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Chemistry of Wine Stabilization: A Review

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Wine derives its general quality and chemical composition from grapes and from the yeast fermentation which converts their juice to wine. The processing, including aging, which new wine undergoes before it becomes esthetically acceptable depends on the wine type. No two wines are exactly the same. The winemaker uses old and new technology coupled with artistic creativity to produce consistently palatable wines. Aside from the expected characteristics such as color, clarity, bouquet, flavor, and taste, modern wines must be physically, chemically, and biologically stable. Worldwide consumption of wine is increasing rapidly. Better wine is produced today because we have a better understanding of its chemical and biochemical nature.

Wine has been part of the diet of civilized man since the early settlements in the Tigris-Euphrates basin and in Egypt. From these areas the grape vine was introduced to the Mediterranean basin countries and Europe by pre-Christian traders where wine became a safe and healthful beverage and an important food adjunct. Since it induced euphoria and pleasing relaxation from the strains of life, wine eventually took on social importance where it was used for religious feasting and celebration as well as for entertaining.

Grape juice was fermented inadvertently into wine by natural yeast. To nomadic tribes, especially, it was welcome since it had flavor reminiscent of the fresh fruit or juice. In addition it could be stored and transported easily and remained drinkable from season to season. Eventually wine found its place as an article of commerce with necessary quality requirements. Not until the time of Louis Pasteur did the scientific foundation of winemaking become established and did enology become the science of wine. Since the early Pasteur experiments and discoveries winemaking has developed from a haphazard, ill understood, and risky

In Chemistry of Winemaking; Webb, A.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1974.

process into a well defined scientific discipline. Likewise, the consumer has become sophisticated, and he will not accept inferior wines. The American wine producer has used his chemical and biological knowledge of grapes and wine to offer a stable beverage produced under exemplary hygienic conditions from grape varieties which have been carefully selected. The ultimate character and quality of wine results from the nature of the raw materials from which it is made, the fermentation process, and the changes which are induced or occur naturally during the post-fermentation period (*1*, *2*, *3*).

General

The term wine refers to the natural beverage produced from the juice of sound and ripe grapes, in strict accordance with federal and state regulations. The stabilization principles discussed will have equal application to fruit wines in general except for tartrate stabilization since tartaric acid, the primary organic acid of grapes, is not found in any other fruits commonly used in winemaking.

Amerine said that the enologist who is unfamiliar with his raw material, grapes, is like a blind painter (*4*). However, it is beyond the scope of this review to discuss the accepted viticultural principles for producing grapes. It will be assumed that grapes delivered to the winery for the purpose of wine production are sound and at their optimum maturity. No amount of technical skill or knowledge can be substituted for the lack of good grapes in the production of wine.

Wine stability is a very relative term and may denote various things to different people. Today's demand is for brilliantly clear wines. The presence of sediment or the development of haze during marketing is generally considered by American consumers as evidence of incipient spoilage or instability (*2*). Berg and Akiyoshi (*5*) defined wine stability as a state or condition such that wine will not, for some definite period of time, exhibit undesirable physical, chemical, or organoleptic changes. These undesirable changes that denote wine instability were listed by them as: (a) browning or other color deterioration, (b) haziness or very slight cloudiness, (c) cloudiness, (d) deposits, and (e) undesirable taste or odor. Modern winemaking depends on controlled pure yeast fermentations (*6*). Grapes, as they arrive at the winery, harbor many types of natural, but undesirable yeasts, lactic bacteria, and occasionally acetic acid bacteria and molds. Soon after crushing, conditions become ideal for the growth of such microorganisms, and these must be inactivated to prevent their proliferation. This can be accomplished by heating the must to a high enough temperature to inactivate all the native microflora which arrive with the grapes. Excessive heating, however, may tend to contribute undesirable cooked flavors to the wine. The method of choice for the inactivation of the native organisms is the judicious use of 100–125 mg/liter of sulfur dioxide at the time of crushing. In addition to its bacteriostatic properties, sulfur dioxide inhibits enzymatic browning and assists in preventing other oxidative changes because of its oxygen scavenging capacity. Sulfur dioxide is not foreign to wines since many yeasts known to ferment well will produce (during normal fermentation) up to 150 mg/liter of sulfur dioxide from other sulfur compounds, primarily sulfate, in grapes. Grapes contain natural pectolytic enzymes which hydrolyze the small amounts of natural pectins in grapes. However, certain grape varieties, particularly the native American species of *Vitis labrusca* (Concord types) contain such high pectin concentrations that they must be enriched with commercial preparations of pectolytic enzymes when crushed. Hydrolysis of grape pectins is essential to free as much of the juice as possible from the pulpy cells of the grape and to facilitate clarification and efficient filtration of juice or wine at some later processing stage. Care must be taken to select pectolytic enzymes on the basis of their efficiency in depectinizing grape juice with minimal side effects, such as oxidation or browning, which may be brought about by impurities in commercial preparations.

