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PROTEIN FINING AGENTS FOR JUICES AND WINES

by
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Winemakers occasionally choose to use protein fining agents for the purpose of color, palatability, stability, and/or clarity modification. These agents include gelatin, casein, isinglass, egg albumin, and the "protein-like" compounds such as polyvinylpyrrolidone (PVP).

Protein fining agents bind principally with wine and juice phenols. These protein compounds can be particularly useful in modifying juice and wine astringency, bitterness, and color. Singleton (1980), as shown in Table 1, estimated the phenol composition of several model wines. It is possible to effect changes in the composition of wine phenols by processing variations, including the use of protein fining agents.

Table 1. Gross phenol composition estimated in mg GAE/L for typical table wines from *Vitis vinifera* grapes.

Phenol class	Source[a]	White wine		Red wine	
		Young	Aged	Young	Aged
Nonflavonoids, total		175	160-260	235	240-500
Cinnamates, derivatives	G, D	154	130	165	150
Low volatility benzene deriv.	D, M, G, E	10	15	50	60
Tyrosol	M	10	10	15	15
Volatile phenols	M, D, E	1	5	5	15
Hydrolyzable tannins, etc.	E	0	0-100	0	0-260
Macromolecular complexes "Protein"-"tannin"	G, D, E	10	5	5	10
Flavonoids total		30	25	1060	705
Catechins	G	25	15	200	150
Flavanols	G, D	tr	tr	50	10
Anthocyanins	G	0	0	200	20
Soluble tannins, derivatives	G, D	5	10	550	450
Other Flavonoids, derivatives	G, D, E, M	?	?	60?	75?
Total phenols		215	190-290	1300	955-1215

[a] D = Degradation Product; E = Environment, Cooperage; G = Grapes; M = Microbes, Yeast.

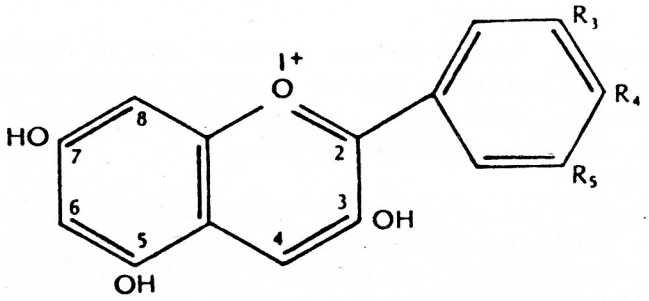
Nonflavonoids. The nonflavonoid phenolics consist principally of cinnamic and benzoic acid derivatives. Hydroxycinnamates and caftaric acid are the major nonflavonoids of grapes. Nonflavonoids are relatively constant in red and white wines because they are easily extracted from the pulp. A large part of the juice phenols consist of tartaric acid acylated with caffeic, p-coumaric, or ferulic acids. Most of the members of this group are present individually below their sensory threshold. Added together, they may contribute to bitterness and perhaps a spicy odor in wines. Tyrosol is produced by yeast, during fermentation, from the amino acid tyrosine and may account for some bitterness in white wines and certainly does account for some bitterness in apple wines (Singleton and Nobel 1976). Tyrosine, hydroxybenzoates, and hydroxycinnamates are known to be bitter (Singleton 1980).

Phenols from oak are almost all hydrolyzable nonflavonoid phenols. One year's storage in new French oak (200L) can contribute at least 250 mg/l GAE nonflavo-

noid phenols (Amerine et al. 1980). American white oak contributes about 1/2 of the above level of phenols.

Flavonoids. The structure of naturally occurring flavonoid compounds consists of two aromatic rings, "A" and "B", linked via an aliphatic 3-carbon chain. Methoxylation and/or hydroxylation of the aromatic components yields such compounds as catechins, Flavan 3,4 diols (leucoanthocyanidins), and anthocyanins. Flavonoids are essentially confined to the skins and seeds. Polymeric flavonoids comprise the major fraction of total phenols in all stages of winemaking.

Flavylium Cation



Catechins (Flavan-3-ols) are precursors to browning in white wines and precursors to bitterness and browning in reds. Catechins account for most of the flavor in light white wines made with no skin contact (Singleton and Noble 1976). These compounds are bitter with no or moderate astringency (Amerine et al. 1980). Their taste threshold in white wine is about 200 mg/1 GAE.

