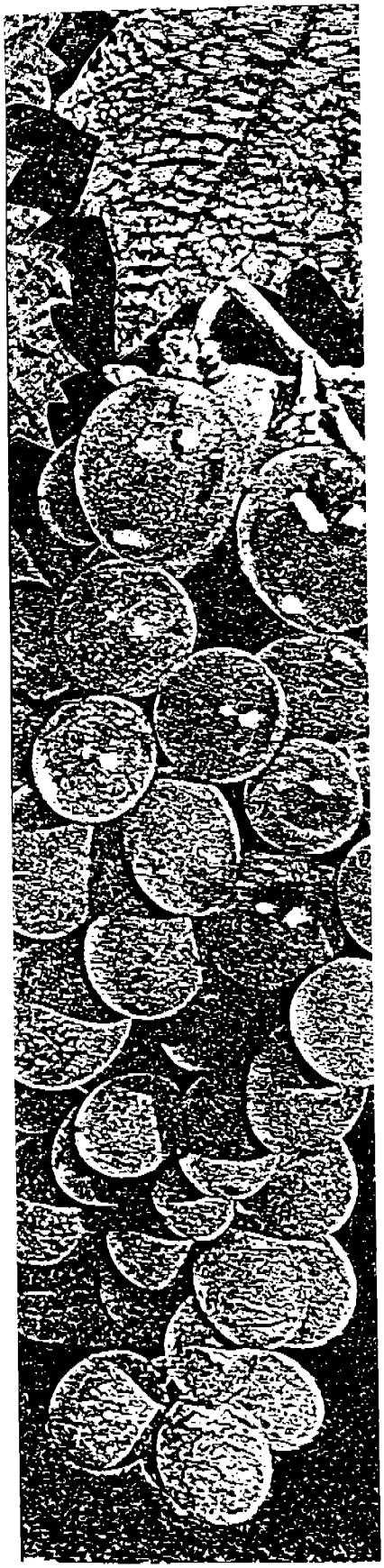

Wine Science

Principles and Applications

Ron S. Jackson
Botany Department
Brandon University
Brandon, Manitoba
Canada

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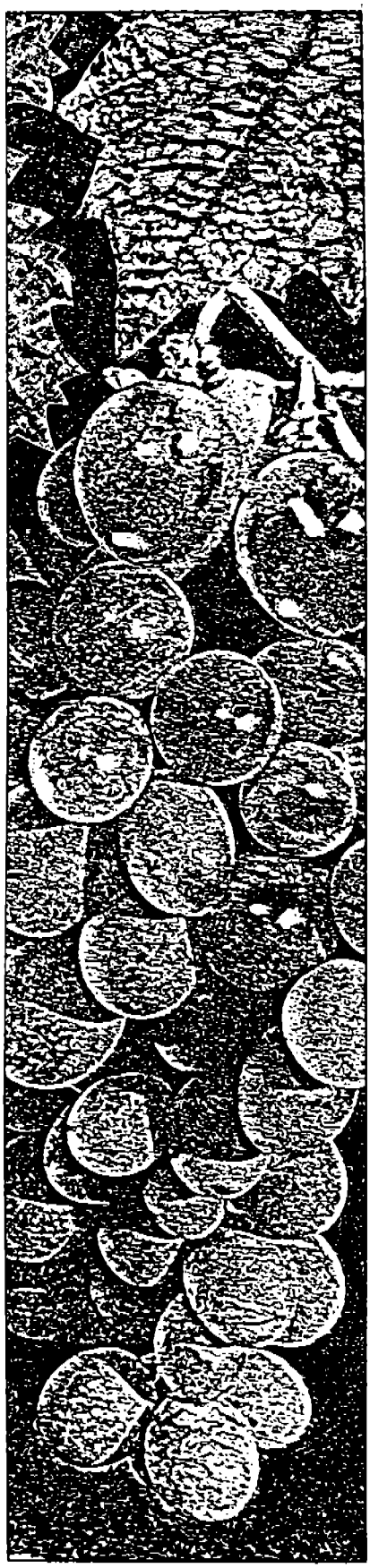
Fermentation



Introduction

The theory and practice of enology have developed enormously since its simple beginnings some six thousand years ago. Although advancements occurred sporadically, the pace of change quickened dramatically in the seventeenth century, reflecting parallel developments in science and technology. Improvements in glass production and the use of cork favored the development of wine styles that benefited from long aging. Sparkling wine also became possible. The research by Pasteur on problems in the wine industry during the 1860s led to solutions to several wine "diseases", and the foundation of our understanding of the nature of fermentation. Subsequent work has perfected wine making skills to their current high standards. Future study should result in premium wines showing more consistently the quality characteristics consumers deserve. In addition, distinctive features, based on varietal, regional, or stylistic differences, hopefully will become more discernible and controlled. Dr. Richard Peterson, a highly respected winemaker in California, has commented that Mother

Nature is a "nasty old lady, who must be controlled." Modern enological and viticultural science is increasingly providing the means by which many of the vicissitudes of Mother Nature can be moderated or controlled.

Basic Procedures of Wine Production

Vinification formally begins when the grapes, or juice, reach the winery. The basic steps in the production of table wines are outlined in Fig. 7.1.

The first step involves removing the stems, leaves, and any other extraneous material (Fig. 7.1). The fruit is then crushed to release the juice and begin the process of **maceration**, which facilitates the extraction of compounds in the seeds and skins. Initially, maceration is activated by the action of hydrolytic enzymes released from cells ruptured during crushing. Enzymatic maceration releases flavor ingredients from the skins, seeds, and pulp and promotes the syntheses of additional flavor compounds. Enzymes also may hydrolyze macromolecules into forms readily utilized by yeast and bacterial cells. In addition, the cytotoxic action of pectic enzymes on undamaged grape cells effects the release of cell contents into the **must** (grape macerate).

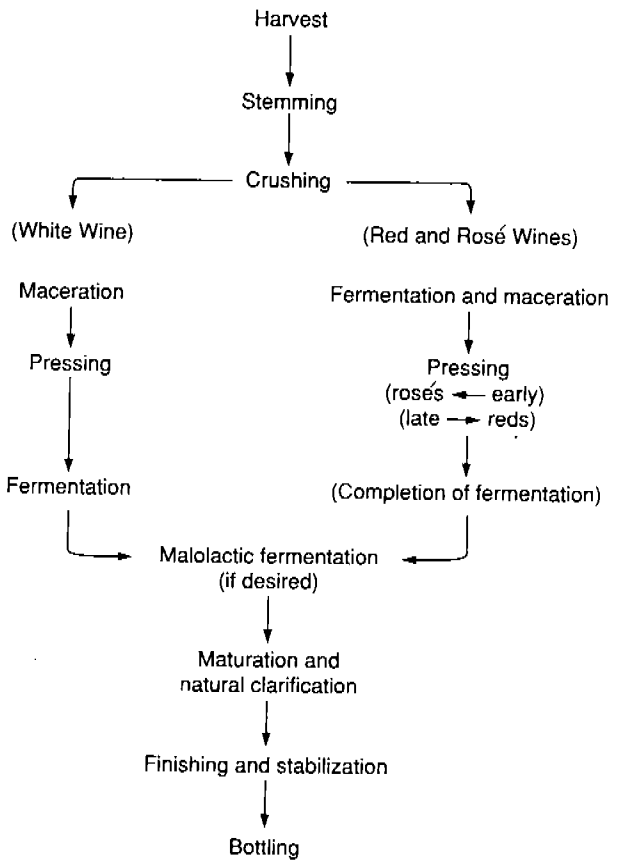


Figure 7.1 Flow diagram of winemaking.

For white wines, maceration either is kept to an absolute minimum or is kept brief, seldom lasting more than a few hours. The juice that runs feely from the crushed grapes (**free-run**) is usually combined with that released on gentle pressing. The combined fractions are then fermented. Subsequent pressings are usually fermented separately.

With red wines, maceration is prolonged and occurs simultaneously with alcoholic fermentation. The alcohol generated by yeast action enhances the extraction of anthocyanin and is crucial to the uptake of tannins from the seeds and skins (**pomace**). The phenolic compounds solubilized give red wines their basic properties of appearance, taste, and flavor. They are also required to give red wines their aging and mellowing characteristics. In addition, ethanol is important in the release of aromatic ingredients from the pulp and skins. After partial or complete fermentation, the **free-run** is allowed to flow away under gravity. Subsequent pressing extracts most of the remaining juice (**press fractions**). Press fractions are commonly added to the free-run in proportions determined by the style of wine desired.

Rosé wines are made from red grapes subjected to a comparatively short maceration on the skins, the duration depending primarily on the intensity of rosé color desired. Owing to the short duration of maceration, it may end before significant alcoholic fermentation has occurred.

Fermentation may start spontaneously due to indigenous yeasts derived from the grape, or picked up from crushing equipment. More commonly, however, the juice or must is inoculated with a yeast strain of known characteristics. Yeasts not only produce alcohol, but also generate the basic bouquet and flavor of wines.

On completing **alcoholic** fermentation, the wine may be encouraged to undergo a second **malolactic** fermentation. Malolactic fermentation is particularly valuable in cool climatic regions, where the acidity reduction improves the taste of the wine. Although most red wines benefit from malolactic fermentation, fewer white wines profit from its occurrence. The milder fragrance of most white wines makes them less able to mask potentially undesirable flavor changes induced by malolactic fermentation. In warm viticultural regions, malolactic fermentation is often unneeded and undesirable; its development usually is discouraged by practices such as the addition of sulfur dioxide, early clarification, and storage under cool conditions.

Newly fermented wine often is protected from or given only limited exposure to air. This is designed to restrict oxidation and microbial spoilage, while the processes of maturation permits the loss of yeasty odors, the dissi-

pation of excess carbon dioxide, and the precipitation of suspended particular matter. Changes in aroma, and the development of an aged bouquet, also commence. Where maturing wines are exposed to air, exposure is usually restricted to that which occurs during racking. Such exposure to air can help oxidize hydrogen sulfide and favors color stability in red wines.

After several weeks or months, the wine is racked. **Racking** separates the wine from solids that settle out during spontaneous or induced clarification. Sediment consists primarily of yeast and bacterial cells, grape cell remains, and precipitated tannins, proteins, and potassium tartrate crystals. If left in contact with wine, they may lead to the production of off-odors, and some may favor microbial spoilage. Racking from small cooperage usually results in the absorption of limited amounts of oxygen.

Prior to bottling, the wine may be **fined** to remove traces of dissolved proteins and other materials that can lead to the development of haziness, especially on exposure to heat. Fining also is used to soften the taste of the wine by removing excess tannins. Wines are commonly chilled and filtered to further enhance clarification and stability.

At bottling, wines are generally given a small dose of sulfur dioxide to prevent oxidation and microbial spoilage. Sweet wines usually are sterile filtered as a further precaution against microbial spoilage.

Newly bottled wines are normally aged at the winery for several months to years before distribution to wholesalers. This period permits wines blended shortly before bottling time to "harmonize". In addition, it allows acetaldehyde that may be produced as a result of slight aeration during bottling to be converted to nonaromatic compounds. As a consequence, "bottle sickness" induced by acetaldehyde usually dissipates before the wine reaches the consumer.