Grape Harvesting, Crushing, Stemming, Pressing, Settling, and Fermentation. The winemaking process must be strictly controlled from grape to glass. Such control involves expert knowledge of the necessary facts and procedures and the ability to modify each in the desired direction when necessary. Knowledge of the microbiological status, chemical composition, and sensory quality at every winemaking stage is essential. Modern wineries apply such knowledge through well-staffed technical departments.

Winemaking begins with the grape harvest. Grapes must be picked at their optimum stage of maturity for the particular type of wine for which they will be used. They should reach the winery in good condition, and they should be processed as soon as possible after picking. Several potential stability problems such as enzymatic and oxidative browning, wild yeast fermentation, acetification, mold proliferation, and associated off flavors, are prevented by careful and speedy handling of the grapes.

Processing grapes into wine begins immediately after delivery when the berries are separated from their stems and possible accompanying leaves. They are crushed to give the must which contains grape juice, skins, and seeds. It is imperative to eliminate all stems and leaves from the must and to avoid disintegration or breaking of the seeds; otherwise the secretion of tannins and other polyphenolics along with leaf and stem flavors, will produce wines which will be astringent, bitter, and grassy.

White wines are more prone to darkening from excessive enzymatic or oxidative browning, and all wines are difficult to clarify following fermentation if proper care is not given in the crushing and destemming operation.

Following destemming, crushing, addition of sulfur dioxide, and possibly pectolytic enzymes, the juice from white grapes must be mechanically separated from the grape skins and pulp solids in the shortest period of time to avoid the excessive extraction of tannins and other polyphenolics. The juice which is freely drained is commonly referred to as free-run and is fermented separately to produce the best quality white wine. The lesser quality press juice, which results from pressing the grape skins, is used to produce white wine of lower overall quality. Such wines may be darker, more astringent, and bitter because of higher concentration of tannins and polyphenolic compounds as a result of longer contact of the must with grape skins and solids. Wines from press juice are frequently distilled for use in brandy. Modern enological procedures call for the separation prior to fermentation of even the small amounts of pulp solids which remain in free-run grape juice after draining. Alcohol acts as a solvent to extract tannins from the remaining grape pulp solids which is not desirable in white wines. Separation of these finely dispersed pulp solids is not easy but may be accomplished to a degree by natural settling, filtration, or centrifugation. All these operations must be accomplished before the onset of fermentation for white wines.

For red (and rosé) wines, fermentation must be induced as soon as possible after the destemming and crushing. Such wines must be fermented in contact with the grape skins since the anthocyanin pigments which give red grapes their color are in the pigmented layers of cells on the inside periphery of the grape skins (except for a few minor varieties where the pigment is in the juice). Grape crushnng or pressing without fermentation will not release enough pigments into the juice for good color. Hot pressing of the must from red grapes has been practiced (especially in the eastern United States), but the practice of choice for producing red and pink wines from the *Vitis vinifera* (European) species has been to ferment the must in contact with the skins until the desired amount of color has been extracted. Extraction of the anthocyanin pigments is enhanced by the solvent action of the alcohol produced during fermentation and by the action of carbon dioxide on the anthocyanin-bearing cells of the grape skins. When sufficient color has been extracted, the free-run red wine is drained off to finish its fermentation separately from the press wine recovered from pressing the grape skins (pomace). Extreme care and skill are required during the color extraction phase of winemaking since the winemaker must extract just enough anthocyanin pigments without extracting too much tannin which would make such wines difficult to age or difficult to ready for consumption within a reasonable time. During the color extraction phase of fermentation the grape skins, buoyed by carbon dioxide, surface and form a cap. This cap must be kept submerged for efficient color extraction and to prevent it from becoming dry, hot, and saturated with air. Exposure to air invites infection with vinegar bacteria which results in high volatile acidity and quality deterioration.