Leucoanthocyanidins (Flavan 3,4 diols) are flavonoid phenols that appear to serve as principle building blocks for tannin polymers. Increased extraction of these compounds is considered responsible for the decreased quality and increased nonharmonious bitter, heavy, or coarse taste in white wines produced from frosted grapes.

Anthocyanins as a group are estimated to have a mild threshold effect on teh richness of young red wine flavor. As red wines age, nearly all of the anthocyanins are incorporated into larger compounds.

The term tannin as it relates to wine phenols causes some confusion. The term was first introduced to describe a group of compounds present in plants which can tan animal skin to produce leather. The word tan is derived from the Latin form of a Celtic word for oak, an extract of oak being a common tanning agent. To be a good tanning agent, a polyphenol should contain enough phenolic hydroxyl groups to form effective cross links with protein - i.e., to be able to tan. Whether or not a phenol exhibits reactions considered as characteristics of tannins [protein precipitation, formation of blue color upon reaction with ferric iron (Fe^{+++}), and astringency] depends primarily upon molecular weight. Phenols eliciting such reactions characteristically have molecular weights from 500 - 3,000. Simple phenols are too small to form effective cross links with most protein fining agents and are therefore not considered tannins.

Rossi and Singleton (1966) showed the relationship between various protein fining agents and phenol reduction. As indicated in Table 1, aging modifies wine phenols by oxidation, polymerization, and precipitation. The phenols of juice and wine can also be attenuated by the use of fining agents. Table 2, from Rossi and Singleton (1967) shows examples of the specificity and capacity of several protein and synthetic polymers for phenol complexing.

Table 2

Examples of the Capacity and Specificity of Precipitants for Phenol Fractions
(Equivalent adsorption, tannic acid g/100 g of agent)

Grape-seed fractions in wine-like model solution

Agent	Amount (mg/l)	Tannin (Polymers)	Anthocyanogens (dimers or larger)	Catechins (monomers)
Gelatin	25	126	112	0
	50	117	110	0
	100	114	86	0
	200	102	77	0
Ammonium caseinate	150	79	80	–
	300	86	61	–
Isinglass	150	100	120	0
	300	91	110	0
Nylon 66	Column	2.8	10.5	7.5
Polyclar AT	Column	1.5	25.0	19.0

The important linkage between phenols and proteins is hydrogen bonding between the phenolic hydroxyls of the phenol (-OH) and the amide bonds of the proteins (R-C). These bonds are relatively weak, 2 to 10 Kcal/mole, compared to C-C covalent bonds, which are about 60 Kcal/mole (Singleton 1967). The strength of the hydrogen bond formed is greater the more acidic the media and the more negative the attracting group. The carbonyl of the amide is the primary attracting group and hydrogen bonds of decreasing strength form with phenolic hydroxyls, alcoholic hydroxyls, and amine-amide hydrogens.

The capacity of a protein fining agent is a function of the number of potential hydrogen binding sites per unit weight. The process of properly hydrating ('swelling') protein fining agents and properly adding them to juice or wine can therefore be seen to affect binding.

The selectivity of a protein-type fining agent for different phenols depends upon optimal type, placement, and geometry of bonding sites to give a stronger total bonding with one phenol over another (Singleton 1967). The phenol-agent combination that results in the strongest total hydrogen bonding usually occurs. The phenols with the msot available hydroxyls (or other hydrogen binding group) per molecule are often absorbed preferentially. As such, larger molecules like tannins are preferentially removed by fining because larger molecules have more total phenolic groups and therefore more hydrogen bonding potential. About 10 H bonds are as strong as one carbon-to-carbon bond. Therefore, a strong complex is usually formed with dimeric and larger tannins and only weak bonds with smaller phenols.

Another important aspect of fining selectivity is the solubility and flexibility to align itself with the phenolic hydroxols of the phenols to form a 'good fit'. Insoluble agents generally have a much lower capacity for phenol removal than soluble agents, owing to less surface available for interaction with phenols (Iwano 1967).

Table 3 (Boulton 1981) shows the relative effect various fining agents have on some important parameters. This listing is dependent upon the age ofthe wine, temperature, etc. For example, small soluble anthocyanins are incorporated into larger molecules as wine ages. If a wine is fined early with a protein fining

agent such as gelatin, there will be a much less dramatic effect on the color than if the fining occurred following aging (Bergeret 1963). The opposite phenomona is observed when fining with bentonite.

