FINING AGENTS PART 1: PROTEINACEOUS FINING AGENTS

Paul K. Bowyer - LAFFORT Australia, 5 Williams Circuit, Pooraka SA 5095, Australia. paul.bowyer@laffort.com.au www.laffort.com

This is the first in a two-part series of LAFFORT inforbriefs on fining agents. In Part 1 we focus on proteinaceous fining agents, which are commonly used for the removal of phenolics. Discussions are presented to support the characteristics of each fining agent.

INTRODUCTION

Fining is a term that is broadly applied, yet not always fully understood in the wine industry. It is a generic term used to describe a range of processes aimed at using the addition of selected agents to a wine in order to refine its stability and/or organoleptic characteristics in terms of appearance, aroma, palate or all of these. Unusually for a winemaking process, the average consumer is familiar with the practice of fining, even if they don't know it. Consider, for example, how many people in the world add milk to their coffee and tea. They do this perhaps because it adds a creamy texture, but importantly it also alters the appearance and reduces the astringency and bitterness of the beverage. How, then, do these processes occur, and what is really happening in wine during fining? In this first article of a two-part series we will delve into the world of proteinaceous fining agents, examining their characteristics and modes of action.

THEORY OF PROTEIN-TANNIN FINING

Protein structure

Proteins have four distinct types of structure (Figure 1; Shiflet, 2002). The primary structure (a) is simply the amino acid sequence of the protein. The secondary structure (b) refers to the shape that the primary structure takes on in three dimensions. The tertiary structure (c) refers to the folding and interactions of various regions of the same protein molecule, whilst the quaternary structure (d) is obtained when different protein molecules interact with one another, such as in haemoglobin.

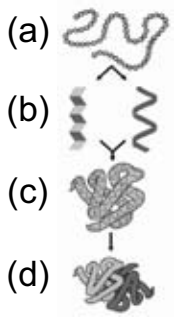

Figure 1. The four components of protein structure: (a) Primary structure; (b) Secondary structure; (c) Tertiary structure; and (d) Quaternary structure.

The formation of the secondary structure is driven in large part by the hydrophobicity, or "water-fearing" sections of the protein chain. The hydrophobic sections tend to align or overlap in space to minimize contact with water molecules, thus generating shapes such as pleated sheets and helices (figure 1b).

Since proteins are comprised of amino acids, and amino acids are responsive to pH changes (being acids), proteins too can alter their physical shape and chemistry as the pH of the medium changes. This is reflected in their solubilitv.

which changes according to pH as indicated in figure 2. The pH at which there is zero nett charge on the protein is called the protein's isoelectric point (pI) (Bowyer and Moine-Ledoux, 2007), and at this pH the protein is least soluble. Thus, the protein pI indicates its solubility in wine. As the medium pH moves away from the pI, solubility increases in concert with the increasing charge on the molecule, which aids aqueous dissolution.

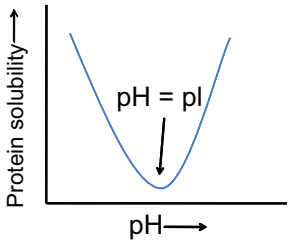

Figure 2. Graph showing the variation in protein solubility with changes in pH of the medium.

Proteinaceous fining agents used in oenology bear positive charges in wine, since their pI's are all above typical wine pH. Care must be used in their preparation, such as the avoidance of high temperatures during preparation and application, to limit structural change and subsequent reduced effectiveness of the fining agent.

Tannin structure

Tannins as a class of chemicals are based on one of two core structures: flavonoid or non-flavonoid. The difference between these core tannin structures is discussed elsewhere (Bowyer, 2002; Bowyer et al., 2007). Loosely, this class of tannins is derived initially from grapes with subsequent modification during winemaking and ageing, and they fall into one of three groups: monomers (ie one discrete flavonoid subunit), oligomers (a small number of flavonoid subunits) and polymers (many flavonoid subunits). The important characteristic of both flavonoid and non-flavonoid tannins is the commonality of the phenolic subunit (figure 3), which is why the term "phenolic" is used as a reference term for a tannic species.