In the United States and in most wine producing countries, the fermentation of both white and red wines is induced by inoculating the must with 2–3% selected pure yeast culture at its exponential stage of growth to provide a minimum of one million yeast cells per ml in the juice to be fermented. Wineries use commercially available pure yeast strains or propagate their own yeast starters under strict control to prevent contamination with wild yeasts or bacteria. Grape juice is an ideal medium for yeast growth, and very rarely if ever is it necessary to rely on yeast foods to promote the primary fermentation of fresh grape must or juice.

Most authorities advocate fermentation of white wines at lower temperatures, 60°–70°F, to preserve their delicate varietal character. Red wines are fermented between 65° and 75°F. All wine fermentations, and certainly those of table wines, must be temperature controlled. When the fermentation temperature is not controlled by refrigeration, the large amounts of heat energy generated during this process will overheat the wine to the point where volatile varietal flavors will be lost by evaporation, cooked off flavors will develop, and the yeast will die, causing a stuck fermentation and leaving behind a wine with residual sugar and low alcohol content. No amount of winemaking skill can salvage any wine so mistreated.

The great strides which have been made in the United States in recent years in improving wine quality are attributed to viticultural efforts to provide the best suited grape varieties for a given area or product, careful grape handling at the winery, pure yeasts, and temperature control during fermentation and aging.

Post-Fermentation Care of Wine. After the desired amount of fermentable sugar has been used during fermentation, the raw wine is ready for clarification, settling, racking (decanting), filtration, aging, cold stabilization, blending, bottling, and distribution.

It is imperative that wine be separated from its fermentation solids (lees) as soon as possible. The lees include yeast, seeds, finely divided grape pulp, and grape skin particles and impair the quality of the wine by prolonged contact. Dead yeast cells, especially, will begin their autolysis under the highly reducing or anaerobic conditions in the new wine, and detrimental off flavors from hydrogen sulfide, mercaptans, and other reduced or yeasty odors will develop.

Immediate filtration is the procedure of choice in separating the new wine from its fermentation lees. Unfortunately, the vast volumes of new wine produced during the relatively short grape crushing period, which is rarely more than 10 weeks during the fall, may not permit the timely filtration of all new wine as soon as fermentation is completed. Most new wines are pumped out of their fermentation vessels into holding tanks and allowed to settle their gross lees and yeast for a few days before they are racked (or decanted).

As soon as a wine leaves its original fermentation vessel, it can no longer be stored for any length of time in anything but full containers. This is absolutely essential in avoiding contact with oxygen which is the greatest enemy of most wines because its high reactivity with wine constituents leads to oxidation flavors, browning, and general quality deterioration when oxidized. Progressive winemakers purge their table wines and vessel headspaces with inert gases such as nitrogen or carbon dioxide to exclude oxygen during all subsequent movements or handling of wine.

Fining and Clarification

Depending on the type of grapes used, length of fermentation, and type of wine produced, the new wine after its first racking and rough filtration may still be cloudy or hazy because of suspended colloidal particles of grape or yeast components, and it may remain so for a long time. It is rare when a sound wine will become brilliantly clear by natural settling. Amerine (7) states that until 50 to 70 years ago most German white wines had to be aged several (five to six) years before they became brilliant on their own; by this time, of course, most of them became brown and deteriorated in quality because of overaging. With present technology, such clouds or hazes caused by grape or yeast proteins, peptides, pectins, gums, dextrans, unstable grape pigments, tannins, and other compounds, may be assisted in their separation from wine by the judicious use of small amounts of fining agents which adsorb or combine chemically and physically with the haze particles or colloids or neutralize their electric charges causing them to agglomerate and gravitate to the bottom in a reasonable time. Such treatment results in a relatively clear wine which tends to become brilliant by subsequent filtration. Bentonite, one of the fining agents most commonly used in winemaking, effectively removes protein or peptide materials. Activated carbon, gelatin, casein, and poly(vinylpyrrolidone) may also be used, and they assist in removing unstable tannins and other pigments. If excess protein is not removed from the new wine at this point, elevated temperatures either at the winery or after distribution may cause a visible haze or actual sedimentation of coagulated protein or its reaction products with other wine constituents such as tannins and inorganic cations.

U.S. regulations (*8*) limit the use of fining agents so that removal of the cloudiness, haze, or undesirable odor or flavor will not change the basic character of the wine or remove wine components that will change its basic character. The criterion for government approval of clarifying agents is that none of the agent remain in the wine. Generally government regulations do not permit the addition of any compounds to grapes or wine that will leave a residue in the wine if such are not already naturally present in grapes or wines.