Table 3

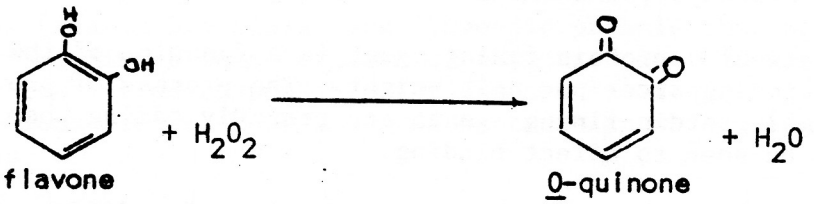
COMPARISON OF FINING AGENTS[1]

Color Removal	Tannin Removal	Quantity of Lees	Tendency to Overfine	Clarity and Stability	Quality Impairment
carbon	gelatin	bentonite[2]	gelatin	bentonite	carbon
gelatin	albumen	gelatin	albumen	ferrocyanide	bentonite
casein	isinglass	casein	isinglass	cufex	casein
albumen	casein	albumen	casein	carbon	gelatin
isinglass	bentonite	isinglass	ferrocyanide	isinglass	albumen
bentonite	carbon	ferrocyanide	cufex	casein	isinglass
ferrocyanide	ferrocyanide	cufex		gelatin	ferrocyanide
cufex	cufex	carbon		albumen	cufex

[1]Most to least effect

[2]Usually twice the lees of other agents

Fining to remove brown pigments from wine often occurs. Brown pigments are initially quinone oxidation products of phenols, which condense and polymerize with other phenols. Quinones cannot hydrogen bond with substances such as nylon. This may be said to be true of Polyclar AT, although such compounds may react with free amino groups (Endres and Hormann 1963).



The changing properties as brown pigments form and polymerize may account for the variable results noticed in fining attempts. Oxidative changes in phenols also help explain why white wines in large containers are observed to clear first near the surface where O_2 contact may occur. White wines are often deficient in tannins capable of precipitating protein. The phenols of young white wines are limited to small, quite soluble molecules. As oxidation converts these phenols to larger molecules, they can soon bind and coprecipitate with proteins. In barrels, presumably both air (O_2) and phenols from the walls of the barrel help explain why barrel finings often clarify more rapidly than tank finings.

Polyphenols in wine have at least two important sensory characteristics - true bitterness and tactile astringency (Amerine et al. 1976). Bitterness and astringency are two wine attributes that are often confused. Astringency is a contracting (puckering), drying mouth feeling involving precipitation of saliva proteins on the mucous membrane surface. Bitterness is a true taste. It is confused with tactile sensation of astringency even by experienced tasters. Small-molecular-weight phenols, such as catechins, are precursors to bitterness; larger polymerized phenolic compounds (tannins) contribute to wine astringency (Arnold et al. 1978). Increased levels of astringency mask bitterness (Single-

ton et al. 1976). Removing a portion of the astringent polymeric phenols with protein fining agents may render a perceivable bitter character to the wien following protein fining. Flavor masking in wine is an important feature that explains several observable phenomena. As young wines age, tannins polymerize or bind together. Astringency increases with an increased degree of polymerization. When polymers reach a density greater than that of the wine, they precipitate from solution. Astringency is proportional to the protein binding ability, with flavonoid tetramers being the most astringent (Singleton 1980). As wines age and lose tannin due to oxidation and precipitation, they become less astringent and may show an unmasking of bitterness (Singleton et al. 1976). Red wines with a total phenol content of over 2500 mg/l GAE are almost always bitter (Singleton et al. 1976). Undoubtedly some bitterness in red wines adds complexity.

Table 4 lists clarifying products and quantities employed in red and white wines. The use of these agents is discussed in detail in the following pages.

Table 4

	Gelatin	Casein	Isinglass	Albumin	PVPP
Suggested levels (wine g/hl					
Red	6-15			5-10	
White		10-100	1.0-2.5		72
Tannin Addition Ratio	1/.5	1/.5	--	--	--
Charge	+	+	+	+	+
Legal Limit	GRAS	GRAS	GRAS	GRAS	6 lb/1000 gal (0.72 g/l)

Gelatin. Gelatin is prepared from collagen, the major structural protein in skin and bones, by heating in the presence of acids or bases. The hydrolysis that results from such heating affects the molecular size and gelling capacity. Gelatin is composed of long polypeptide chains in which glycine and proline are the most common amino acids.

