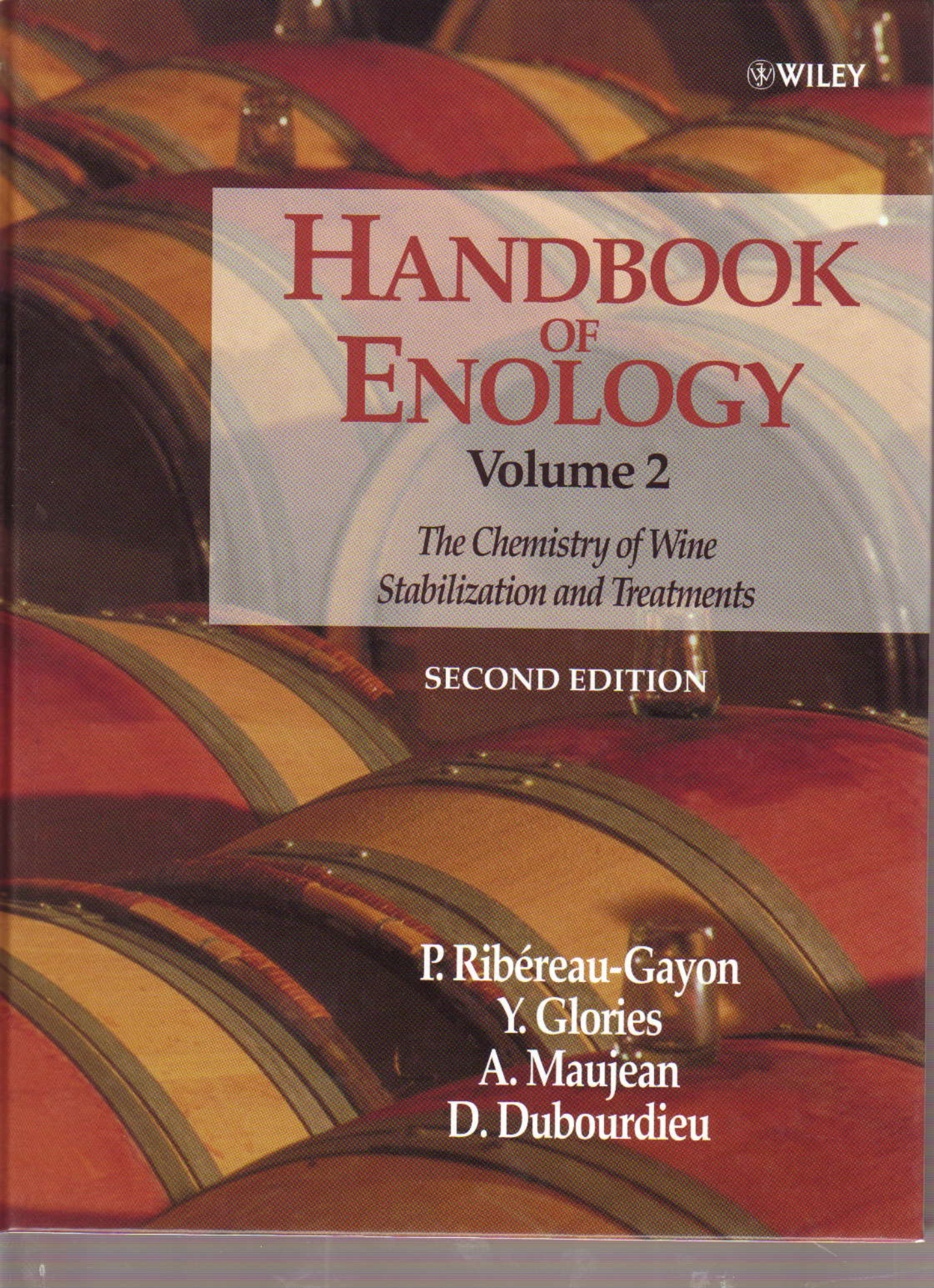
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The Chemistry of Wine
Stabilization and Treatments
SECOND EDITION
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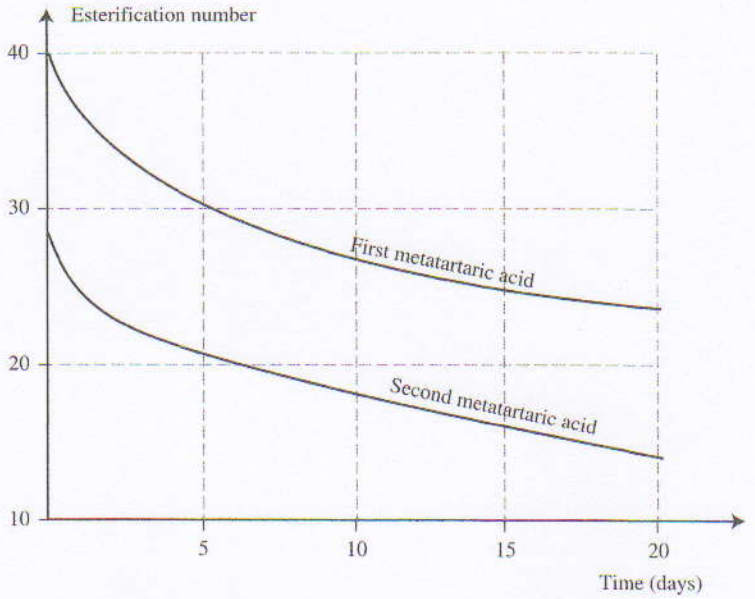