The amount and type of fining agents used depend on the nature of the haze or cloud forming colloidal matter and the type and composition of the wine. Winemakers carry out small-scale fining tests in the laboratory before attempting to clarify commercial quantities of wine to determine the efficiency of the fining agent, its effect on the quality and flavor of the wine, and particularly, to establish accurately the minimum quantity of fining agent required to provide acceptable clarification (*see* Ref. *2*).

Non-Microbial Disorders of Wine

Tartrate Instability. Tartaric acid is not in any of the major carboxylic acid cycles of plant metabolism. Aside from its presence in tamarinds, only grapes, among the economically significant fruit crops, contain relatively high concentrations of tartaric acid. In grapes it normally constitutes over 50% of the total organic acids. Grapes are also rich in potassium, and significant concentrations of potassium bitartrate, cream of tartar, are present in grape juice. After fermentation, wine, because of its alcohol content, becomes supersaturated with natural potassium bitartrate, and removel of the excess is necessary to avoid sedimentation after the wine is bottled. Potassium bitartrate is not harmful, but the American consumer has been conditioned to suspect any product with sediment, especially wines, and is inclined to discriminate against such products. The procedure of choice for stabilizing wine against the sedimentation of potassium bitartrate after the wine has been bottled and sealed is the cold stabilization technique where wine is chilled just above its freezing point (this will vary from 26°F to 15°F depending on alcohol and soluble solids content) and held there to precipitate the excess potassium bitartrate. The time required to hold the wine at these near-freezing temperatures depends on the type of wine and its alcohol content, total acidity, pH, potassium, total tartrates, etc. This time will vary between a few weeks and several months. When it has been established by chemical analysis that no more potassium bitartrate is dropping out of solution, the wine is filtered at low temperature to separate it from the crystalline potassium bitartrate. Filtering the wine before it has a chance to rise in temperature appreciably is essential if redissolving any of the potassium bitartrate into the wine is to be avoided. Wineries use various cold temperature tests to determine empirically whether wines have achieved their desired potassium bitartrate stability. One such test combines either freezing of the wine at about 0°F for 4–6 hrs after which the wine is thawed slowly at room temperature and observed for potassium bitartrate crystals at the point when the last floating trace of ice melts away or a less severe cooling of the wine to 32°F where it is kept for a period of several weeks and checked while cold for the presence or absence of potassium bitartrate crystals at weekly intervals. If a wine does not show any signs of potassium bitartrate precipitation by these tests the winemaker can be reasonably certain that severe climatic conditions during shipment or abuse of the wine by improper storage by the retailer or the consumer will not precipitate potassium bitartrate during the sales portion of the life of the wine.

Protective colloids, which prevent the crystallization of excess potassium bitartrate in a reasonable time, make a wine resistant to cold stabilization, even by prolonged refrigeration. In such cases ion exchange treatment of a small portion of the wine will remove enough potassium to render the entire lot of wine potassium bitartrate stable when blended back. The work of Berg and Keefer (9) provides the modern enologist with useful data from which to calculate the relative stability of potassium bitartrate in wines of various alcohol contents under different conditions of pH, temperature, and time. The tables they presented for using approximate concentration products to calculate potassium bitartrate stability should be used by winemakers to determine how much of the potassium and tartrate of the wine must be removed to cold stabilize wine while avoiding excessive ion exchange which may cause undesirable taste and quality defects. The possibility of using electrodialytic techniques to remove excess potassium and tartrate from wines has been explored. Initial experiments have proved successful in achieving cold stabilization of wines, but problems still remain with the irreversible loss in capacity of the electrodialysis membranes which are commercially available. Electrodialysis more closely resembles refrigeration for tartrate stabilization of wines since it removes the components present in supersaturation whereas no new components are introduced to the wine. With further research in the technology of the electrodialysis membrane field, it is expected that this process will become an important tool for wine stabilization.

In addition to deposits of crystalline potassium bitartrate, infrequent calcium tartrate deposits occur in wines. The calcium level of carefully produced wines is seldom high enough to cause stability problems. Occasionally, however, wines may extract calcium from improperly prepared filter materials. Prolonged storage in uncoated concrete tanks also will release calcium into wine.