Gelatin has an isoelectric point of pH 4.7, well above the normal pH of juice or wine. Therefore, it occurs in solution as a positively-charged entity capable of reaction with negatively-charged species such as tannins vai hydrogen bond formation. For a discussion on protein isoelectric points and their significance, see Zoecklein 1984 (P.W. Vol. V No. 1. 1984). Gelatin finds its principle application in clarification as well as in modification of overly astringent wines.

Commercial gelatin is available in several forms and grades. It is usually rated according to purity as well as "bloom". The bloom number is determined by allowing a 6.66% gelatin solution to age at 10°C for exactly 18 hours. The number of grams needed to force a 0.5-inch diameter stamp 4 mm into the gel gives the bloom number (Hahn et al. 1977). The bloom number of the gelatin molecule relates to gelatin's ability to absorb large quantities of water, usually on the

order of 6-10 times its weight. Generally, the greater the bloom, the greater the binding capacity of the gelatin. A good fining gelatin for wine ranges from 80-150 bloom and averages 15,000 to 140,000 molecular weight (Recht 1984). The size of the gelatin molecule (number of available binding sites) can have a profound affect on phenol removal. Fining with a gelatin of reduced molecular size is reported to reduce the rate of precipitation but allows for better clarification and compaction (Chimiciperdomine Spa 1984).

In terms of phenol adsorption capacity, gelatin is usually similar to albumin (see Table 3). Gelatin preferentially binds with larger molecules (Singleton 1967); thus, the fining agent has a more dramatic effect on color and tannin reduction in older wines than in younger products. Older wines have a greater percentage of large polymeric phenols. Gelatin is occasionally employed to help remove the harshness and perhaps color of press juice prior to fermentation. Gelatin can strip wine character as well as remain suspended in solution. Like egg albumin, gelatin is often added to soften wien astringency; however, unlike albumin, it may remove too much fruit character from the wine.

The quantities of gelatin needed to achieve the desired level of clarification may reduce wine astringency to undesirably low levels. Most white wines have such a low precipitable phenol content that an exogenous source of tannic acid or (-) kieselsol is needed for reaction with gelatin. If this is not done, gelatin may remain suspended in the wine. If tannin is used, additions are often made 24 hours prior to gelatin fining of whie wines. The ratio of tannin to gelatin, for optimal results, is often 1/0.5 (by weight) but will vary depending on the individual wine and the source of the tannin (Gustavson 1956).

Some producers prefer to fine with (-) kieselsols instead of tannin in conjunction with gelatin. The replacement of tannin with kieselsol is said to make the activity of the gelatin on wine flavor more gentle, require less gelatin, reduce the lees volume, and improve clarity and the speed of clarity (Hahn and Possman 1977). Gelatin that is soluble at elevated temperatures (i.e., 80-130 bloom) is recommended to be used in conjunction with most kieselsols (Mobay 1976). When fining rose' and white wines with kieselsol, the amount of gelatin used seldom exceeds 3 g/hl (Recht 1983). For a further discussion on gelatin-kieselsol interaction, see Zoecklein (1984a, 1984b).

Gelatin fining is employed occasionally in conjunction with bentonite. The negatively charged bentonite can coprecipitate with the positively charged protein, thus enhancing clarification. Such a procedure may aid in compaction of bentonite lees. Residual gelatin remaining in wine may increase the likelihood of copper casse formation. Winemakers may elect to counterfine with bentonite after gelatin addition.

In using gelatin as a clarification agent, over-#/or under-fining adversely affects filtration, a problem that may be more significant with use of this as compared with other fining agents (Akiyoshi et al. 1974). Gorinstein et al. (1984) confirmed that low temperature, as would occur during conventional potassium bitartrate stabilization, aids gelatin precipitation. Additionally, precipitation of protein fining agents is generally enhanced by low juice or wine pH's.

Table 5 shows the results of fining on a 1-year-old red wine with 30% liquid gelatin.

Table 5

THE EFFECT OF GELATIN ON WINE PHENOLS

	Control Wine	Wine Treated with g/10 L of Gelatin
-Total polyphenols (Folin met.) mg/l	1680	1500
-Anthocyanins mg/l	55	53
-Leucoanthocyanins mg/l (Flavon 4 diols)	137	117
-Flavanoid polyphenols mg/l	1050	930
-Polyphenols with a high molecular weight (tannins) mg/l	300	150

Source: Soluzione Stabile De Gelatina per il pronto impiego-Chimici Spa.