Prefermentation Practices

Stemming and crushing are commonly conducted as soon as possible after harvesting. During the harvest, some grapes are unavoidably broken and their juice released, while others may be bruised. Thus, oxidative browning often begins before the grapes reach the winery and crushing begins. The juice also becomes "field-inoculated" with the yeast and bacterial flora present on the grape surface. If the berries are harvested during the heat of the day, undesirable microbial contamination can rapidly develop. To minimize this occurrence, grapes may be sulfited on harvest, and they are picked during cool parts of the day.

Left in containers, harvested fruit quickly warm owing to endogenous metabolic activity. This can aggravate contamination by speeding microbial activity. In addition, warming may necessitate cooling to bring the temperature down to an acceptable prefermentation value.

Stemming

The modern trend is to separate the processes of stemming and crushing. Removal of the stem, leaves, and grape stalks before crushing has several advantages. Notably, it minimizes the excessive uptake of phenols and lipids from vine parts. Extraction of stem phenols is of potential value only when dealing with red grape varieties low in phenol content. Stem phenols generally produce more astringent and bitter tastes than phenols released by the seeds and skins.

In the past, stems were often left with the must throughout fermentation, especially in the production of red wines. Presence of the stems made pressing easier, presumably by creating drainage channels along which the wine could escape. Modern improvements in press design have made stem retention unnecessary. The higher tannin contents derived from a prolonged contact with the stems gave red wines made during poor vintage years extra "body" and improved color density by stabilizing the limited anthocyanin content (see Chapter 6).

In addition to facilitating phenol extraction and pressing, maceration with the stems may increase the fermentation rate. This appears to be due to the increased uptake of oleanolic acid (Bréchot *et al.,* 1971). This is especially valuable under cool cellar conditions by favoring complete fermentation.

Leaf removal before crushing is beneficial as it limits the production of C_6 ("leaf") aldehydes and alcohols generated during the enzymatic oxidation of linoleic and linolenic acids. The aldehydes and alcohols can taint wine with a grassy to herbaceous odor, but in small amounts can contribute to the typical aroma of some wines. High leaf content also may result in the considerable uptake of quercitin. If the wine is bottled shortly after fermentation, quercitin can lead to the production of a yellowish haze in white wines (Somers and Ziemelis, 1985). When the wines are matured sufficiently, quercetin precipitates before bottling. High flavonol contents also can produce bitterness in white wines.

For convenience and efficiency, stemming and crushing often are performed by the same machine. Stemmers usually contain an outer perforated cylinder that permits berries to pass through but prevents the passage of stems, stalks, and leaves (Fig. 7.2). Often there are a series of spirally arranged arms, possessing flexible paddle ends, situated on a central shaft. Shaft rotation draws grape clusters into the stemmer, forces the fruit through the perforations, and expels the stems and leaves out the end. When crusher–stemmers are working optimally, the fruit is removed largely unbroken. Expelling

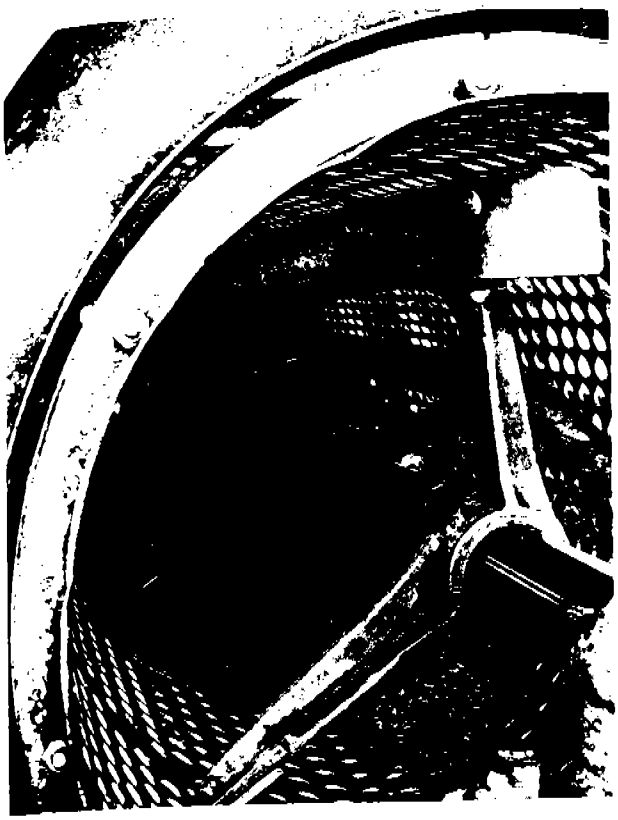


Figure 7.2 Internal view of a crusher–stemmer. (Photograph courtesy of the Wine Institute.)

the stems and leaves in a dry state avoids juice loss and facilitates their disposal. The stems may be chopped for subsequent soil incorporation.

Crushing

Crushing typically follows immediately on stemming because stemming unavoidably crushes some of the fruit. The juice so released is highly susceptible to oxidative browning and microbial contamination. Crushing the fruit without delay permits fermentation to commence almost immediately, limits microbial contamination, and permits better control of oxidation.

Crushing is accomplished by any one of a number of procedures. Those generally preferred involve pressing the fruit against a perforated wall or passing the fruit through a set of rollers. In the former, the berries are broken, and the juice, pulp, seeds, and skins pass through openings to be collected and pumped to a retaining tank or vat. In the latter process, berries are crushed between a pair of rollers turning in opposite directions. The rollers usually have spiral ribbing or contain grooves with interconnecting profiles to draw the grapes down and through the rollers. Spacing between the rollers usually can be adjusted to accommodate the variation in berry size found among different cultivars. It is important to avoid crushing the seeds to preclude contaminating the must with seed oils, the oxidation of which could produce rancid odors. Crushed seeds also provide an additional and undesirable source of bitter tannins.

Crushing also can be achieved using centrifugal force. In centrifugal crushers, the fruit is flung against the sides of the crusher. Because they tend to turn the fruit into a pulpy slurry, centrifugal crushers generally are undesirable. Clarification of the juice is difficult, and seeds are commonly broken.

Although grapes are customarily crushed prior to vinification, there are a few exceptions. Juice for sparkling wine production is commonly obtained by pressing intact grapes. Special presses extract the juice with a minimum of pigment and tannin extraction. The absence of pigments and tannins is particularly important where white sparkling wines are made from red-skinned grapes.

Botrytized grapes also may be pressed, rather than crushed. The gentler separation of the juice minimizes the incorporation of fungal dextran polymers (β-glucans) into the juice that can plug filters used in clarification. In the production of the famous botrytized wine Tokaji Eszencia, juice is derived solely from the liquid that drains freely from heavily infected grapes; no pressure other than the weight of the fruit promotes juice release.

In the production of wines employing carbonic maceration, such as *vino novello* and beaujolais, it is essential that most of the fruit initially remain uncrushed. Only within intact berries does the internal grape fermentation occur that develops the characteristic fragrance shown by the wines. After a variable period of autofermentation, berries that have not broken under their own weight are pressed to release the juice. Fermentation is completed by yeast action.

Supraextraction

An alternative to crushing being investigated in France is supraextraction (Defranoux *et al.*, 1989). It involves cooling the grapes to −4°C, followed by warming to about 10°C before pressing. Freezing causes both grape cell rupture and skin splitting that facilitate juice escape during pressing. While increasing the extraction of sugars and phenolics, supraextraction reduces total acidity and raises the pH. The latter may result from induced crystallization of tartaric acid.

Maceration

Maceration refers to the breakdown of grape solids following crushing of the grapes. The rupture and release of enzymes from grape cells facilitates the liberation and

solubilization of compounds bound in cells of the skin, flesh, and seeds. While maceration is always involved in the initial phase of red wine fermentation, until recently the trend has been to limit maceration in white wine production. However, there is a shift back to limited maceration for white wines, along with slight juice oxidation before fermentation.

The major factors influencing the extraction and types of compounds released during maceration are the temperature and duration of the process. Extraction may be a linear function of the temperature and length of skin contact. For example, cool temperatures and short duration minimize flavonoid uptake (Fig. 7.3), and thereby limit wine bitterness and astringency. Occasionally, the concentration of extracted compounds decreases with prolonged maceration, presumably owing to precipitation or degradation. Extraction also varies markedly with the class of compounds involved. For example, flavonoid phenols from the skin are more rapidly solubilized than nonflavonoids from seeds (Fig. 7.3).

In addition to phenolic compounds, the concentration of many nutrients and flavorants in juice and wine is influenced by maceration. For example, amino acid, fatty acid, and higher alcohol contents rise, while total acidity falls (Ramey *et al.*, 1986; Soufleros and Bertrand, 1988). The decline in acidity appears to be due to the extraction of potassium that induces tartrate salt formation. Other changes result from indirect effects on yeast metabolism. For example, increased amino acid availability has been correlated with a reduction in the production of hydrogen sulfide (Vos and Gray, 1979).

Occasionally, a short exposure (15 min) to high temperatures (70°C) greatly increases the release of volatile compounds, such as monoterpenes (Marais, 1987). Although the concentrations of most monoterpenes increase on short high-temperature maceration, not all follow this trend. For example, the concentration of geraniol decreases.

Maceration temperature also may affect the subsequent production of flavor compounds during fermentation. Production of volatile esters may increase with a rise in maceration temperature up to 15°C, whereas it decreases at higher temperatures. Synthesis of most alcohols is reduced following maceration at warm temperatures (Fig. 7.4), except for methanol. Synthesis of methanol is probably spurred by the increased action of grape pectinases, which release methyl groups from pectins.

The sensory influence of maceration can be affected by the degree of simultaneous oxidation. Oxidation speeds phenol polymerization, the products of which can cause browning and increase bitterness and astringency. However, polymerization also aids early tannin precipitation, leaving the wine less sensitive to subsequent in-bottle oxidation.