The cold stability tests used by wineries are of short duration and do not predict potential calcium tartrate instability which may occur several months after a wine has been packaged. Berg and Keefer (*10*) have published data on the solubility product of calcium tartrate in alcohol–water solutions at various pH values, and this information can be used to predict the probability of calcium tartrate instability in wines. They warn that such data must be interpreted with care since other components of wine can form complexes with calcium and tartrate ions. However, such complexing with other ionic moieties in wine will tend to eliminate a certain amount of calcium and tartrate ions from active participation in the formation of calcium tartrate crystals. Therefore, the prediction of stability/instability on the basis of solubility product calculations from Berg and Keefer data, collected from alcohol–water model systems will be conservative, and a wine predicted to be of borderline stability will prove to be stable and not subject to calcium tartrate crystallization in actual practice. Yamada and DeSoto (*11*), using Berg and Keefer data (*9, 10*) studied a wide range of wine types and determined the maximum concentration products that would permit a wine to remain tartrate stable for prolonged periods under commercial conditions. Wines which may defy prediction of their potential calcium tartrate instability on the basis of the above considerations are those where the natural dextrorotary tartrate has undergone autoracemization to yield optically inactive or racemic tartrate, of which the calcium salt is much less soluble according to the following data (*50*):

D-Calcium tartrate = 230 mg/liter
L-Calcium tartrate = 250 mg/liter
Racemic calcium tartrate = 30 mg/liter

The autoracemization of dextrorotary tartrate in wines is quite slow and requires elevated temperatures for prolonged periods. It can be assumed that under U.S. wine production practices, sherry, because of its prolonged heating to develop the desired baked or nutty character and flavor, would be one of the few wine types which could undergo auto-

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racemization of tartrate. Martini (*12*) has proposed adding racemic tartaric acid prior to refrigeration to remove excess calcium from wine.

Protein Cloudiness. Of the various colloids known to be present in wines, peptides and proteins are of particular importance because of their influence on wine stability and clarification (*13*). According to Amerine and Joslyn (*2*) proteins serve as nuclei about which copper, iron, and salts deposit and form hazes, clouds, suspensions, or sediments on denaturation by heat, cold, or prolonged aging of wines. Proteins were shown by Ribéreau-Gayon (*14*) to be associated with ferric phosphate and cupric sulfide sediments, and this was confirmed by Kean and Marsh (*15, 16, 17*) and by Lukton and Joslyn (*18*). Protein clouds or hazes can also be the result of protein–tannin complex formation in the presence of traces of heavy metals, especially tin according to Krug (*19*). Kielhöfer and Aumann (*20*) reported tin–protein precipitates in tin-containing wines. The levels of protein present in wine as reported by various investigators and summarized by Amerine (*27*), varied from 0.015 to 0.143% of nitrogen as protein. Several studies on the nature of the proteins in German table wines have been cited by Amerine and Joslyn (*2*). These show higher protein concentrations in the wines of warmer seasons, and large variations are manifested in wines made from different grape varieties. Fermentation, heating, bentonite fining, and filtration removes the bulk of proteins from wine according to Moretti and Berg (*22*). Holden (*23*) proposed a combined heat and cold treatment for the protein stabilization of wine.

Unfortunately, in spite of the published literature on wine proteins, we do not know the actual protein levels at which table or dessert wines are stable. The changes in protein content during production and processing of wines are still not known with sufficient accuracy to predict their behavior. The winemaker has to depend on empirical tests if he is to produce protein stable wines. Early separation of new wines from their fermentation yeast greatly improves their chances for protein stability by decreasing the release of yeast autolysis products into the wine.

Iron and Copper Cloudiness. Wine cloudiness caused by colloidal complex formation involving cations, particularly copper and iron, are referred to in the enological literature as *casse*. Under normal conditions of vinification, the copper and iron content of wines as it originates from grapes is not high enough to cause stability problems, according to Thoukis and Amerine (*24*). They reported that up to 90% of the copper and up to 70% of the iron initially present in grape juice is eliminated during fermentation mainly by yeast assimilation. It is generally believed that wines containing an excess of 0.5 ppm copper or 10 ppm iron may be susceptible to clouding or sedimentation as well as flavor deterioration by the strong catalytic and oxidative properties of these cations. Modern wineries which use stainless steel or coated mild steel in all equipment contacting grapes, grape juice, or wine have eliminated the incidence of copper and iron cloudiness which was common in the pre-World War II wine industry.