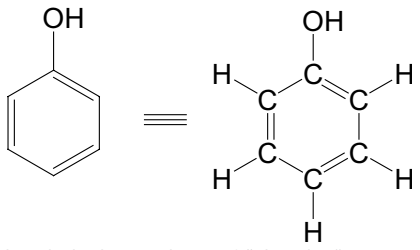

Figure 3. Phenol, the basic subunit of "phenolics" compounds, including tannins.

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The phenolic subunit is also relatively hydrophobic, and it is this factor that limits the solubility (and therefore the extractability) of grape tannins early in the fermentation. More tannin is progressively extracted as the alcohol content of a red must increases, since the polarity (Bowyer, 2003) of the must medium changes from entirely aqueous (ie no alcohol, all water and very polar) to partially alcoholic (some alcohol, less water, less polar). The tannins, being less polar than water, are thus extracted to a greater extent as more of the similarly less polar alcohol is produced. It is for this reason that winemakers wishing to produce red wines of softer tannin profile often press their wines off with some residual sugar remaining, below the maximum alcohol production, in order to limit the extraction of tannin (particularly seed tannin).

Protein interaction with phenolics: protein fining

The interaction of tannins and proteins initially involves a two-stage process (figure 4). Firstly, the hydrophobic regions on the tannin and protein move into close proximity in order to exclude water and lower the energy of the system. Secondly, hydrogen bonds (Bowyer, 2003b) are formed, which serve to lock the two structures together. At this stage the process is reversible, and excessive energy being applied to the system (e.g. heating of the wine) is likely to lower the effectiveness of the fining process.

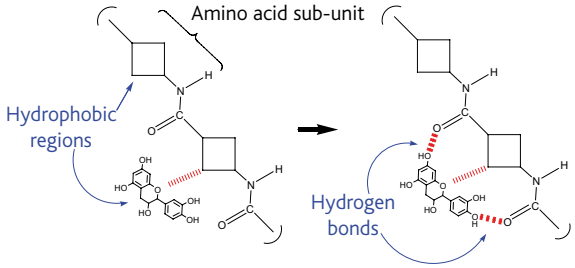

Figure 4. The two stages of tannin-protein interaction: hydrophobic association followed by hydrogen bonding.

Once the protein-tannin association is complete flocculation follows, where the associated complexes aggregate, which is in turn followed by precipitation. This process is, in part, governed by the concentration of the added protein. When this concentration is low, simple association occurs. When the protein concentration is high, cross-linking occurs between sites of association, affecting the overall reactivity and function of the fining agent.

COMMON PROTEINACEOUS FINING AGENTS

Gelatine

Gelatine (figure 5) is perhaps the most technical of the proteinaceous fining agents, and there are several different types available on the market. Gelatine is derived from the hydrolysis of collagen, a triple-helical structural component, from the bones and skins of animals, typically cattle (bovine) or pigs (porcine), and it is used in many industries, even outside of food and beverage production. This results in a distribution of protein sizes in the gelatine, which in turn affects the effectiveness of the fining action, and explains its relatively broad activity towards tannins of various sizes. It is perhaps one of the most widely-used and controversial fining agents. Gelatine can be used on juice or wine.

Figure 5. Examples of liquid and finely granulated commercial gelatines, in this case **LAFFORT Gecoll Supra®** (liquid) and **Gecoll®** (powder).

Relatively few types of gelatine are developed specifically for use in winemaking (only 1-5% of the total gelatine product pool), and not all are suitable for wine application (Ribéreau-Gayon et al., 2006a). The two factors having the largest impact on the effectiveness of a given gelatine solution are the charge density on the proteins (the higher the charge, the greater the fining effect) and the mass distribution of the proteins (Ribéreau-Gayon et al., 2006a).