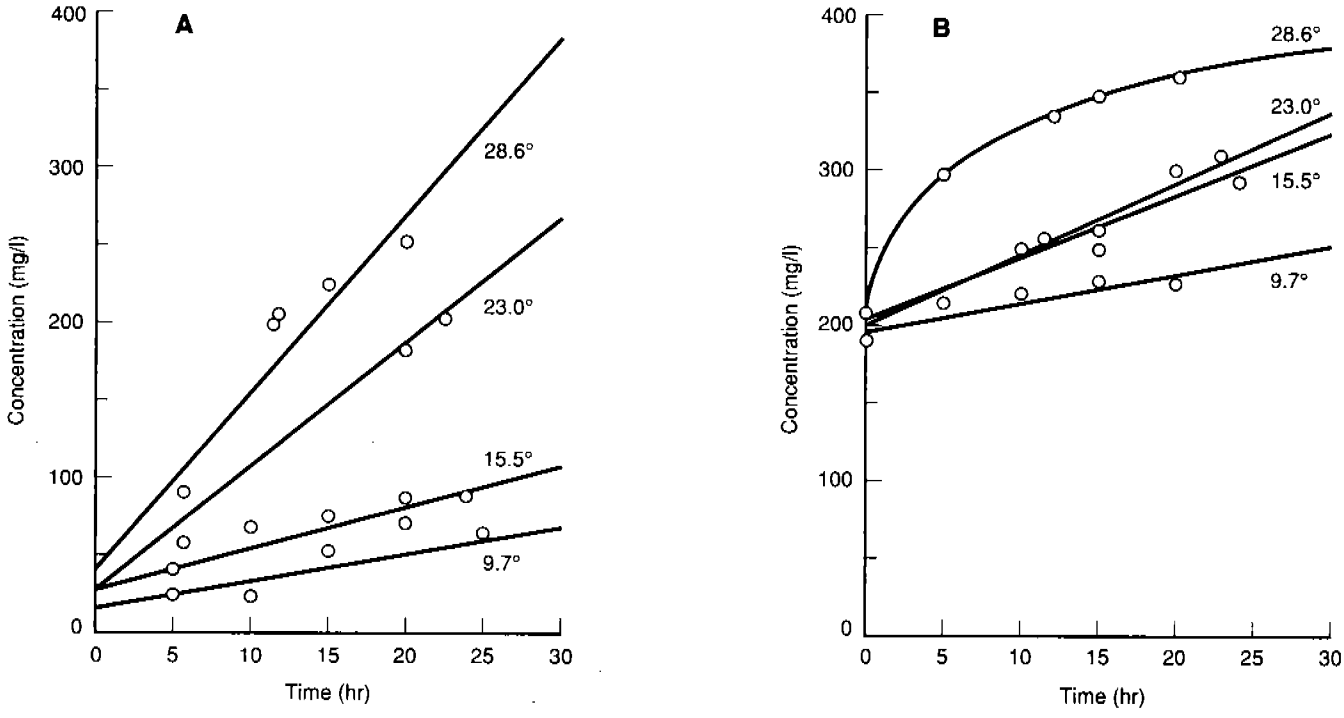


Figure 7.3 Flavonoid (A) and nonflavonoid (B) phenol content in 'Chardonnay' must during skin contact. Temperatures are in °C. (From Ramey *et al.*, 1986, reproduced by permission).

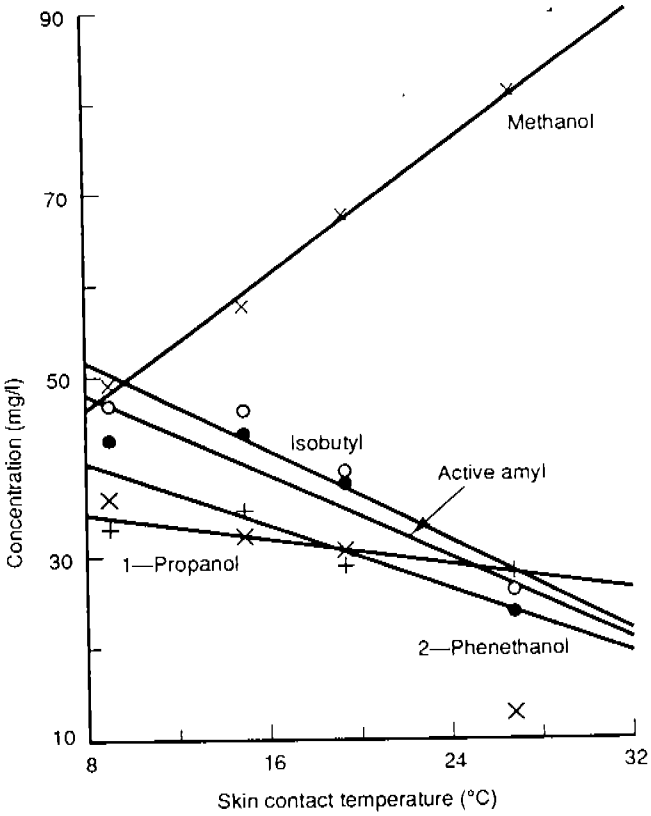


Figure 7.4 Concentration of various alcohols in 'Chardonnay' wine as a function of skin contact temperature. (From Ramey *et al.*, 1986, reproduced by permission.)

Maceration directly and indirectly improves juice fermentability (Ollivier *et al.*, 1987). Part of this effect is due to the release of particulate matter into the juice. Particulate matter is well known to increase microbial growth. The solids provide a surface for yeast and bacterial growth, adsorption of nutrients, binding of toxic carboxylic acids, and the escape of carbon dioxide. The latter is thought to increase must agitation and, thereby, promote more uniform nutrient distribution. Skin contact facilitates the extraction of unsaturated lipids, such as oleanolic, linolenic, and linoleic acids. The lipids are important in permitting yeast cells to synthesize essential steroids and build cell membranes under anaerobic fermentation conditions. The small amounts of oxygen absorbed during crushing, and during other prefermentation cellar activities, likely activate the synthesis of sterols by yeast cells.

Although the oxygen absorbed during crushing (~6 mg O_2/liter at 20°C) benefits certain wines, most winemakers prefer to avoid contact between the juice and oxygen during maceration.

Sulfur dioxide is added to the juice, depending on the health of the crop and the maceration temperature. Even small amounts of moldy fruit can significantly increase microbial contamination. The laccase concentration tends to rise with the degree of grape infection. Sulfur dioxide also may be added to restrict the growth of indigenous grape flora in the juice at warm maceration temperatures. In contrast, juice from healthy grapes, chilled and macerated at cool temperatures, seldom requires the addition of sulfur dioxide. The rapid disruption of cell membrane function by sulfur dioxide may be useful in speeding the release of grape constituents. In addition, sulfur dioxide can inhibit the action of grape polyphenol oxidases, delay the inception of alcoholic fermentation, and retard the onset of malolactic fermentation. Whether these effects are desirable depends on grape maturity, the cultivar involved, and the wine style desired. Addition of sulfur dioxide, at commonly used concentrations (~50 mg/liter), does not markedly affect the residual SO_2 content of the wine.

Minimal maceration at cool temperatures often leads to the production of young, fresh, fruity wines. Longer, warmer maceration typically produces a wine deeper in color and of fuller flavor. The latter may age more quickly and develop a more complex character than wines produced with minimal skin contact (Ramey *et al.*, 1986). Thus, varietal characteristics (Singleton *et al.*, 1980), fruit quality, equipment availability, and market response all influence the decision of the winemaker on whether and how to conduct maceration.

A new means of complementing or replacing maceration is called **cell-cracking** (Bach *et al.*, 1990). Cell-cracking involves forcing must through narrow gaps separating steel balls positioned in a small bore.

MACERATION: RED WINES

In red wine production, maceration studies have focused primarily on the extraction of pigments and tannins. Both the style and consumer acceptance of the wine can be dramatically altered by the duration and conditions of maceration. Thus, maceration provides one of the primary means by which winemakers can adjust the character of a wine. Short macerations (<24 hr) commonly produce a rosé wine. For early consumption, red musts are commonly pressed after 3 to 5 days. This provides good coloration but avoids the undue extraction of tannins. Wines for long aging may be macerated on the seeds and skins from 5 days to as long as 3 weeks. Long maceration may result in a decline in free anthocyanin content (see Fig. 7.9), but enhance color stability and aging potential.

Because of the importance of phenol solubilization during alcoholic fermentation, little attention has been given to the extraction of aromatic compounds. In one of the few studies on the subject, the "berry" aspect of 'Cabernet Sauvignon' was increased on prolonged mac-

eration, while the less desirable canned bean/asparagus aspects were diminished (Schmidt and Noble, 1983).

Dejuicing

Dejuicers are especially useful when dealing with large volumes of must. The capacity of presses can be used more economically to extract only the remaining juice (**pressings**).

Dejuicers often consist of a tank sealed at the exit by a perforated basket. Gravity forces much of the juice from crushed grapes pumped into the tank into the basket, from which the juice flows into a receiving tank (sump). Carbon dioxide pressure may be used to speed the separation. When drainage is complete, the basket is raised to ease pomace discharge for transport to a press.

Dejuicers of simpler design may consist of a sloped central cylinder containing perforations that permit juice escape, but retain most of the pomace. The crushed grapes are moved up the cylinder by rotation of a central screw. The dejuiced grapes are dumped into a hopper for loading in a press. The upward flow of the crush in the dejuicer supplies the gravitational force needed to speed juice release.

Pressing

If the crush has not been previously dejuiced, the must may be allowed to rest in the press for several minutes during which juice runs out under its own weight.

One of the first major advances in press design involved the use of hydraulic force. It replaced muscle power with mechanical force. The use of a removable bottom permitted easier pomace discharge. Previously, presses had to be dismantled, or the pomace shoveled out, at the end of each press cycle. Both tasks were unpleasant, time consuming, and labor intensive.

Increasing drainage surface area has been one of the modern goals of press design. This not only speeds juice release, but also reduces the flow path for juice escape. Increasing the area over which the pressure is applied also has been a major design improvement. By reducing the force required for juice extraction, the presses diminish the release of grape tannins, and pigments.

Placing the press on its side (horizontally) permitted additional improvements. Because the length (former height) of the press could be increased considerably, the surface area over which juice could escape was greatly increased. A horizontal orientation also permitted a section of the press to be hinged, providing access for convenient filling and emptying. By suspending the press on heavy gears, the press could easily be rotated for pomace **crumbling** (tumbling) and inverted for emptying. Crumbling breaks the compacted pomace produced during pressing and helps entrapped juice escape on subsequent pressing. Previously, chains or manual mixing were used to acheive crumbling. This had the disadvantages of both crushing seeds and increasing juice clouding, owing to the greater release of solids into the juice.

Another major innovation was the development of the continuous screw press. By permitting uninterrupted operation, such equipment avoids time-consuming filling and emptying cycles. This is especially valuable when large volumes of must, or wine, need to be pressed in a short period.

Because presses produce juice and wine fractions of differing physicochemical properties, winemakers can influence the character by the choice of press. The degree of fining and blending of the various press fractions provides additional means of adjusting the final character of the wine.

Brief descriptions of the three major types of presses in current use are given below. Figure 7.5 compares vertical, horizontal, and pneumatic presses.

HORIZONTAL PRESSES

A well-known press of horizontal design is that produced by Vaslin (Fig. 7.6). Both crushed and uncrushed grapes, as well as fermented juice, are effectively pressed in Vaslin-type presses.

Loading occurs through an opening in the upper, raised end of the press. Pressing is conducted by moving one or both end plates inward. The rate at which pressure is applied can be modified to suit the needs of the grape variety involved and characteristics of the press fraction desired. Fluid escape occurs between the slats of the pressing cylinder. Chains and/or rotation of the press break the pomace cake between successive pressings. Once pressing is complete, inversion of the press places the exit port downward for convenient dumping of the pomace.

The primary drawback to horizontal presses is the progressive reduction in drainage surface area during pressing. Consequently, the force required to maintain a rapid discharge increases during pressing.