Fig. 1.20. Hydrolysis rate of two qualities of metatartaric acid in 2% solution (t = 18–20°C), followed by a decrease in the esterification number (Ribéreau-Gayon *et al.*, 1977)

One year to eighteen months at temperatures varying between 10°C in winter and 18°C in summer
Three months at 20°C
One month at 25°C
One week at 30°C
A few hours between 35 and 40°C

Metatartaric acid instability accounts for initially surprising observations concerning wines treated in this way. One sample, stored at 0°C in a refrigerator, had no precipitation, while calcium tartrate precipitation occurred in another sample stored at 20–25°C when it was no longer protected due to hydrolysis of the metatartaric acid.

The conditions for using metatartaric acid depend on its properties. A concentrated solution, at 200 g/l, should be prepared in cold water at the time of use. As metatartaric acid is strongly hygroscopic, it must be stored in a dry place.

Metatartaric acid is added after fining, as there is a risk of partial elimination due to flocculation. It is particularly affected by bentonite and potassium ferrocyanide treatments. Although there was some cause for concern that high-temperature bottling would reduce the effectiveness of metatartaric acid, in fact, under the actual conditions where it is used, this technique has little or no negative impact (Section 12.2.4). Incidentally, a slight opalescence may be observed after a wine has been treated, especially when the most efficient products, with high esterification numbers, have been used. It is therefore recommended that metatartaric acid be added before the final clarification.

1.7.7 Using Yeast Mannoproteins

It is well known that wine, especially red wine, naturally contains macromolecules that act as protective colloids (Section 9.4.2). At concentrations present in wine, these substances tend to hinder tartrate crystallization, but do not completely inhibit it (Section 3.6.5). Little research has been done into isolating these crystallization inhibitors in wine and making use of their stabilizing properties. On the contrary, for many years, major efforts were made to eliminate these colloids, by drastic fining and filtration, as they reduce the effectiveness

of physical stabilization treatments, especially cold stabilization.

It is known, however, that the traditional practice of barrel-aging white wines on yeast lees for several months often gives them a high level of tartrate stability, so that cold stabilization is unnecessary (Section 12.3.2). Although, in practice, this phenomenon is very widespread, very little mention of it has been made until now in enology theory. Thus, in Bordeaux, most dry white wines aged on the lees are not stable in March after their first winter, but become stable by June or July without any further treatment. When the same wines are not aged on the lees, they must be systematically cold-stabilized to protect them from tartrate crystallization. As it was known that white wines are enriched with mannoproteins released by the yeast during aging on the lees, it was reasonable to suppose that these macromolecules contributed to the tartrate stabilization of wine.

Yeast mannoproteins were first found to have a certain inhibiting effect on tartrate crystallization in a model medium by Lubbers *et al.* (1993). However, these experiments used mannoproteins extracted by heat in alkaline buffers, under very different conditions from those accompanying the spontaneous enzymic release of mannoproteins during aging on the lees. Furthermore, the effectiveness of mannoproteins extracted by physical processes in preventing tartrate precipitation has not been established in most wines, despite demonstrations in a model medium.

The discovery of the crystallization-inhibiting effect of mannoproteins extracted by the enzymic treatment of yeast walls (Dubourdieu and Moine-Ledoux, 1994) adds a new dimension to this subject. The mannoprotein preparations are obtained by digesting yeast walls with an industrial preparation of β-(1–3)- and β-(1–6)-glucanases (Glucanex™), permitted in winemaking as a clarifying enzyme for improving the filtrability of wines made from botrytized grapes (Sections 3.7.2 and 11.5.2). These preparations inhibit tartrate crystallization in white, red and rosé wines, whereas the same dose (25 g/hl) of heat-extracted mannoproteins does not have this stabilizing effect (Moine-Ledoux and Dubourdieu, 1995).