Iron clouds forming in wine depends on a number of factors including the concentration of iron, the nature and concentration of the predominating organic acids, the pH, the oxidation–reduction potential, and the concentration of phosphates and tannins. Ribéreau-Gayon (*14*) made an extensive study of the conditions under which iron clouds will occur in wines and concluded that when these conditions are not at their optimum, iron clouds will not occur even with fairly high iron contents. According to Marsh (*25, 26*) the iron in wine exists in several ionic forms depending on the physical and chemical constitution of the wine. Usually, under reducing conditions, the ferrous (Fe^{2+}) state predominates, but under oxidative conditions the ferrous form is converted to the ferric (Fe^{3+}) state. Both of these ionic forms of iron may exist in their free state or as soluble complexes with wine constituents such as citrate. Under certain conditions, *i.e.*, low acidity or considerable amounts of tannin or phosphate, the ferric state of iron formed by exposure of the wine to air will combine with the tannins and/or the phosphates to yield insoluble or colloidal ferric complexes. Under California conditions ferric tannate *casse* is quite rare whereas the ferric phosphate *casse* occurs primarily in white wines in the pH range 3.0–3.6.

The mechanism of copper *casse* and the factors influencing its development in wines have been investigated by Joslyn and Lukton (*18, 27*), Kean and Marsh (*15, 16, 17*), and Peterson *et al.* (*28*).

In heavily sulfited white wines containing over 0.5 ppm copper and stored in sealed containers, a reddish-brown deposit may form. This occurs in the absence of oxygen and ferric ions but redissolves readily upon exposure to oxygen. Its formation may be accelerated by exposure to sunlight or heat, and it is believed to consist of colloidal cupric sulfide (*14, 29*). More commonly, copper *casse* may arise from reactions between copper and sulfur-containing amino acids, peptides, and proteins (*15, 16, 17*).

Methods for removing excess copper and iron cations to prevent copper and iron turbidities in wine have been proposed by Joslyn and Lukton (*30*) and Joslyn *et al.* (*31*). One of the most efficient techniques for removing excess copper and iron from wines is to add potassium ferrocyanide, a technique developed in Germany by Moslinger around 1905 and described by Ribéreau-Gayon (*32*). This treatment is called blue-fining, and its use is permissible in most wine producing countries including Germany, France, Italy, Austria, U.S.S.R., Yugoslavia, Hungary, and Luxembourg. When properly used, it is successful in achieving wine stability. Using potassium ferrocyanide as such to treat wines is not permitted in the U.S. although none remains in the wine when properly used. It is possible to decrease the copper and iron content of wines by ion exchange using selective resins. However, excessive copper and iron in wines is the result of careless contact of wine, after fermentation and during aging or processing, with mild steel or copper alloy surfaces. Eliminating such equipment from winery operatons will solve any problems of copper or iron instability which still persists in the wine industry.

Oxidation Defects. During clarification, filtration, aging, blending, and bottling, wine must be protected against aeration, except for types such as sherry, Marsala, and Madeira where controlled oxygenation is indicated. Table wines, and all white wines in particular, must not be unduly exposed to air to avoid discoloration, haze formation, or loss of flavor because of the oxidation of alcohol, tannins, pigments, and other wine constituents. Partially filled tanks, leaky pumps, use of compressed air in mixing, or improper transfer of wines contribute to oxygen pick up.

Oxidasic *casse* has been defined by Amerine and Joslyn (*2*) as the clouding of wines and their change of color on exposure to air. Red wines become brown, and white wines turn various shades of yellow, orange, or brown. Such wines may also develop a cooked or rancid flavor which may result from increased acetaldehyde content. These changes are aggravated by phenolase or polyphenoloxidase which, in small concentrations, is normally present in grapes. Its activity may be inhibited by moderate amounts of sulfur dioxide or by flash pasteurization. Grapes with high mold count will produce juice and wine with abnormally high concentrations of polyphenoloxidase, but this condition is more common in Europe than in the U.S. In California, some wine producers use third party grape inspections conducted by the State Department of Agriculture to determine and eliminate excessive mold counts from grapes. Berg (*33*) demonstrated a clear correlation of grape variety to the browning susceptibility of white wines, and this was confirmed by Berg and Akiyoshi (*5*).

Stripping with nitrogen or other inert gases (*34, 35, 36*) may prevent the wine from picking up oxygen during the processing stages. Bonetti (*37*) pointed out that air-tight tanks are needed to store table wines under a blanket of nitrogen, and he stressed the utility of glass-lined or stainless steel tanks for this purpose.

Microbial Disorders of Wine

Microorganisms can cause various degrees of haze, cloudiness, or sediment formation as well as changes in the composition of wine constituents by metabolizing components of the wine.

Only a few types of microorganisms are able to survive and proliferate under the relatively high alcohol and low pH conditions which characterize most wines. Of these, yeast, acetic acid bacteria, and lactic acid bacteria are the most common. No pathogenic organisms are able to survive in wine.