PNEUMATIC (TANK OR MEMBRANE) PRESSES

Pneumatic presses, such as those produced by Willmes, Diemme, and Bucher, come in forms that effectively press crushed or uncrushed grapes as well as fermented must. The press is filled through an elongated opening in the top. Once filled and closed, the press is inverted to allow the free-run juice or wine to escape. Gas forced into the press between the sack and the cylinder wall compresses the grape mass against perforated plates that project into the central cavity (Fig. 7.7). Alternately, grapes or wine are placed between the cylinder wall and a central sack. Gas forced into the sack forces

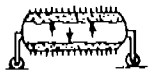
	Vertical	Horizontal	Pneumatic
Press type			
Size of the basket (cm)	113 x 90	215 x 73	215 x 73
Volume (m^3)	0.9	0.9	0.9
Pressure area (m^2)	1	0.42	4.95
Pressure per 1 cm^2 (MPa)	1.25 – 1.6	1.2	0.6
Pressure over the whole area (MPa)	12,500 – 16,000	5000	29,700
Pressure per 1 dm of pomace (MPa)	13.9 – 17.8	5.6	33.0
Average size of the cake (cm) at one half of the original volume	113 x 18	73 x 43	215 x 239 x 3.3
Shape of the cake			
Flowing out of the must (time)	long	short	very short
Time of one pressing (min)	100 – 120	100 – 120	50 – 90
Number of pressings	2	1	1
Total time of pressing (hr)	3 – 4	2	1

Figure 7.5 Comparison of various types of presses. (From Farkaš, 1988, reproduced by permission.)

the grape mass against the slatted sides of the press. In either case, a fairly constant surface for drainage is maintained throughout pressing. Crumbling of the pomace cake is achieved by rotating the pressing cylinder. Opening of the filling trap and inversion discharge the pomace.

Small volume presses (5 to 22 hl) also are being constructed by producers such as Willmes. These presses are or particular value when small lots of high quality juice or wine need to be kept separate.

Both horizontal and pneumatic presses yield high quality pressings. The pressings are relatively low in suspended solids, and press operation neither crushes the seeds nor extracts high amounts of tannins. A common drawback involves the time associated with the repeated filling and emptying, and fairly fixed press cycle (~1–2 hr).

CONTINUOUS SCREW PRESS

Continuous-type presses have the advantage of running uninterruptedly. While working best with fermented must, they can be adjusted to handle crushed, nonpulpy grapes. They do not function adequately with uncrushed grapes.

Crushed grapes as well as fermenting or fermented must are pumped into the press via a hopper at one end of the press (Fig. 7.8). A fixed helical screw forces the material into a pressing chamber whose perforated wall allows the juice or wine to escape. Pressed pomace accumulates at the end of the pressing cylinder, where it is periodically discharged through a port opening.

The primary disadvantage of the continuous press is the poorer quality of the released juice or wine. This is particularly noticeable in older models, where separation of different press fractions was not possible. Newer models permit such separations. The first fractions (closest to the intake) possess characteristics similar to free-run material. Fractions obtained nearer the end of the pressing cylinder progressively resemble the first, second, and third pressings of conventional presses. Slower pressing

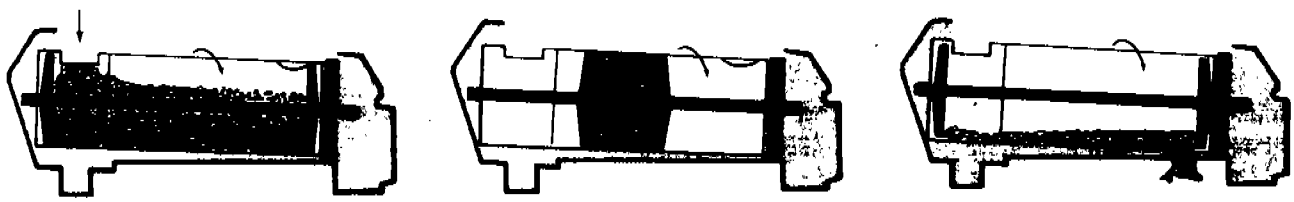


Figure 7.6 Schematic diagram of the operation of a horizontal press. (Courtesy of CMMC, Chalonnes-sur-Loire, France.)

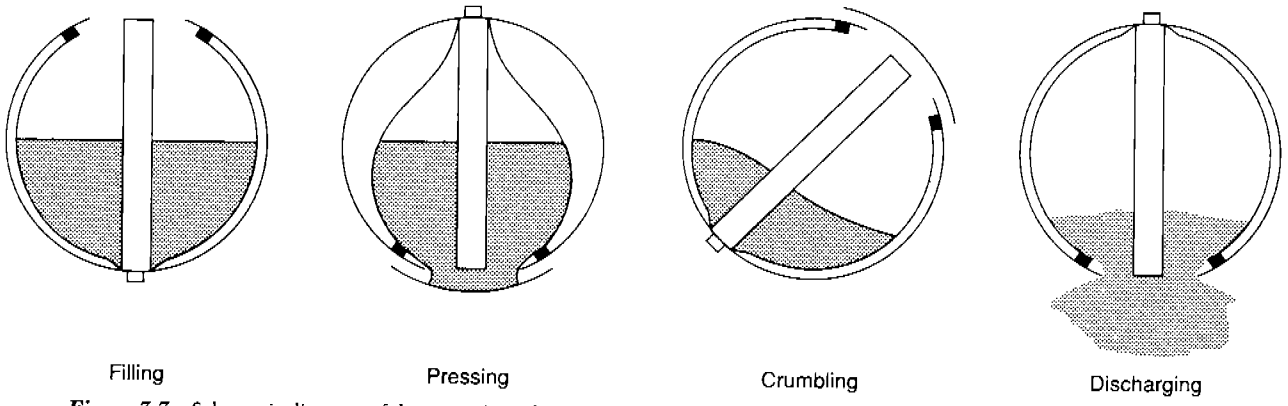


Figure 7.7 Schematic diagram of the operation of a pneumatic press. Note the centrally located perforated plates for drainage and the inward moving bladder membrane. (Courtesy of WILLMES.)

decreases the incorporation of suspended solids that diminish juice or wine quality, but also reduces the principal advantage of continuous-type presses, namely, speed.

Because different presses and press fractions produce fluids of distinct physicochemical properties, they can influence the sensory properties of the wine produced. For example, the production of fruit esters during fermentation tends to be lower in juice derived from continuous screw presses. The amount of tannins, pigments, and particulate matter also can vary considerably between fractions.

Pressing aids, such as cellulose or rice hulls, may be added to improve extraction. Occasionally, though, the addition has been noticed to influence fragrance development in the wine. Addition of pectinase to the crush also has a marked effect in improving juice release, especially with slip-skin (*V. labrusca*) or other pulpy cultivars.

Must Clarification

White must typically is clarified before fermentation to favor the retention of a fruity character. Loss of fruitiness may result from the excessive production of fusel alcohols, associated with juice containing high amounts of suspended solids. The largest particles in the solids fraction seems to be the most active in inducing higher alcohol synthesis (Klingshirn *et al.*, 1987). In addition, much of the polyphenol oxidase activity is associated with the particulate material. Thus, early removal of suspended material is important in minimizing enzyme-catalyzed oxidation. High levels of suspended solids also are reported to increase hydrogen sulfide production (Singleton *et al.*, 1975).

While high amounts of suspended solids in the juice are generally undesirable, highly clarified juices are also unsuitable, owing to increased susceptibility to "stick" during fermentation. For example, filtration and centrifugation can remove more than 90% of the higher fatty acids from must (Bertrand and Miele, 1984). Because suspended solids favor early malolactic fermentation, retention of small amounts of suspended solids in the juice can be beneficial. Although concentrations of suspended solids between 0.1 and 0.5% seem desirable for several white grapes (Groat and Ough, 1978), the optimal value can vary with the cultivar and the style of wine wanted. Within the range noted above, juice fermentation usually goes to completion and is associated with the production of desirable amounts of fruit esters and higher alcohols. The precise reasons for these benefits are poorly understood but may involve factors such as the adsorption of toxic carboxylic acids produced during fermentation and the availability of essential nutrients.

White juice commonly is allowed to settle spontaneously for several hours (~12 hr) before racking. Bentonite may be added to facilitate settling and subsequent protein stability. When used, bentonite is commonly added after an initial period of spontaneous settling. This avoids the production of voluminous amounts of loose sediment, and the associated loss of juice. Occasionally, some of the precipitate may be left with the juice during alcoholic fermentation. This permits vital nutrients, such as sterols and unsaturated fatty acids, to remain available. Although bentonite is commonly used, its effects on

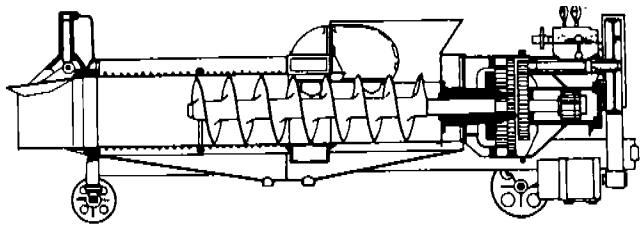


Figure 7.8 Schematic representation of a continuous press with hydraulic control. (Courtesy of Diemme.)

wine quality are still contentious (Groat and Ough, 1978).

To speed clarification, the juice may be centrifuged. By removing only suspended particles, centrifugation affects the chemical composition of the juice the least of any clarification technique. Although centrifugation equipment is expensive, minimal juice loss and speed have made it particularly popular.

Filtration with diatomaceous earth also may be used to clarify must prior to fermentation.

Adjustments to Juice and Must

ACIDITY AND pH

Juice and must failing to possess the desired acidity and pH may be adjusted before fermentation. Acidification of low acid juice or must often occurs before fermentation because it limits the growth of spoilage microorganisms and may be illegal after fermentation in some jurisdictions. In contrast, deacidification typically occurs after fermentation, when its effect on acidity is known. Deacidification can be based on actual rather than projected need. Flavor production also is generally better in musts fermented at a low pH. Finally, postfermentative deacidification permits the process to be delayed until spring, when other winery activities are less urgent.

One of the oldest procedures of adjusting the pH of juice, known as plastering, is rarely used today. The addition of gypsum acts by converting some of the potassium bitartrate to the free acid form:

$$CaSO_4 + 2\,KH(C_4H_4O_6) \longrightarrow K_2SO_4 + H_2(C_4H_4O_6) + Ca(C_4H_4O_6)$$

Gypsum (Calcium sulfate)	+ Potassium bitartrate	→ Potassium sulfate	+ Tartaric acid	+ Calcium tartrate

The procedure has fallen out of favor not only because it increases the sulfur content of wine, but also because organic acids are readily available, inexpensive, and do not markedly effect the chemistry of wine.

Currently, the high pH usually associated with low total acidity is corrected by the addition of organic acids (**acidification**) (Buechsensteing and Ough, 1979). Tartaric acid often is preferred because of its relative insensitivity to microbial decomposition and its ability to increase pH by inducing the precipitation of excess potassium as a bitartrate salt. Citric acid may be substituted because of its iron stabilization properties, but it is susceptible to microbial degradation.

Deacidification of excessively acidic juice low in pH may involve blending with juice of lower acidity but higher pH. Alternately, some of the acid may be neutralized by the addition of calcium carbonate, potassium carbonate, or Acidex.