The differences in mass distributions of commercial gelatines is of great importance to fining, as is it well known that the reactivity of tannins towards gelatine varies dramatically with the size of the protein strand (Yokotsuka and Singleton, 1987). No linear relationship exists between the concentration of the gelatine, it's effectiveness in winemaking nor it's impact on wine sensory characteristics, as these factors are directly dependent on the raw materials used, the way in which the gelatine solution is produced and the tannins that the gelatine is interacting with in the wine (Ribéreau-Gayon et al., 2006b). Figure 6 indicates the protein mass distribution of two commercial gelatines of equal protein concentration. The significant difference in the distribution of protein sizes clearly means that each product will react with the tannin in a given wine in different ways. LAFFORT's Gecoll Supra®, for example, is produced by first selecting raw materials within a tightly-defined specification, then refining and formulating specifically and only for oenological application. The protein fractions in Gecoll Supra® are so active in terms of oenologically-appropriate mass distribution and charge density that if the product is made in higher concentration or it is chilled it gels. High protein concentrations also make it far more difficult to ensure complete dispersion in the total volume of the wine before the fining reaction (which is quite rapid) occurs. Thus, quality control, method of production and product development are extremely important factors in the production of gelatine products for winemaking. Table 1 illustrates the difference in the average molecular weights of a series of commercial gelatine preparations, and is indicative of how different commercial gelatine products can be in terms of composition and, by extension, how different their fining characteristics will be.

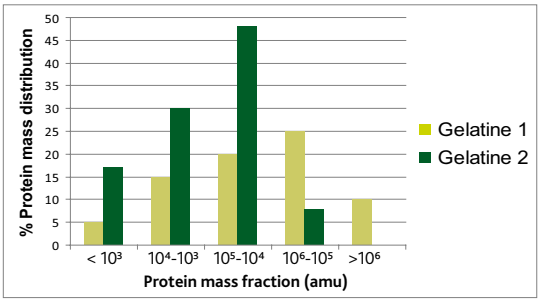

Figure 6. The protein mass distribution of two commercial gelatine solutions with the same protein concentration Given that the size of the protein strands in each product is so different, the impact of each in terms of fining effectiveness and wine organoleptic characteristics will clearly be different.

Commercial gelatine	Mean molecular weight of component proteins (amu)
A	129,300
B	44,500
C	42,600
D	13,000
E	7,500
F	15,400

Table 1. Mean molecular weights (atomic mass units) of a series of commercial gelatines.

In white wine fining the co-fining agent silica gel is often used, the purpose of which is to avoid over-fining. Typically the silica gel colloidal solution is added to the wine prior to the gelatine solution. Over-fining occurs when the fining activity is too localised, interacting heavily with one part of the wine in a tank and not the total wine volume in an even manner. Thus, gelatine over-fining is more likely as the concentration of the gelatine solution increases, and consequently high-concentration gelatine solutions must be used with caution and high speed of application.

Aside from over-fining, solubility is also a problem with gelatine. As gelatine is highly soluble in wine, it is plausible that fractions that are less reactive towards phenolics will remain in the wine, with potentially detrimental impact on wine quality. Gelatine is also thermally stable, and so residues will not be detected in a heat test (Boulton et al. 1999). Bovine spongiform encephalopathy (BSE) is another concern, hence bovine-derived gelatine products must be certified BSE-free. A simple solution to this is to usegelatines derived from pork.

Isinglass

Isinglass (figure 7) is derivative of the support and connective tissue collagen, and is made from certain fish species. Typically the swim bladder is used, but sometimes the source is skin and structural tissue. Unlike gelatine, it denatures at a lower temperature and so retains more of its original collagen-like structure, being degraded into larger protein fragments of the collagen sub-structure tropocollagen. This in turn affects the target tannin species in a fining operation: since the molecular weight of isinglass is very high, it tends to react with smaller tannins (oligomers), and over-fining is less likely. Isinglass is typically only used on white wines and can be frustrating to prepare as a stock solution, as dissolution is poor and the end result is nearer to a gel, making additions difficult. Poor quality isinglass is said to deliver a "fishy" odour to the wine, but it is prized by some winemakers for the low addition rates and brilliance that it imparts to the wine with the penalty of slowly formed diffuse lees.