Yeast Disorders. Many genera and species of yeasts are found on grapes. Undesirable yeasts must be inhibited as soon as the grapes are crushed, and the primary fermentation must be controlled by inoculating the must with pure strains of yeast. This ensures a clean fermentation in a reasonable time with efficient conversion of the grape sugars to alcohol. Selecting pure yeasts is a matter of preference for individual wineries, but the preference in the United States has been for yeasts which do not produce undesirable by-products.

Rankine (*6, 38*) believes that the differences in the secondary products formed in wines during fermentation by various yeasts are quantitative rather than qualitative, and careful selection of pure yeast strains can eliminate wine disorders caused by large amounts of undesirable by-products such as hydrogen sulfide, mercaptans, acetaldehyde, acetic acid, ethyl acetate, higher alcohols, etc.

Hydrogen sulfide in wines is aggravated by elemental sulfur (from the grapes) in fermenting media as reported by Thoukis and Stern (*39*), Rankine (*40*), and Acree *et al.* (*41*). When elemental sulfur is not available, yeasts differ widely in their ability to produce hydrogen sulfide from sulfate, sulfur dioxide, sulfur containing amino acids, and other sulfur compounds. Selecting yeasts on this basis has largely eliminated this disorder in Australian wines (*36*).

Ethyl acetate, above threshold levels, is considered a spoilage defect by most enologists. This condition is often attributed to acetification of wine by vinegar bacteria, but quite often it is the result of contamination with spoilage yeasts belonging to the genera *Kloeckera, Hansenula, Pichia,* and *Saccharomycodes* in the early stages of alcoholic fermentation.

During storage and aging of wine, various spoilage yeasts from genera such as *Pichia, Hansenula, Torulopsis,* and *Candida* may grow as surface films and cause quality deterioration by by-product formation. Because of their affinity for oxygen and their limited tolerance for alcohol and sulfur dioxide, film forming spoilage yeasts seldom grow in the main body of wine. Their growth as surface films can be discouraged by keeping wine containers full or by preventing air from headspaces above the wine by sweeping with an inert gas.

The Spanish sherry type of film yeasts tolerate high alcohol concentrations (up to 16 percent) and cause compositional and taste changes which are considered to be serious defects in nearly all wines except Spanish-type flor sherries. The most obvious compositional changes in wines contaminated with flor sherry yeasts of the genus *Saccharomyces* are the large increases in acetaldehyde, acetal, diethyl succinate, and 2-phenethyl alcohol (*42*) and the insipid or oxidized flavor and taste characteristics which accompany these changes in table wines.

Wine spoilage by various genera of yeasts after bottling is one of the most serious problems that challenges winemakers in every wine producing country. Such yeasts may be those which brought about the initial fermentation of the wine and were not completely removed, or they may be spoilage yeasts which infected the wine during fermentation, aging, or bottling operations. Marketing such wines becomes impossible since the consumer will reject them on the basis of their cloudiness, sediment, and in the case of wines with residual reducing sugar, fermentation, and bottle breakage. The general quality of such wines is usually impaired to the point where they must be withdrawn from commercial distribution. Important research in the area of sterile filtration and aseptic bottling technology is continuing.

Acetic Acid Bacterial Disorders. A small amount of acetic acid is produced by yeasts during alcoholic fermentation and new wines normally contain about 0.02 to 0.03 gram of acetic acid per 100 ml. Higher concentrations of acetic acid in wines are usually the result of contamination with various species of *Acetobacter* which may invade the wine during fermentation or aging. Acetic acid bacteria require a great deal of oxygen to proliferate and to oxidize ethanol to acetic acid. With pure yeast fermentations and storing wine in full containers to ensure minimal contact with air, acetification of wines is no longer the disorder it once was. A sound wine will not undergo acetification after bottling because the high oxygen demand of *Acetobacter* cannot be satisfied.

Lactic Acid Bacterial Disorders. The widely distributed acid-tolerant organisms which account for widespread spoilage of wines are gram-positive bacteria which produce lactic acid. The lactic acid bacteria which are involved in wine spoilage belong to the general group comprising *Pediococcus, Leuconostoc,* and *Lactobacillus* (*6*) and may be either hetero- or homofermentative, depending their ability to form carbon dioxide from glucose.

Lactic acid bacteria isolated from wine may use residual sugars or alcohol, or decompose organic acids as a source of carbon for growth and energy. Malic, citric, and tartaric acids may be metabolized, depending on conditions.