The most difficult situation occurs when juice shows both high acidity and high pH. This situation is particulary common in cool climatic regions where grapes may possess both high malic acid and potassium contents. Nagel *et al.* (1988) suggest adding tartaric acid to adjust the malic/tartaric ratio to unity. This is followed by precipitation of the excess potassium with Acidex and acidification with tartaric acid to a desirable pH/acidity.

Amelioration is a means of deacidification involving the dilution of juice acidity by the addition of water. Because dilution also reduces juice sugar content, sugar addition is required to readjust the °Brix upward. Although amelioration is illegal in most countries, it has the advantage that it little affects juice pH. This results because of the dicarboxylic nature of tartaric acid and its low dissociation constant. The dilution of H^+ that results from the addition of water is counterbalanced by the increased dissociation of tartaric acid. Thus, acidity falls but pH is only slightly affected. While reduced color, body, and flavor are usually undesirable consequences of the use of amelioration, the effects may not all be undesirable in intensely flavored varieties, such as *V. labrusca* cultivars. However, the greatest disadvantage of amelioration is its consumer image. The addition of sugar, and especially water, is commonly viewed as unscrupulous behavior.

Acidity and pH adjustment of wine are discussed in Chapter 8.

SUGAR CONTENT

The sugar content (**total soluble solids**) of juice is commonly measured with a hydrometer in units variously called **Brix, Balling, Baumé,** and **Oechsle** (Appendix 6.1). Because sugars constitute the major component of grape soluble solids when over 18° Brix (Crippen and Morrison, 1986), °Brix is a fairly accurate indicator of the capacity of the juice to support alcohol production. More precise measurements of sugar content are available, but the hydrometer determinations are usually adequate early in the winemaking process. In the field, refractometer readings are often used to assess grape sugar content.

As fermentation progresses, hydrometer readings become imprecise measures of sugar content. This results because the alcohol produced during fermentation independently affects specific gravity, the property measured by the hydrometer. Although specific gravity is an adequate indicator of the termination of fermentation in dry table wines, correction tables are necessary for use with sweet fortified wines (Amerine and Ough, 1980).

Following the completion of fermentation, precise chemical analysis of the residual sugar content of the wine usually is required (Zoecklein *et al.*, 1990). Even small amounts of residual sugars can affect the microbial

stability of the wine and, therefore, how the wine should be treated up to and during bottling.

When juice °Brix is insufficient to generate the desired alcohol content, chaptalization may be used. **Chaptalization** usually involves the addition of a concentrated solution of sugar to the juice or must. It was first advocated by Dr. Chaptal in 1801 to improve the stability and character of wines produced from immature or rain-swollen grapes. The increased alcohol content generated by the added sugar improved both features.

Chaptalization is typically illegal in regions or countries where warmer growing conditions obviate its need, but it often is permissible in areas where cool climates may prevent full ripening of the grapes. Where permissible, chaptalization usually occurs under strict governmental regulation.

Although many factors influence the conversion of sugars to alcohol (Jones and Ough, 1985), 17 g of sucrose (i.e., cane sugar) typically yields about 10 g of ethanol. The sugar is first dissolved in grape juice and added near the end of the exponential phase of yeast growth (commonly 2 to 4 days after the commencement of fermentation) (Ribéreau-Gayon *et al.*, 1987). By this time, yeast multiplication is essentially complete, and the sugar does not disrupt fermentation. Simultaneous aeration of the fermenting juice or must is recommended.

In addition to elevating the alcohol content, chaptalization slightly augments the production of certain compounds in wine, for example, glycerol, succinic acid, and 2,3-butanediol. Synthesis of some aromatically important esters also may be increased, while that of others is decreased (Fig. 7.21). However, these influences do not make up for the lack of varietal character found in immature grapes or grapes diluted by rains. In some varietal wines, such as 'Riesling,' chaptalization can diminish the "green" or "unripe" taste derived from immature fruit (Bach and Hess, 1986).

Various techniques are under investigation to improve the character of wines produced in poor vintages without the addition of sugar. **Reverse osmosis** is one such technique (Duitschaever *et al.*, 1991). Although first designed as an economical means of obtaining fresh water from salt water, reverse osmosis has found many applications in other industries, from sewage treatment to fruit juice concentration. It is the latter application that has attracted the attention of enologists. In addition to offsetting some of the problems of poor vintages, reverse osmosis can concentrate fruit flavors in the juice.

Reverse osmosis operates by forcing water out of the juice through a membrane that retains most of the sugars and flavoring components. The principles of the operation are discussed more fully in Chapter 8.

Although effective, reverse osmosis has its limitations and drawbacks. Presently, it concentrates the juice only up to about 30° Brix. During concentration, acids may accumulate to a degree requiring deacidification. More significantly, important aroma components may be lost. Small, highly volatile, water-soluble compounds such as esters and aldehydes are the most likely to be lost. The addition of untreated juice to the concentrated juice can partially alleviate this problem. Concentration of volatiles removed with water and reintroduction into the treated juice constitute another possible solution. Development of filters with improved selective permeability may eliminate this problem.

Cryoextraction is another technique being investigated to overcome deficiencies in sugar and flavor content (Chauvet *et al.*, 1986). As with reverse osmosis, cryoextraction can be used with immature grapes or berries swollen with water after rains. It also may be used to augment the sugar and flavor content of grapes in the production of sweet table wines. Cryoextraction is the technical equivalent of *eiswein* production, except that overmature grapes are not used. Cryoextraction involves freezing and subsequent crushing and pressing of the frozen grapes.

As water in the grapes forms ice, dissolved substances become increasingly concentrated in the remaining liquid juice. Because berries of greater maturity (sugar content) freeze more slowly than immature grapes, preferential extraction of juice from the more mature grapes can be achieved. Although temperatures down to −15°C increase solute concentration, temperatures between −5° and −10°C are generally sufficient to remove unwanted water. Cryoextraction appears not to produce undesirable sensory consequences.

Another technique under investigation is the Entropie concentrator (Froment, 1991). It involves juice concentration under vacuum and at moderate temperatures (~20°C).

Brix adjustment usually is designed to generate a higher alcohol content in the wine. There is, however, a growing market for low alcohol wines. Reduced alcohol contents are usually produced following alcoholic fermentation by dealcoholization. A new technique offers the possibility of diminishing the capacity of juice to support alcohol production (Villettaz, 1987). The process involves the action of two enzymes, glucose oxidase and peroxidase. Glucose oxidase converts glucose to gluconic acid, a nutrient that yeasts cannot ferment. Hydrogen peroxide, produced as a by-product of glucose oxidation, is destroyed by peroxidase. The two reactions are as follows:

$$2\ \text{Glucose} + 2\ H_2O + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} 2\ \text{gluconic acid} + 2\ H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{peroxidase}} 2\ H_2O + O_2$$

With glucose oxidase, alcohol production can be reduced by about half, equivalent to the proportional concentration of glucose in the juice. Thus, ethanol production is dependent on the remaining fructose content of the juice.

Because a steady supply of oxygen is required for enzymatic dealcoholization, the juice becomes oxidized and turns brown. While much of the color compounds formed precipitate during fermentation, the wine is still left with a distinct golden color. The effects of this, and other factors, on the sensory quality of the wine have yet to be fully assessed.

COLOR EXTRACTION: THERMOVINIFICATION

The grapes of several red varieties seldom produce a dark red wine using standard vinification techniques, for example, 'Pinot noir.' Standard procedures may extract only about 30% of the anthocyanin content of the grapes. Poor color also may result from the action of fungal polyphenol oxidases such as laccase. **Thermovinification** is one technique of improving the color of red wines.

Thermovinification involves the heating of intact or crushed grapes to between 50° and 80°C. Some versions involve rapid heating of whole grapes with steam or boiling water. Such treatments are typically short (~1 min) and heat the outer pigment-containing layers of the fruit to about 80°C. Other procedures involve heating some or all of the pomace, or both the pomace and juice. The juice and pomace are typically heated to about 70°C for 30 min. Where this treatment damages subtle varietal aromas, temperatures as low as 50°C may be used. For especially delicate varieties such as 'Pinot noir,' heating may be as low as 32°C for 12 hr (Cuénat **et al.**, 1991). Only mold-free grapes can safely be treated at temperatures below 60°C, as laccase activity increases up to this temperature. Heating may be conducted with or without continuous stirring. Subsequent vinification may be conducted in the presence or absence of the seeds and skins. Each variation influences the attributes of the wine generated from the must.

The heat dramatically increases anthocyanin extraction (Fig. 7.9), and temperatures above 60°C inactivate laccases. Thermovinification is used primarily to produce wines designed for early consumption. In addition to generating a rich red color, thermovinification improves juice fermentability (both alcoholic and malolactic), produces wines low in astringency, and reduces varietal aroma. Although not normally considered beneficial, diminished varietal aroma can be desirable with strongly flavored cultivars. Low astringency contributes to a soft mouth-feel, appropriate for wines designed for early consumption. Rapid completion of fermentation has numerous benefits, including the liberation of fermentors for additional fermentations. However, it increases the need for temperature control during fermentation.

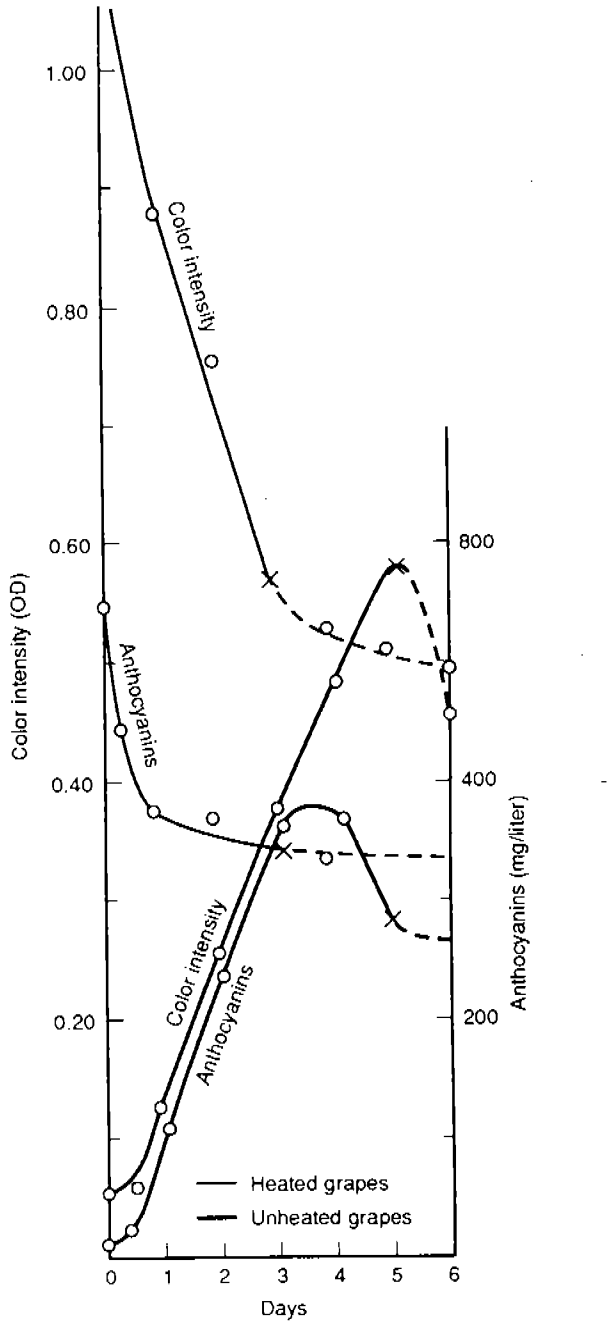


Figure 7.9 Development of color intensity (OD) and level of anthocyanins (mg/liter) during fermentation; × indicates the end of alcoholic fermentation. (After Ribéreau-Gayon *et al.*, 1976, reproduced by permission.)