Gelatin is sold in sheets, pearls, and powders as well as liquids. Dry gelatin is prepared by either expanding it in cold water for several hours and then dissolving the resulting gelatin by heating the solution to 30°C or by dissolving the gelatin in 45°C water while constantly stirring. Prolonged or excessive heating of the gelatin slurry results in the hydrolysis (breakdown) of the gelatin molecular. Laboratory tests must be performed using the same material that will be used in the cellar.

Liquid gelatins are produced by hydrolysis to reduce molecular weight (some as low as 2000) by the addition of salts, heat, or both. Such procedures prevent gelatinization at high concentrations and allow these products to remain liquid. Liquid gelatins usually range from 30-46% gelatin and are stabilized with benzoates, sulfur dioxide, or both.

Addition levels range upward from about 1/16 pound gelatin per 1000 gallons. The level in red wines is often in the range of 6-10 g/hl (0.4 to 0.8 pounds per 1000 gallons). If employed in white wines, the range is often 6-10 g/hl following a tannin addition of approximately 10-15 g/hl of kieselsol, depending upon the nature of the wine and the desired response. Much larger doses are often employed in juice fining, particularly press juice fining. Fining heavy press juice with 4 pounds per 1000 gallons gelatin, 10 pounds of kieselsol, and 1-3 pounds of bentonite is an effective means of removing both harshness (astringent phenols) and color (Zoecklein 1984a).

Casein. Casein is the principal protein in milk (1 lb of milk contains 30 grams of casein adn 10-15 grams of albumin). Casein exists as a positively charged macro-molecule with a molecular weight of 275,000. It is available to the wine industry in several forms. Purified milk casein is insoluble in acid but sol-

uble in an alkali solution. Potassium caseinates are water soluble and mildly soluble in acidic or low-pH solutions such as wine. Most caseinates (casein salts) can be simply dissolved in water prior to use. Milk casein, on the other hand, must be hydrated in water with a pH greater than 8.0. Some proprietary compounds contain potassium bicarbonate, which enhances the water solubility of both casein and its salts. Additionally, some proprietary caseins are mixtures of finely powdered casein and bicarbonate.

Milk casein is produced by acidifying fresh fluid skim milk. The precipitated casein is thoroughly washed and then dried and ground to a specific mesh size. The standard common mesh size is 30 mesh (Western Dairy Bulletin 1977). Pulverizing casein enhances solubility.

Regardless of the particular casein or casein preparation employed, it should be hydrated in water, never juice or wine, and allowed to fully hydrate prior to use.

Upon addition of the casein slurry to wine, the relatively low wine pH causes the casein to flocculate with the resulting precipitate adsorbing and mechanically removing suspended material as it settles from solution. Casein has an important property of precipitating in acid solution and therefore, contrary to other protein fining agents, can fine white wines containing few large phenols without the danger of leaving protein in solution.

Casein finds its principle application in white wines for the removal of color or color precursors, oak character, perhaps bitter components, and infrequently to enhance clarification. Like isinglass, casein can bring forward the fruit character.

Casein can bind with and precipitate oxidized phenols, thus helping to remove and prevent brown formation. Casein can also be an aid in preventing pinking in susceptible wines such as Pinot blanc. Casein is known to be a 'body stripper', a fact that warrants careful laboratory trials. Caseins can be used as substitutes for decoloring carbon in decoloring white wines. Although not as effective in color removal as carbons, caseins avoid the oxidative degradation often associated with carbons. Casein is frequently used for the removal of dark color and cooked flavor from sherries.

Caseins are reported to remove as much as 45% of the copper and 60% of the ferric iron from must or wines (A.E.B. 1982). These metals can hasten wine aging by acting as oxidative catalysts.

As with gelatin, white wines fined with casein often must be preceded by a tannic acid or kieselsol addition. If tannic acid is used prior to casein addition, approximately one-half the expected casein weight is employed.

Clarification with casein is not always optimal. When used for clarification, it should be employed in a solution of at least 25 g/l concentration prepared in water. This helps assure slower flocculation with finer flakes, thus giving better clarification. Casein is on occasion coprecipitated with bentonite. Previous treatment of wine with tannin may aid casein fining as well as fining at 'cellar temperatures' (O'Neal et al. 1951). As with other fining agents, casein solutiosn must be mixed intimately with the wine to assure the desired results.

Pure casein is often used in white wines in the range of 5 to 10 g/hl. Sometimes more is used because it precipitates due to the effect of acidity alone and will not remain in the wine (Watts et al. 1981).

Egg Whites. Egg whites are a common fining agent employed in red wine production, used principally to soften by reducing astringency. Thsi fining material is seldom employed in white wines because of the significant amount of tannin needed for flocculation.