The bacterial conversion of malic acid to lactic acid and carbon dioxide has been recognized since 1890 and is referred to as the malolactic fermentation. This conversion has been promoted under controlled conditions in the cooler viticultural regions of the world where grapes mature with excessive amounts of malic acid which causes taste imbalance because of high total acidity. Lüthi (*43*) emphasized that malo-lactic acid fermentation is necessary for the production of acceptable Swiss wines.

The extensive literature relating to the historical development of our knowledge of the bacteria involved in and the chemistry of the malo-lactic fermentation has been cited by Amerine and Joslyn (*8*). Vaughn and Tchelistcheff (*44*) reported methods to control the malo-lactic fermentation in California. Under California conditions, the desirability of promoting the malo-lactic fermentation has not been established clearly, and the nature of any improvement in sensory quality of such wines is not fully evident. Pilone and Kunkee (*45*) demonstrated differences in the sensory characteristics of wines fermented with several strains of malolactic bacteria, but their taste panel was not able consistently to rank wines with or without malo-lactic fermentation in order of their quality. Amerine and Joslyn (*2*) point out that if the activity of such bacteria were limited to a net decrease in acidity, this would rarely be desirable under normal California conditions. Amerine (*46*) stressed that the malo-lactic fermentation should be prevented in California wines of low total acidity. In spite of this, Ingraham and Cooke (*47*) reported that their study of 144 California commercial wines revealed that over half had undergone malo-lactic fermentation which was 75% more common in red table wines. Likewise, Kunkee *et al.* (*48*) found that most commercial wines from southern California had undergone malo-lactic fermentation which, they suggest, may have been caused by bacteria associated with the grapes rather than from winery contamination. Metabolic products of the malo-lactic fermentation, other than lactic acid, may include esters of lactic acid as well as acetoin (acetylmethylcarbinol) and its oxidation product, diacetyl(2,3-butanedione), which is considerably more odorous than acetoin (*48, 49*). Rankine (*6*) reported the level of diacetyl to be higher in wines which had undergone malo-lactic fermentation, and that too much of this by-product is undesirable since its butterlike aroma dominates and lowers the quality of the wine.

If the malo-lactic fermentation does not occur during the primary alcoholic fermentation or during subsequent storage and aging of the wine, malo-lactic bacteria in the wine after bottling will make the wine susceptible to the malo-lactic fermentation during warehousing or distribution. If this happens, the wine will develop haze or sediment along with sauerkraut odor, a decrease in acidity caused by the decarboxylation of malic acid to lactic acid, an increase in pH, a build up of pressure caused by the carbon dioxide released from the decarboxylation of malic acid. The wine will therefore be unacceptable to the consumer on the basis of aesthetic and flavor deterioration.

Control of Microbial Disorders

The most obvious method of controlling microbial wine disorders is to prevent contamination of the wine. Yeasts which have been used for the alcoholic fermentation must be removed or inactivated before bottling. The same restriction applies to acetic- or lactic acid bacteria which may have entered the wine during or after fermentation.

The judicious use of 100–125 ml per liter of sulfur dioxide at crushing time, prior to inoculation with active yeast cultures, will control the growth and proliferation of spoilage (wild) yeasts and acetic acid and lactic acid bacteria during the primary fermentation. Pasteurization of grape juice before inoculation with selected pure yeasts will accomplish similar inactivation of the grape microorganisms. This is rarely practiced because of the economic and logistic difficulties involved in heating and cooling vast quantities of grape juice. Also, heating grape juice may bring about flavor loss or introduce a burnt or cooked flavor.

Removing yeasts and other solids, soon after fermentation by racking and rough filtration will prevent further proliferation of the fermentation yeast or invitation of acetic or malo-lactic contamination and spoilage. Periodic tasting and chemical analysis of all wines in storage is mandatory to detect such problems. Refrigerating white wines during storage will prevent the growth of microorganisms. Red wines which are stored at cellar temperatures for faster aging are more prone to bacterial spoilage if infected. Centrifugation is not widely used in California for controlling microorganisms.

Prior to bottling, wines must be polished filtered followed by either pasteurization or sterile filtration. To preserve quality, wineries are abandoning pasteurization as a method of destruction of microorganisms and have adopted cold-bottling techniques which have gained popularity since the early 1960's when inert membranes for sterile filtration became available to the wine and other beverage industries. The known and uniform pore sizes of these membrane filters guarantee yeast and bacteria removal. Controlled sanitation before bottling provides a good assurance that the wine will be free from microbial disorders for its entire shelf life.

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