Occasionally, thermovinification generates undesirable bluish colors and "cooked" flavors. These usually can be avoided by appropriate adjustments to the technique. Difficulties with clarification and filtering also may be experienced, but they often can be corrected by the addition of pectinase. Some proprietary pectinase

combinations are reported to enhance color extraction as well.

OTHER ADJUSTMENTS

The addition of nitrogen (usually as an ammonium salt) is uncommon, but can improve the fermentation of highly clarified white juice.

BLENDING

For white wine production, it is common to combine free-run juice with that from the first pressing. Occasionally, the second pressing also is added. Other pressings usually are too tannic and difficult to clarify to be used in making white wine. However, several finings and centrifugations may permit later pressings to be incorporated with the other fractions. Alternately, late pressings and the pomace may be fermented to obtain alcohol for distillation, or they may be sold for vinegar production.

For grape varieties such as 'Riesling,' pressings may contain two to five times the concentration of terpenes found in the free-run juice (Marais and van Wyk, 1986). Because of the importance of terpenes to the distinctive aroma of the cultivars, the addition of pressings improves wine quality. The distribution of individual monoterpenes within the fruit varies with the cultivar, grape maturity, and the free versus bound state of the terpenes (Park *et al.*, 1991). Thus, the addition of pressings may affect both the quantitative and qualitative aspects of wine aroma.

DECOLORIZATION

If necessary, decolorization is normally conducted after fermentation. However, addition of the enzyme **anthocyanase** permits decolorization before fermentation. Anthocyanase removes the sugar component from anthocyanins, converting them to anthocyanidins. The lower solubility of anthocyanidins promotes their precipitation during fermentation. Because anthocyanase is inactivated by sulfur dioxide, ethanol, and high temperatures, treatment normally follows juice clarification. At this point, the free sulfur dioxide content is likely to be low and little alcohol will have been produced by the indigenous yeast inoculum.

Alcoholic Fermentation

Fermentors

Fermentors come in a wide variety of shapes, sizes, and technical designs. Most differ little in design from those used centuries ago. However, some are highly complex and designed for specific functions.

Most fermentors are straight sided or have the form of slightly inverted cones. **Tanks** are differentiated from vats by being closed; **vats** have open tops. Tanks commonly double as storage cooperage, while vats can be used only as fermentors.

BATCH-TYPE FERMENTORS

During the fermentation of red wines, carbon dioxide released by yeast metabolism becomes entrapped in the pomace. This causes the pomace to rise to the top, where it forms a **cap.** However, the entrapped carbon dioxide prevents contact between the pomace and most of the juice, retarding the extraction of anthocyanins and other compounds from the skins and pulp.

With vats, periodic submerging (**punching down**) of the cap into the fermenting must is adequate. In addition to improving color removal, punching down can aerate the fermenting must, limit the growth of spoilage organisms in the cap, and help equalize the temperature throughout the fermenting must. Before our own era, there were no simple, convenient methods of achieving the benefits of punching down other than manually.

With modern developments, tanks have almost completely replaced vats for the fermentation of all type of wine. By having a closed top, tanks prevent must exposure to air-borne contaminants and oxygen. Thus, tanks can act as fermentors during the harvest period and as storage cooperage during the rest of the year.

Since the 1950s, there has been a move away from wooden tanks (oak, chestnut, redwood, etc.) to more impervious and inert materials. Cement is favored in some regions, but stainless steel is probably the most generally preferred material. Fiberglass tanks are becoming more popular because of their light weight and lower cost. Nevertheless, stainless steel has one distinct advantage over other materials, namely rapid heat transfer. This property can be used to cool the fermenting juice. This can be achieved by flushing water over the sides of the tank, with evaporating water acting as the coolant. However, double-jacketed tanks, circulating a coolant between the inner and outer walls, can provide more versatile temperature regulation of the fermenting juice.

Although cement is a poor insulator, the rate of heat transfer of cement is usually insufficient to prevent excessive heat buildup during fermentation. Thus, the juice may be passed through a heat exchanger to achieve temperature control. Cement is also more difficult to surface-sterilize. While an epoxy coating helps, it requires frequent maintenance. However, cement tanks do have the initial advantage of being less expensive to construct than equivalent stainless steel tanks.

Fermentors for white wine production are generally of simple design. The primary technical requirement is for

efficient temperature control. If not initially cool, the juice may be chilled before yeast inoculation, and the ferment is maintained at cool temperatures (8° to 15°C) throughout vinification.

Fermentors for red wine production may be even simpler in design. Cap formation and a higher phenol content give fermenting red wines considerable protection from oxidation. Also, if the cellar is cool, and the fermentor volume relatively low (50 to 100 hl), cooling during fermentation may be unnecessary. Red wines generally are fermented at, or allowed to warm to, 25° to 28°C. Punching down slightly cools the fermenting must.

The shift to tanks for red must fermentation has demanded means of replacing manual punching down. This requirement has spawned an incredible array of solutions. One of the more novel is the *pileage* fermentor with mechanical cap punchers to simulate the action of manual punching down (Anonymous, 1983). Other solutions include pumping the must over the cap for about an hour several times a day. This may be combined with passing the wine through cooling coils for temperature control. If the headspace over the cap does not contain an inert gas (N_2 or CO_2), pumping over produces some aeration. By submerging the cap, growth of potential spoilage organisms in the cap is limited. **Pumping over** may be manual or automated, as well as periodic or continuous. Other devices designed to mix the pomace with the must consist of large mechanical stirrers.

Autofermentors are specifically designed to facilitate the extraction of pigments from the pomace. They generally possess two superimposed fermentation chambers. The lower, main chamber contains two traps into the upper smaller chamber. An elongated, perforated cylinder descends from one trap into the main chamber. As fermentation progresses, carbon dioxide accumulation increases the must volume. At a certain point, the pressure forces this trap open and a portion of the carbon dioxide and fermenting juice escapes into the upper chamber. Here, the carbon dioxide escapes and the fermenting juice cools slightly. The weight of the juice forces the other trap to open downward. The flush of juice back in the main chamber ruptures the cap and temporarily disperses it into the fermenting must. Perforations in the cylinder prevent pomace from escaping with the juice into the upper chamber.

The cap in autofermentors is normally submerged. A simpler system of achieving a submerged cap involves a grill located below the surface of the must. Although autofermentors avoid the need of punching down, additional agitation is still required to achieve adequate extraction of color from the cap.

Fermentors of modern design typically include some system to ease pomace discharge. For this purpose, sloped bottoms with trap doors are often used. Removable bottoms are another solution.

CONTINUOUS FERMENTATION AND RELATED PROCEDURES

Most fermentors for winemaking are of the **batch** type. In other words, separate lots (batches) are individually fermented to completion. In most industrial fermentations, continuous fermentation is the norm. In continuous fermentation, substrate is added constantly, or at frequent intervals. Equivalent volumes of the fermenting liquid are removed to maintain a constant volume. Continuous fermentors may remain in uninterrupted operation for weeks or months. For the industrial production of single metabolic products, synthesized primarily during a particular phase of colony growth, continuous fermentation has many economic advantages. The technique is less compatible with wine production, however, especially with high quality wines showing subtle and complex associations of hundreds of compounds.

Despite the potential advantages of continuous fermentation, it is rarely used in the wine industry. Because of their design, and expense, continuous fermentors are economically feasible only if used year-round. This in turn demands a constant supply of must. With the seasonal character of the grape harvest, this requires the storage of must under sterile, nonoxidizing conditions. These requirements demand more sophisticated storage than would be needed to store the corresponding volume of wine. Thus, technical and financial concerns generally outweigh the benefits of product uniformity and the easier alcoholic and malolactic fermentations achieved by the use of continuous fermentors.

A new technique under investigation is the repeated use of yeast in fermentation. After each fermentation, the yeast is removed and used to inoculate successive fermentations. Removal is achieved either by filtration, centrifugation, or spontaneous sedimentation. In addition to cost saving, there are further benefits to what is called **cell-recycle batch fermentation** (Rosini, 1986). The duration of fermentation is considerably reduced, and the conversion of sugars to ethanol is slightly improved. There also is a reduction in the synthesis of sulfur dioxide by yeast cells, but an increase in volatile acidity. Although yeast multiplication continues at progressively reduced rates, continual monitoring for contamination by undesirable yeasts and bacteria is necessary. Periodic assessment to determine that the genetic character of the yeast population has not changed is required.

FERMENTOR SIZE

Optimal fermentor size has more to do with the volume of juice or must typically fermented than almost any other factor. When the volumes are large, immense fermentors are both needed and economically appropriate. When modest volumes need to be fermented, suitably small fermentors are required. Specially designed tanks

of 50 to 60 hl capacity are produced by several European manufacturers that mix the pomace and fermenting juice automatically (Rieger, 1993). They have been particularly valuable for poorly colored varieties such as 'Pinot noir.' Traditional use of carbonic maceration (see Chapter 9) also requires the use of special, shallow, small volume fermentors.

Small must volumes may result from limited land holdings or when wine is made from small lots of special quality fruit. The latter situation can develop when grape harvesting occurs at different times or states of maturity. This is particularly striking with the higher level Prädikat wines of Germany. Separate fermentation is essential to maintain the individuality of the different grape lots. In these situations, fermentation often is conducted in barrels (~225 liters), puncheons (~500 liters), or fuders (~1000 liters).

In addition to permitting the separation of small lots of juice or must, "in-barrel" fermentation has a number of potential advantages. Because cooling during fermentation occurs only by heat radiation through the sides of the cooperage, fermentation may occur at temperatures higher than currently recommended, especially for white wines. As a result, fruit-smelling acetate esters may dissipate more readily along with escaping carbon dioxide (Fig. 7.25) and varietal aromas achieve a clearer expression in the wine.

As the wine usually is left on the lees (dregs) longer than in larger fermentors, there is an increased likelihood of malolactic fermentation. There also is an increased risk of off-odor production which can be countered by using yeast strains with a low potential for hydrogen sulfide synthesis. In addition, periodic mixing of the lees and wine provides aeration that further decreases the generation of sulfide odors. Furthermore, yeast viability is enhanced by slight aeration. This may permit the enzymatic oxidation of lipid precursors needed to maintain membrane function. Enhanced yeast viability is viewed as contributing to a better integration of oak flavors and tannins from the cooperage wood and to improved mouth-feel.