Egg whites contain 12.5% of their fresh weight as protein. This corresponds to 3-4 grams of active product per white (Peynaud 1984). The main proteins are albumin adn globular proteins. Albumin is a common protein found in whites of eggs, blood, and milk. Albumins are water soluble, while the globulin fraction is insoluble in water but soluble in diluted neutral salt solutions. Egg whites contain 7-8 grams of sodium chloride per liter, which causes the egg white to be transparent. If egg whites are diluted with water prior to addition into wine, the natural salt content will be lowered, thus making globulins insoluble and giving a muddy looking solution. In order to solubilize the entire protein content and thus maximize fining efficiency, the egg white, if diluted with water, should have a pinch of potassium chloride added. The use of sodium chloride is not permitted in the U. S. The salt permits solubilization of the globulins, thus allowing for a more rapid flocculation and better clarification. The Federal Register gives the following guidelines for albumin (egg white) use: a brine may be prepared by adding 1 oz (28.35 grams) potassium chloride, 2 lbs (907.2 grams) egg white, 1 gallon (3.7851) of water. The usage of this solution may not exceed 1.5 gallons per 1000 gallons of wine. This practice does produce a more voluminous sediment. The presence of salt is necessary for flocculation, but too high a content will slow flocculation. Wines contain a sufficient quantity of salts to allow for proper flocculation.

When fresh egg whites are employed, they should be gently homogenized with a whisk. Egg whites are suspended in very thin-walled cells. This type of tissue imparts a gelatinous consistency. Excessive mixing facilitates the dissolution, but the snowy foam which results does not mix well with the wine. It often remains on the surface where it floats. This portion not only doesn't participate in the fining, but it leaves some coagulated egg white at the surface, which falls in time.

Egg albumin in the form of frozen or dried flakes is available. In addition to being somewhat expensive, these often do not have the same fining ability as fresh egg whites. Fresh egg whites have a larger phenol adsoprtion capacity (usually twice as much as frozen whites). Presumably, this is the result of protein denaturation.

Egg whites are added in the range of 6-10 g/hl, or 5-8 eggs per barrel (225.1). Approximately 33 grams of egg white are contained per medium size egg.

Isinglas. Isinglass is a positively charged protein fining agent produced from fish collagen (Sturgeon swim bladders).

Isinglass is available in two forms. Sheet isinglass often must be laboriously hydrated, rinsed, and rerinsed many times to remove the fish odor.

Flocked isinglass, because of its purity, generally needs only to be hydrated and perhaps rinsed. Flocked isinglass must be stored cool prior to hydration. Hydration of flocked isinglass should be performed using cold water 60°F (16°C).

If prepared in hot water, it undergoes partial hydrolysis that results in a reduction of its molecular weight from 140,000 to between 15-58,000 (Rankine 1984). Such a reduction in the molecular weight significantly affects the fining ability of isinglass - rendering its activity more 'gelatin like'. According to Joslyn (1953), isinglass acts like gelatin except it has a lower proportion of its proline hydroxylated and dissolves at lower temperatures.

A typical preparation method for flocked isinglass is given by Rankine 1984: To a 250 l vessel, add 60 liters of 10°C water, 500 grams of citric acid, 140 grams of potassium metabisulfite. Mix and add slowly 1 g of powdered isinglass. Continue mixing until uniform. This solution is allowed to stand overnight at no greater than 15°C. The next day, the solution volume is raised to 200 liters. Further mixing may be required. If particle size is excessive, the isinglass solution is rubbed through a low-mesh screen. This solution contains 5 grams of isinglass per liter, 2.5 grams citric acid per liter, and 350 mg/l sulfur dioxide. If stored cool, this solution can last for some time. It should be noted that some flocked isinglass already contains both citric acid and potassium metabisulfite.

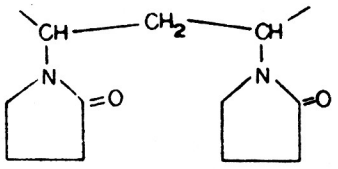
In white wines, isinglass if often used to unmask or bring forward fruit flavors while not significantly affecting tannin levels. Isinglass removes more leucoanthocyanidin but fewer condensed polyphenols are principally those responsible for astringency. Therefore, isinglass has a less dramatic effect on both wine astringency and body reduction than other protein fining agents (see Table 3). Color removal of isinglass is inferior to both gelatin and casein. Isinglass requires little tannin to precipitate (see Table 3). According to Ribereau-Gayon et al. (1972), tannin addition with isinglass fining is seldom used. Most wines possess sufficient quantities of endogenous tannin to aid isinglass precipitation. Colvite is a French isinglass solution used in conjunction with Boltane (an enological tannin) as a riddling aid in methode champenoise. The addition levels range from 500-750 milliliters per 1000 gallons of each. Generally, slightly more Boltane (1-3%) than Colvite is utilized. As indicated in Table 4, the quantities of isinglass used is often very low.