The inhibiting effect of mannoproteins extracted from yeast on tartrate crystallization is not due to compound MP32, the invertase fragment responsible for protein stabilization in wine (Section 5.6.4) (Dubourdieu and Moine-Ledoux, 1996). The mannoproteins in question are more highly glycosylated, with an average molecular weight of approximately 40 kDa. They have been purified (Moine-Ledoux *et al.*, 1997) from the same mannoprotein preparations, obtained by the enzymic treatment of yeast walls.

Furthermore, it has been demonstrated that these mannoproteins share covalent bonds with glucane (Moine-Ledoux and Dubourdieu, 1999). They remain in the cell walls treated simultaneously with sodium dodecyl sulfate (SDS) (which cuts the hydrogen bonds) and β-mercaptoethanol (Figure 1.21), which do not affect osidic bonds.

The presence of peak 2, corresponding to elution of the mannoprotein responsible for tartrate stabilization, confirms that the bond is covalent. Some of the mannoproteins that share covalent bonds with glucane also have a special type of glycosylation, leading to a glycosyl-phosphatidyl-inositol (GPI). The use of a mutant strain (FBYII), deficient in GPI-anchored mannoproteins when cultured at 37°C (FBYII-37), showed that the mannoproteins responsible for tartrate stabilization had this type of glycosylation. Two types of mannoprotein extracts were obtained by enzyme hydrolysis of yeast cell walls (FBYII), cultured at 24°C or 37°C.

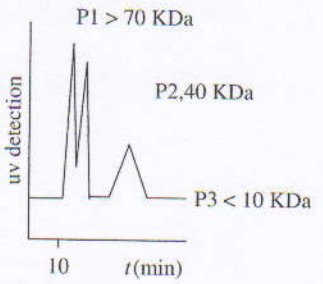

Fig. 1.21. HPLC analysis of molecular-screened mannoprotein extract obtained by enzyme digestion of cell walls treated simultaneously with SDS and β-mercaptoethanol

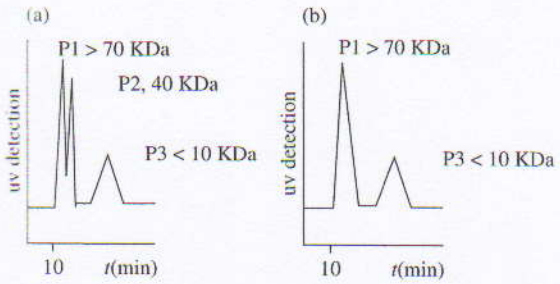

Fig. 1.22. HPLC analysis of molecular-screened mannoprotein extract obtained by enzyme digestion of (a) FBYII-24 and (b) FBYII-37 yeast cell walls, cultured at 24°C and 37°C, respectively

HPLC analysis of these two extracts (Figure 1.22) showed that peak 2 was absent when the cell walls came from yeast cultured at 37°C, i.e. deficient in GPI-anchored mannoproteins. These results: (1) show that the mannoproteins responsible for tartrate stabilization are GPI-anchored and (2) explain why they are only extractible by enzyme digestion.

An industrial preparation (Mannostab™) has been purified from yeast-wall mannoprotein. It is a perfectly soluble, odorless, flavorless, white powder. This product has been quite effective (Table 1.20) in preventing tartrate precipitation in white wine samples taken before the normal cold stabilization prior to bottling. Initial results show that Mannostab™ inhibits potassium bitartrate crystallization at doses between 15 and 25 g/hl. However, in certain wines in Table 1.13 (1996 white Bordeaux and 1996 white Graves), larger quantities apparently reduced the stabilizing effect. A similar phenomenon has been reported with a protective colloid used to prevent protein precipitation (Pellerin *et al.*, 1994). The dose of Mannostab™ necessary to stabilize a wine must be determined by preliminary testing. It is very clear that the use of excess amounts of this additive is inefficient.

The addition of this product could replace current stabilization methods (Moine-Ledoux *et al.*, 1997). With this in mind, its effectiveness has been compared to that of two other tartrate stabilization methods: continuous contact cold stabilization and the addition of metatartaric acid (Table 1.21). This comparison was carried out by measuring spontaneous crystallization after the addition of KHT (Section 1.6.4). The values obtained indicate the effectiveness of protective colloids, even if they do not necessarily correspond to the instability temperatures. The addition of 15 g/hl of Mannostab™ to wine 2 and 25 g/hl

Table 1.20. Tartrate stabilization of various wines by adding Mannostab™. Visual observation of potassium crystallization after 6 days at −4°C (Moine-Ledoux *et al.*, 1997)