On the negative side, more effort is involved in sterilizing, cleaning, topping, and maintaining small wood fermentors. There also is increased risk of oxidation and acetic acid bacterial activity during maturation (Stuckey *et al.*, 1991). In small amounts, acetaldehyde and acetic acid and the uptake of oak flavors can increase wine complexity, but excess amounts can mar wine flavor. Barrel reuse increases the risks of microbial spoilage.

Another complicating factor with the use of small wooden cooperage is the loss of water and alcohol by evaporation. Depending on the relative humidity of the cellar, the wine may either lose more water or alcohol. While this results in a loss in wine volume, evaporation from barrel surfaces also influences the concentration of most wine flavorants.

For many premium wines, fermentors commonly range in size from 50 to 100 hl. Such a volume appears to strike an appropriate balance between economics and ease of operation and the desire to maintain individuality. For standard quality wines, the economics of size shift the balance toward fewer but larger fermentors. In this case, fermentors in capacities from 200 to more than 2000 hl (~50,000 gal) become preferable. Computers have proved useful in monitoring and regulating the course of fermentation in mammoth fermentors.

Associated with increased fermentor volume are temperature control problems. In large fermentors, passive heat dissipation via the surface is insufficient to prevent excessive heat buildup during fermentation. As a result, overheating of the must is likely, and the fermentation will "stick." However, the economics of size permit the use of sophisticated cooling systems to maintain a favorable fermentation temperature.

Fermentation

Fermentation is an energy-releasing form of metabolism where both the substrate (initial electron donor) and by-product (final electron acceptor) are organic compounds. It differs fundamentally from respiration in not requiring the involvement of molecular oxygen. Although many fermentative pathways exist, *Saccharomyces cerevisiae* possesses the most common, **alcoholic fermentation.** In it, ethanol acts as the final electron acceptor (by-product), while glucose is the preferred electron donor (substrate). Although *Sacch. cerevisiae* possesses the ability to respire, it preferentially ferments even in the presence of oxygen.

Although most organisms are able to ferment sugars, they do so only when oxygen is lacking. This partially results from the toxic action of the usual end products of fermentation, lactic acid or ethanol. In addition, fermentation is an inherently inefficient mode of energy release. Its converts only about 6 to 8% of the chemical bond energy of glucose into readily available metabolic energy, (ATP, adenosine triphosphate). Much of the eneregy remains bound in the terminal by-product of electron acceptance, ethanol.

The two main organisms involved in vinification, *Saccharomyces cerevisiae* and *Leuconostoc oenos,* are somewhat unusual in selectively employing fermentative metabolism. They are also atypical in withstanding moderately high ethanol concentrations.

The combined properties of alcohol tolerance and preferential alcoholic fermentation endows *Sacch. cerevisiae* with the ability to dominate rapidly in grape must in the absence of oxygen. The mechanism(s) by which

Sacch. cerevisiae avoids ethanol toxicity is incompletely understood but may involve the rapid diffusion or export of alcohol out of the cell.

Leuconostoc oenos is less well adapted to growing in grape juice or must than *Sacch. cerevisiae*. It typically grows slowly in wine after *Sacch. cerevisiae* has completed alcoholic fermentation and has become inactive. In most ecological habitats, production of lactic acid by lactic acid bacteria lowers the pH and excludes competitive bacteria. Lactic acid bacteria are one of the few acid-tolerant bacterial groups. However, the high acidity of juice and wine often retards or inhibits their growth. Thus, the metabolic conversion of malic to lactic acid, a weaker acid, has the result of increasing the pH and favoring bacterial growth. Malolactic fermentation also makes excessively acidic wines more acceptable to the human palate and may improve microbial stability by removing residual fermentable substrates.

As noted above, wine is usually **batch fermented.** Thus, nutrient availability is maximal at the beginning of fermentation, and progressively declines thereafter. By the end of fermentation, most sugars have been metabolized, leaving the wine "dry."

Batch fermentations generally show a growth pattern consisting of four phases: lag, log, stationary, and decline. Immediately following inoculation, cells need to adjust to the new environment. Because some cells do not make the adjustment successfully, the number of new cells produced approximates the number that die. Thus, there is no net increase in the number of viable cells. This is called the **lag** phase.

Once adjustment is complete, most cells begin to multiply at a steady rate until conditions become unfavorable. Because of the unicellular nature of most microbes, the growth curve approximates an exponential equation. This phase is appropriately called the **exponential** or **log** (logarithmic) phase. During this period, the population of viable cells rapidly increases to its maximum value.

Under batch conditions, the nutrient content progressively falls and toxic metabolic by-products accumulate. Thus, after a period of rapid growth, the rate of cell division (growth) declines and approaches the rate at which cells die. The culture is now said to have moved into the **stationary** phase. As nutrient conditions continue to deteriorate and the concentration of toxic metabolites escalates, more cells die than divide. At this point, the culture enters a **decline** phase. Because most viable cells are not replaced, the colony eventually perishes, or the remaining cells become dormant.

Although basically similar, the population growth pattern displayed by yeast growth in grape juice shows several variations from the norm (Fig. 7.10). Typically the lag phase is short or undetectable; the exponential growth phase is relatively short (seldom amounting to more than eight divisions); the stationary phase may be short and commence long before nutrients become limiting; and the decline phase is atypically long and stabilizes at a high population level. As much as 40% of the sugar metabolized to alcohol occurs during the decline phase (Ribéreau-Gayon, 1985).

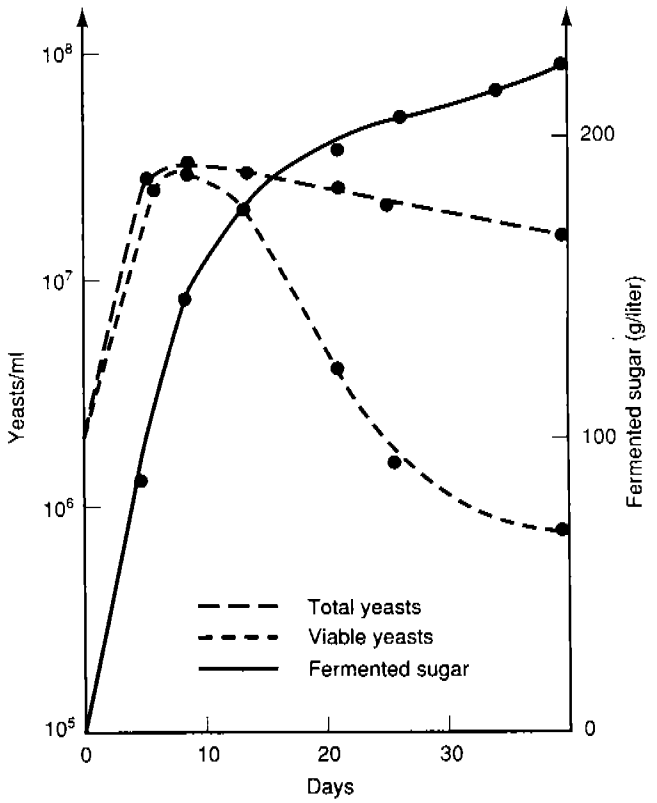


Figure 7.10 Growth cycle of yeasts and fermentation kinetics in grape must with a high sugar content (320 g/liter). (From Ribéreau-Gayon *et al.*, 1976, reproduced by permission.)

The brevity or apparent absence of a lag phase in yeast growth may result from the preadapted state of the cells initiating fermentation. Active dry yeast commonly used for juice or must inoculation comes from cultures grown exponentially in aerated media. Thus, the cells possess the enzymatic capacity necessary to commence exponential growth almost immediately. Equally, the indigenous yeast population on grapes may require little enzymatic adaptation to commence rapid cell growth. However, the absence of a noticeable lag period with spontaneous fermentation also may be an artifact. Indigenous yeasts are commonly bathed in juice released from broken grapes and may pass through the lag phase before fermentation officially begins in the winery. In addition, yeasts growing on berry skins may exist under minimal, but concentrated, nutrient conditions.

Although physiological adjustment to growth in juice appears to be minimal, a lag phase may be observed

cinate is generated. This probably explains why succinate is one of the major by-products of fermentation.

Replacement of TCA cycle intermediates lost to biosynthesis, or secreted as succinate, probably comes from pyruvate. Pyruvate may be directly channeled through acetate, carboxylated to oxaloacetate, or indirectly routed via the glyoxylate pathway. The involvement of biotin in the carboxylation of pyruvate to oxaloacetate may partially explain its requirement by yeast cells.

The accumulation of another major by-product of fermentation, glycerol, also is probably explained in terms of maintaining a favorable redox balance. The reduction of dihydroxyacetone phosphate to glycerol 3-phosphate can oxidize the NADH generated in the oxidation of glyceraldehyde 3-phosphate in glycolysis (Fig. 7.15). However, coupling of the two reactions does not generate net ATP production. This is in contrast to the net production of 2 ATP molecules during fermentation to ethanol.

The increased production of glycerol in the presence of sulfur dioxide is probably explained by the need to regenerate NAD^+. The binding of sulfur dioxide with acetaldehyde inhibits its reduction to ethanol, the usual means of NAD^+ regeneration during alcoholic fermentation.

Throughout fermentation, yeast cells adjust continuously to the changing conditions in the juice to produce adequate levels of ATP, maintain favorable redox and ionic balances, and synthesize necessary metabolic intermediates. Consequently, the concentration of yeast by-products in the juice changes continually during fermentation (Figs. 7.13 and 7.14). Because several of the products are aromatic, for example, acetic acid, acetoin, and succinic acid, their presence can affect bouquet development.

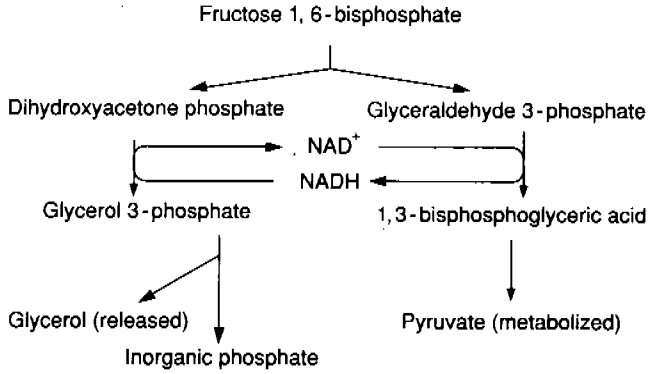


Figure 7.15 Simplified pathway showing how NADH derived from the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglyceric acid is used in the reduction of dihydroxyacetone phosphate to glycerol. As a consequence NADH is unavailable to reduce acetaldehyde to ethanol.

Although different strains of *Sacch. cerevisiae* possess similar enzymes, the relative proportions and catalytic activities may vary. The differences probably depend on the precise functioning of the regulatory systems of the cells and the number of copies of each gene. Thus, no two strains are likely to respond identically to the same set of environmental conditions. This variability in response undoubtedly explains much of the subtle, and not so subtle, differences between fermentations conducted by different yeast strains.

INFLUENCE ON GRAPE CONSTITUENTS

Yeasts have their major effect on the sugar content of the juice or must. If fermentation goes to completion, only minute amounts of fermentable sugars remain (preferably $\leq$2 g/liter). Small amounts of nonfermentable sugars, such as arabinose, rhamnose, and xylose, also remain (~0.2 g/liter). The small quantities of sugars are imperceptible and leave the wine tasting dry.