Isinglass has several advantages over gelatin for white wine fining. Weaker doses often result in better clarification and greater brilliance than gelatin (Ribereau-Gayon et al. 1972). Additionally, isinglass requires less tannin to precipitate. Therefore, it has a less dramatic affect on the overall wine character. Clarification with gelatin is enhanced at low temperatures. Isinglass clarification appears to be much less temperature related (Ribereau-Gayon et al. 1972).

Isinglass has some significant disadvantages for the winemaker. The weak density of the flakes that form following its addition into the wine can give voluminous sediment (>2%). Additionally, particles may have a tendency to attach themselves to the sides of casks or barrels and take a long time to fall. This difficulty can usually be minimized by counterfining with bentonite. Depending upon the source and age of the fining agent, laborious rinsing may be required to remove the fishy odor of the slurry. Consequently, it is essential that only isinglass of the highest purity be utilized.

Isinglass can be utilized anytime during the wine making process. Many producers fine with isinglass after aging - before bottling - leaving the white wine clean to the taste and sparkling clear without stripping newly formed bouquet. The amount of isinglass used ranges from approximately 1/8 to 1/3 lb/1000 gallons.

Polyvinylpolypyrolidone (PVPP). PVPP is a synthetic, high-molecular-weight fining agent composed of cross-linked monomers of polyvinylpyrrolidone (PVP).



PVP

These compounds behave like proteins with respect to phenol binding. Hydrogen bonds are formed between the carbonyl groups of the polyamide (PVPP) and the phenolic hydrogens of the polyphenols. Soluble proteins such as gelatin, isinglass, albumin, etc., are flexible and can essentially 'snuggle up' to phenols so that these agents are able to hydrogen bond with a number of the phenolic hydroxyls, thereby removing large molecules. Insoluble PVP on the other hand is able to contact only a relatively few hydroxyls of the larger molecules. As such, PVP binds with smaller phenols which conform to the PVP particle.

As selective phenol adsorbents, these synthetic fining agents are available in several forms. Polyclar 10 is chemically equivalent to Polyclar AT. The differences lies in the particle size, with 10 being smaller than AT. The smaller particle size of 10 results in greater surface area and therefore greater adsorption per gram of material. However, for use in the winery, the cost-performance benefits of Polyclar 10 must be weighed against the ease of settling and filtration of Polyclar AT.

The activity of Polyclar AT is similar to protein-type fining agents. It is specific for small-molecular-weight phenols such as catechins (G.A.F. 1975). See Table 2. Catechins are precursors to browning in white wines and precursors to browning and bitterness in red wines (Boulton 1980). Polyclar AT can be effective in 'toning down' bitterness or the potential of bitterness in wines. It is used sparingly in red wines due to color removal. Ough (1960) found that Polyclar AT removed more tannin and anthocyanin than gelatin.

Polyclar AT find its principle application in white wines to remove browning and pinking precursors. As an aid to reduce browning or pinking, it is best added to young wines, although it can be used at any point in the winemaking process, including in juice. Juice fining with Polyclar AT is not common. Phenol reduction in juice may be inhibited due to fouling caused by proteins, etc. When used to remove brown color, it is often used in conjunction with decolorizing carbon. In cofining with carbon, Polyclar AT can aid in carbon precipitation.

PVPP has the ability to strip complexities from wines. It is therefore desirable to fine with this compound prior to the development of aging bouquet. Because of the inert nature of Polyclar AT, it imparts no off flavors or aromas to juice or wine.

Recommended use levels for Polyclar AT range from 1 lb/1000 gallons to 6 lbs/1000 gallons. Federal law states that this material must be removed from the wine by filtration. Currently under study is the use of immobilized PVPP and methods of recovery and regeneration.