Wines		Mannostab™ (g/hl)				
		0	15	20	25	30
1996 Blanc de Blanc	Visual test	[a]	0	0	0	0
	$\Delta(K^+)$ (mg/l)	52	72	17	0	0
White *vin de table*	Visual test	[a]	0	0	0	0
	$\Delta(K^+)$ (mg/l)	104	53	33	0	0
1996 white Bordeaux	Visual test	[a]	0	0	0	0
	$\Delta(K^+)$ (mg/l)	62	21	0	0	21
1996 white Graves	Visual test	[a]	[a]	0	0	0
	$\Delta(K^+)$ (mg/l)	155	52	0	0	62
1996 white Bordeaux	Visual test	[a]	0	0	0	0
	$\Delta(K^+)$ (mg/l)	51	0	0	0	0
1996 Entre Deux Mers	Visual test	0	0	0	0	0
	$\Delta(K^+)$ (mg/l)	52	0	0	0	11

[a] precipitation; 0, no precipitation.

Table 1.21. Effect of different treatments on the spontaneous crystallization temperature of various wines (Moine-Ledoux *et al.*, 1997)

Stabilization treatments	Wine 1	Wine 2
Control	−10°C	−11°C
Mannostab™ (15 g/hl)	−21°C	−18°C
Mannostab™ (25 g/hl)	−31°C	−13°C
Continuous contact cold	−28°C	−17°C
Metatartaric acid (10 g/hl)	−40°C	−40°C

Wine 1, 1996 Entre Deux Mers; Wine 2, 1996 white Bordeaux.

to wine 1 produced the same spontaneous crystallization temperature, i.e. a stability comparable to that obtained by continuous cold stabilization (Table 1.21). The addition of metatartaric acid, however, considerably reduced the crystallization temperature.

However, metatartaric acid is hydrolyzed in wine, and loses its effectiveness, while adding tartaric acid may even facilitate potassium bitartrate crystallization. Under the same conditions, mannoproteins are stable and have a durable protective effect on tartrate crystallization. To demonstrate this difference, white wines treated with metatartaric acid or Mannostab™ and kept at 30°C for 10 weeks were then subjected to a cold test. Crystallization occurred in the sample treated with metatartaric acid, while the Mannostab™ sample remained stable (Table 1.22).

This new treatment process to protect wines from tartrate precipitation has been used experimentally in France since 1997 (Moine-Ledoux and Dubourdieu, 2002). Mannoprotein preparation treatment of white wine is registered in the OIV

Table 1.22. Influence of keeping a white wine supplemented with metatartaric acid or Mannostab™ at 30°C for 10 weeks on the tartrate stability, estimated by the decrease in potassium concentration after 6 days at −4°C (Moine-Ledoux *et al.*, 1997)

	$\Delta(K^+)$ mg/l, after 6 days at −4°C
Control	200
Metatartaric acid (10 g/hl)	260
Mannostab™ (25 g/hl)	0

International Code of Oenological Practice. Their findings are likely to lead to the authorization of this type of treatment in the near future.

1.7.8 The Use of Carboxymethylcellulose

Carboxymethylcellulose (CMC) is a polysaccharide. Like metatartaric acid and mannoproteins, its polymer structure gives it "protective colloid" characteristics. It is obtained by priority etherification of the primary alcohol functions of the glucopyranose units (Figure 1.23) linked by β-type stereochemical 1–4 etheroxide bonds. A CMC is, therefore, characterized partly by the degree of etherification of its alcohol functions, known as the degree of substitution (DS), and partly by its degree of polymerization (DP), i.e. the average number of glucopyranose units per polymer molecule. This mean number indicates that a given CMC, such as metatartaric acid, is a polymer with a dispersed molecular weight.

A DS of 0.65 means that, out of 100 glucopyranose units, 65 have been etherified by sodium

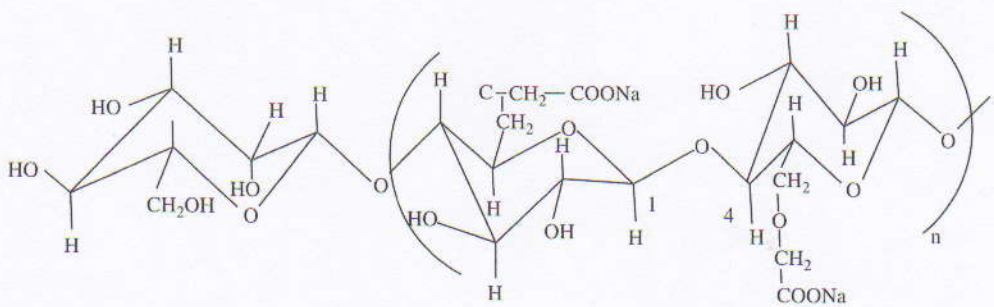

Fig. 1.23. Structure of a carboxymethylcellulose (CMC) chain