity µS/cm 20 °C	Day 0	1702	-	1718	-
	Day 1	1717	0	1735	0
	Day 2	1719	0	1737	0
	Day 3	1702	*	1761	0
	Day 4	1654	**	1746	0
	Day 5	1620	**	1738	0
	Day 6			1734	0
	Day 7			1724	0
	Day 8			1739	0
	Day 9			1739	0
	Day 16			1719	0

0: no crystal
**: suspicion of cristal*
***: visible crystals*

***Table 3**:*
conductivity and visual observation at -4 °C as a function of time and ISTC 50 after 12 months stored at 18 °C.

Stability of wine over time

The stability of the Sauvignon was checked after 12 months storage at 18 °C. The results are presented in Table 3. Visual observation, conductivity measurements and ISTC 50 were repeated.

The results indicate that even after 12 months storage the wine treated with Claristar™ remained stable (ISTC 50 > 300min and no crystals were observed after 16 days at - 4 °C). In conclusion, Claristar™ was highly effective in long term stability to KHT crystallisation.

Conclusions

These studies summarise the compounding evidence behind the new mannoprotein ingredient, Claristar™.
This specific mannoprotein fraction proved to be very efficient at preventing tartrate crystallisation across all the wines tested for a period of at least 18 months.
Stability trials on this wine continue to this day. Furthermore, Claristar™ did not impact wine composition nor taste.
Studies are continuing with INRA including benchmarking against other existing tartrate stabilisation techniques.
Results to date confirm that Claristar™ is a highly effective and easy to use solution for the problem of tartrate precipitation. Further results will be published later this year.

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