Figure 7. An example of powdered isinglass, in this case **LAFFORT Ichtyocolle®**. Note the slightly coarser grain compared with the gelatine (figure 5).

Casein

Like isinglass, casein (figure 8) is typically only used in white wines. It is a very high molecular weight protein isolated from fat-reduced milk, and some winemakers still persist in using skim milk directly. The use of skim milk is likely to decline, however, given that it does not come with certification (certificate of analysis, non-GMO etc). Also like isinglass, casein can be difficult to use, since the pI is very close to wine pH. The result of this is virtual instant insolubility on addition to wine, and rapid flocculation. The implication for treating large volumes of wine with casein is that adequate and very fast dispersion is required, but difficult to achieve in practice. The potassium salt form of the protein is often used to aid in the preparation process, as it is far more soluble and therefore user-friendly than the protein itself. Casein is noted for its ability to remove oxidative browning, often being used on juice for this specific purpose and over-fining with casein is difficult due to its poor solubility in wine. Solution: Choosing thermally tolerant yeast strains and attending the rehydration phase.

Figure 8. An example of granular potassium caseinate, in this case **LAFFORT Casei Plus®**.

Egg albumin

Albumin (ovalbumin) is the major protein of many found in egg whites. Egg albumin a medium weight protein and is classically associated with the fining of red wines, due to its noted lack of reactivity towards smaller anthocyanin-tannin complexes and therefore lower colour removal. It is not typically used on white wines or on youthful red wines.

Figure 9. An example of powdered egg white, in this case **LAFFORT Albumine d'oeuf®**.

PRATICAL APPLICATION OF PROTEINACEOUS FINING AGENTS

An historical knowledge of vineyard fruit characteristics is a valuable tool when it comes to wine production and, specifically, fining activities, as it allows the winemaker to make an educated guess in terms of the most appropriate fining agent, dosage rates and likely sensory outcomes. Table 2 provides a roug guide for the use of the proteinaceous fining agents, nevertheless the manufacturer's recommendations for dosage rates should be adhered to.

Fining agent	Phenolic target size	Dosage
Gelatine	Medium-large	40-100 mL/hL [l]
Isinglass	Small	1.25-2.5 g/hL [p]
Casein	Small-medium + oxidative browning	10-50 g/hL [pk]
Egg albumin	Medium-large	5-15 g/hL [p]

Table 2. Approximate target tannin sizes and approximate dosage rates for proteinaceous fining agents. l = liquid; p = powder; pk = powder of potassium salt. Suggested dosage rates from (Ribéreau-Gayon et al., 2006)

Also as a general guide, table 3 provides some relative information between the proteinaceous fining agents in terms of wine phenolic impact.

	Colour removal	Phenolic removal	Tendency to overfine
Highest impact	Gelatine	Gelatine	Gelatine
	Casein	Albumin	Albumin
Lowest impact	Albumin	Isinglass	Isinglass
	Isinglass	Casein	Casein

Table 3. A rough comparison of proteinaceous fining agent characteristics in terms of decreasing impact on the wine. More accurate relativities cannot be provided due to the variable nature of products from different manufacturers and the wines being fined.

SUMMARY

Deciding which proteinaceous fining agent will work best in a given wine or juice depends directly on many factors, but ultimately on what the goals of the winemaker are. Fining trials must always be performed to firstly elucidate the most appropriate fining agent(s) to use and secondly to determine the rate of application. It is becoming increasingly difficult for winemakers to use products that do not have appropriate certification and traceability, hence the future use of non-certified products like fresh egg white and skim milk is dubious.

The next article will discuss non-proteinaceous fining agents.

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Products discussed in this article are available in Australia through LAFFORT, phone 08 8260 7974 and in New Zealand from Oenological Resources (Greg Wilkin: greg@oenological.co.nz), phone 0213 22290. www.laffort.com

Dr Paul Bowyer is the Technical Manager for LAFFORT, and in that role he provides technical information and advice to the wine industry in the Australasian region. He can be contacted on 08 8260 7974 or by email at paul.bowyer@laffort.com.au

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