Yeast Fining. Consisting of approximately 30% protein, the yeast cell wall may play an important role in complexation of wine and must polyphenols. Singleton and Essau (1969) report that 10 grams of active brewers yeast may adsorb up to 1 gram of flavan 3, 4 diol (leucoanthocyanidin). They go on to state that yeast production during fermentation is on the order to 10 grams per liter of product. They conclude by stating that at this level, up to 1000 mg/l of tannin may be removed due to adsorption by yeasts.

In addition to their adsorbant activity, actively fermenting yeasts have been used to revitalize oxidized wines. As such, yeast fining has been found to have application in reduction of excessive browning and other oxidative characters in problem wines.

Yeast fining involves the addition of yeast to a wine with subsequent removal by centrifugation or filtration. The yeast cells adsorb color and off odors. This procedure has been employed for the removal of the herbaceous character of certain cultivars and off odors due to mold and frosted grape damage. The utilization of yeast as fining agents has been replaced by other agents, particularly in this county.

Research regarding the addition of yeast cell walls (yeast hulls) to must and wine is currently underway. Work by S. Lafon-Lafourcade (et al. 2984 AD) indicates that addition of yeast hulls can be of benefit in the following cases:

1) fermentation of high sugar juices
2) fermentation of heavily classified juices
3) fermentation of juices containing pesticide residues
4) fermentation of partially spoiled grapes
5) to help complete fermentation of overheated and stuck fermentations

The presence of fatty acids during alcoholic fermentation diminishes sugar consumption due to their toxic affect on the yeast hull. Yeast hulls have the ability to adsorb fatty acids, such as decanoic and octanoic acids, thus allowing fermentations to go to completeness.

Wine Stability. Wine fining can have a significant effect on wine stability by removing complexing factors. These refer mainly to complexes formed between polyphenols and tartaric acid in red wines and proteins and tartaric acid in whites. There is, therefore, an intimate relationship between wine fining and potassium bitartrate stabilization. For example, it is known that condensed polyphenols interfere with potassium bitartrate precipitation (Amerine and Joslyn 1970). This suggests that the use of fining agents, particularly protein fining agents, can have an effect on potassium bitartrate stability.

If one compares the fining abilities of filtered vs. unfiltered wines, there is often a striking difference even if the unfiltered product is brilliantly clear. This is the result of protective colloids. It is these protective colloids which make fining of young wines and wine produced from botritized grapes very difficult. Gelatin is particularly sensitive to this phenomenon, albumin less sensitive, and casein and isinglass are least effected by protective colloids in wines (Ribereaue-Gayon et al. 1972). For this reason, if initial fining does not give sufficient clarification, a second one following racking often leads to improved results.

The affects of protein fining agents can be unpredictable; therefore, carefully controlled laboratory fining trials must be performed and evaluated prior to cellar treatment. The greater the level of protein fining, the greater is the phenol removal; although, as noted in Table 6, this relationship is not linear.

Table 6

Initial phenols conc.	Albumin addition	Final phenol conc.
mg/l GAE	mg/l	mg/l GAE
1000	25	976
	100	938

Source: Ribereaue-Gayon et al. 1972.

Tables 7 and 8 from Ribereaue-Gayon et al. 1972 demonstrate the relationships between phenol removal and wine pH.

Table 7

			Removed	
50 mg/l	Casein	6.5 mg/l	phenol GAE	pH 2
		10.5 mg/l	phenol GAE	pH 3
		13.0 mg/l	phenol GAE	pH 4

Table 8

			Removed	
75 mg/l	Albumin and	60 mg/l	phenol GAE	pH 2
200 mg/l	Tannin	107 mg/l	phenol GAE	pH 3
		384 mg/l	phenol GAE	pH 4

A lowering of the temperature augments the quantity of phenols removed by a given weight of protein fining agent. Fining at around 0°C, according to Ribereaue-Gayon et al. 1972, removes the maximum quantity of wine or juice phenols.

It is generally accepted that young wines are much more 'forgiving' than older wines to the action of protein fining agents.

Due to their phenol binding abilities, protein fining agents can have a dramatic affect on color, texture, body (front, middle, and finish), astringency, bitterness, the nose characteristics in general, the fruit, the finish, the aging potential, stability, lees production, lees compaction, and overall palatability. It is therefore essential that careful fining trials be performed and evaluated prior to use in the cellar. Naturally, exactly the same lot must be used in the laboratory as will be employed in the cellar. Protein fining agents must be of the highest purity, properly hydrated, and properly added in the cellar to be effective.

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AEB spa via V. Arici 92 s. Polo. bentogram microdose bentonite granules.

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