Yeasts may increase the pH by metabolizing malic acid to lactic acid. However, the proportion converted is highly variable in *Saccharomyces cerevisiae* and can differ among strains from 3 to 45% (Rankine, 1966). In addition, some *Sacch. cerevisiae* strains liberate significant amounts of malic acid during fermentation (Farris *et al.*, 1989). In contrast, *Schizosaccharomyces pombe* completely decarboxylates malic acid to lactic acid. It has been little used in juice deacidification as the sensory impact on wine is generally negative. Undesirable flavors, such as hydrogen sulfide, often mask the fragrance of the wine. Delaying inoculation until after *Sacch. cerevisiae* has been active for a few days, or has completed fermentation, apparently reduces the negative impact of *Schizosacch. pombe* on wine quality (Carre *et al.*, 1983).

During fermentation, the release of alcohols and other organic solvents helps extract compounds from seeds and skins. Quantitatively, the most significant of these are the anthocyanins and tannins found in red wines. The extraction of tannins is especially dependent on the solubilizing action of ethanol. Anthocyanin extraction often reaches a maximum after 3 to 5 days, when the alcohol content produced during fermentation has reached about 5 to 7% (Somers and Pocock, 1986). As the alcohol concentration continues to rise, color intensity may begin to fall. This can result from the coprecipitation of anthocyanins with grape and yeasts cells, to which they bind. Nevertheless, the primary reason for color loss is the disruption of weak anthocyanin complexes present in the juice. Freed anthocyanins may change into uncolored states in wine (see Chapter 6). Although removal of tannic compounds occurs more slowly, tannin content often reaches higher values than that of anthocyanins. Extraction of tannins from the stems (rachis) may reach a plateau after about 7 days. Tannins from the seeds are

the slowest to be liberated. Accumulation of seed tannins may still be active after several weeks (Siegrist, 1985).

Ethanol also extracts various aromatic compounds from grape cells. Regrettably, little is known about the details of the effects. Conversely, ethanol decreases the solubility of other grape constituents, notably pectins and other carbohydrate polymers. The pectin content may fall by upward of 70% during fermentation.

The metabolic action of yeasts, besides producing many of the most important wine volatiles, notably higher alcohols, fatty acids, and esters, also degrades some grape aromatics, notably aldehydes. This potentially could limit the expression of the herbaceous odor generated by C_6 aldehydes and alcohols produced during the grape crush.

Yeasts

Classification and Life Cycle

Yeasts are classified taxonomically among the fungi. However, the unicellular habit, the possession of a chemically distinct cell wall, the budding or fission form of cell division, and the presence of a single nucleus per cell make yeasts a unigue fungal group. Although characterized by a distinctive set of properties, yeasts are not a single, evolutionarily related group. The yeastlike growth habit has evolved independently in at least three major fungal taxa.

The members of only two yeastlike groups occur in wine. These are the ascomycete and imperfect yeasts. Most imperfect yeasts are derived from ascomycete yeasts that have lost the ability to undergo sexual reproduction. Under appropriate conditions, the cells of ascomycete yeasts differentiate into asci. For *Saccharomyces cerevisiae,* this often means culturing on acetate-containing media. Asci are the structures in which haploid spores are produced through meiosis and cytoplasmic separation. In *Sacch. cerevisiae,* four haploid spores are produced (Fig. 7.16). On breakdown of the ascal wall, spores may germinate to produce new vegetative cells. Cells of opposite mating type typically fuse shortly after germination to reestablish the diploid state. The diploid cells divide by budding until appropriate conditions induce ascal development and spore production. Although wine strains of *Sacch. cerevisiae* possess the potential for sexual reproduction, the property is rarely expressed in must or wine. Ascal development is suppressed by high concentrations of carbon dioxide and either glucose or ethanol.

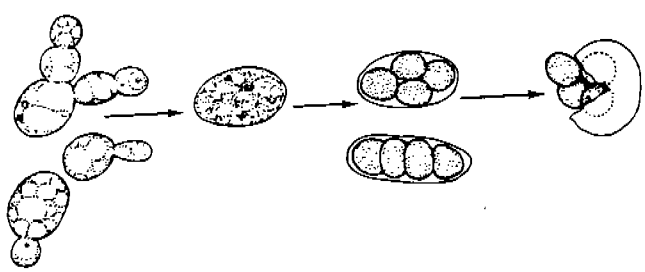


Figure 7.16 Phases of yeast sporulation. From left to right: budding vegetative cells, cessation of budding, development of ascospores, and spore release from the ascus. (From Ribéreau-Gayon *et al.*, 1975, reproduced by permission).

Until the late 1970s, yeast classification was based primarily on physiological properties and the few morphological traits readily observable under the light microscope. To these have now been added protein serology, amino acid sequence analysis, nucleotide sequence analysis, and DNA–DNA hybridization. The new procedures are likely to permit taxonomists to develop more stable classification based on evolutionary relationships.

In the most recent major taxonomic treatment of yeasts (Kreger-van Rij, 1984), many named species of *Saccharomyces* have been reduced to synonyms of *Sacch. cerevisiae* or other genera. This does not deny the reality of the differences used to distinguish the former species, but rather indicates that they were either minor and/or genetically unstable. Most of the former species are now viewed as physiological variants of recognized species. For example, *Saccharomyces fermentari* and *Sacch. rosei* are considered to be strains of *Torulaspora rosei.* The taxonomic treatment presented in Kreger-van Rij (1984) is followed in the text.

A listing of currently accepted names and synonyms of some of the more commonly found yeasts on grapes or in wine is given in Appendix 7.1. Differences between some of the physiological races of *Sacch. cerevisiae* are noted in Appendix 7.2.

Ecology

Saccharomyces cerevisiae is undoubtedly the most important yeast to mankind. In its various forms, it functions as the wine yeast, brewer's yeast, distiller's yeast, and baker's yeast. Laboratory strains are extensively used in industry and in fundamental studies on genetics, biochemistry, and molecular biology. For all the importance of *Sacch. cerevisiae,* its original habitat in nature is uncertain (Phaff, 1986). Strains similar to those used in winemaking are rarely if ever isolated from natural sources. *Saccharomyces cerevisiae* var. tetrasporus isolated from oak tree exudate may be the ancestral form. Although *Sacch. cerevisiae* is occasionally isolated in nature from the intestinal tract of fruit flies (*Drosophila* spp.), the importance of insects in the dispersal of *Saccharomyces* is unclear (Phaff, 1986; Wolf and Benda, 1965). *Saccharomyces cerevisiae* is usually absent or rare

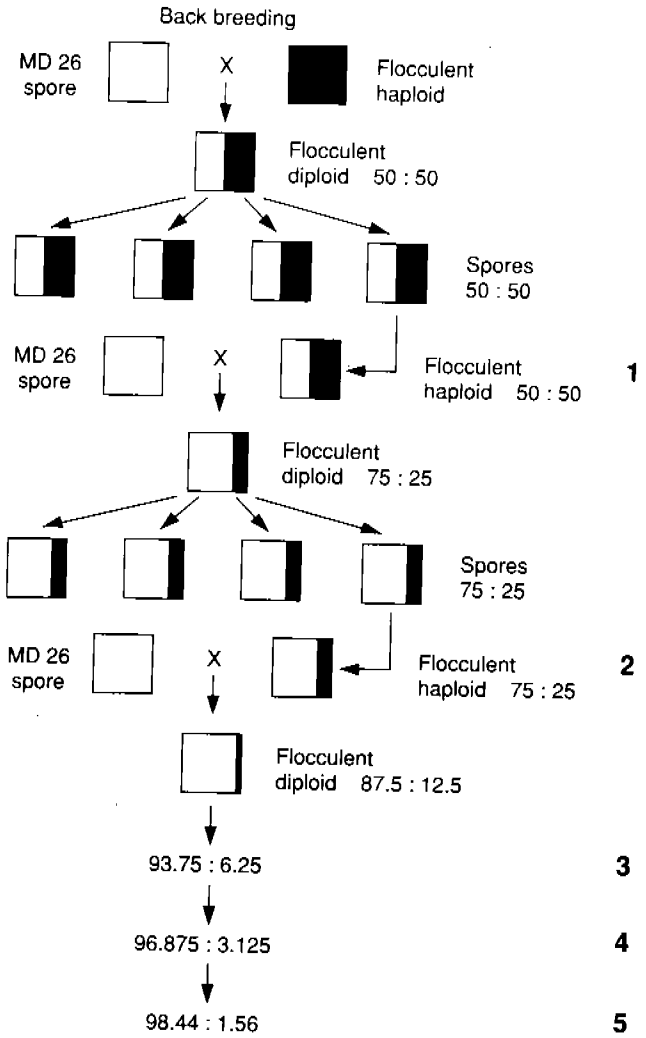


Figure 7.19 Use of backcrossing to eliminate undesired genetic information following the crossing of two yeast strains. In the example, a nonflocculant (recipient) strain is crossed with a flocculent (donor) strain to obtain the flocculant trait. While some haploid progeny will express the flocculant property, the hybrid cells possess only 50% of the genes from the recipient (MD 26) parent. Haploid flocculant cells of the hybrid strain are backcrossed to spores of the parental MD 26 strain. This reduces the proportion of genes from the flocculant parent to 25%. Haploid flocculant progeny from this backcross are again backcrossed (2) to parental MD 26 spores. Several similar backcrossings of flocculant progeny to MD 26 (3, 4, and 5) essentially eliminate all but the desired flocculant genes derived from the flocculant strain. (From Thornton, 1985, reproduced by permission.)

tion containing DNA from the donor organism. Incorporation requires uptake of the DNA containing the gene and its insertion into a yeast or plasmid chromosome. Plasmids are circular, cytoplasmic DNA segments partially controlling their own replication. Although frequently found in *Saccharomyces cerevisiae*, plasmids are not essential to yeast existence.

Using transformation, the malolactic gene from *Leuconostoc oenos* has been transferred into *Sacch. cerevisiae* (Snow, 1985). As the gene functions in *Sacch. cerevisiae* at about 1% of its normal value, the example is only illustrative of the potential of the technique. Incorporation of genes improving malate uptake, or improving expression of the malolactic gene, may produce a commercial strain capable of inducing both malolactic and alcoholic fermentations. Incorporation of desired genes into the naturally occurring yeast **2μm plasmid** as a vector may ease the incorporation, expression, and maintenance of foreign genes.

A requirement for all useful strains, new and old, is genetic stability. Although a property of most yeast strains, genetic stability is not a property of all. For example, flocculant strains often lose the ability to form large clumps of cells and settle out as a powdery sediment. Loss of a genetic property is thought to be due to factors such as aneuploidy or mutation.

Environmental Factors Affecting Fermentation

CARBON AND ENERGY SOURCES

The major carbon and energy sources for fermentation are glucose and fructose. Other nutrients may be utilized, but they either are present in small amounts (amino acids), are poorly incorporated into the cell (glycerol), or can only be respired (acetic acid and ethanol). Sucrose can be readily fermented, but it is seldom present in significant amounts in grapes. It may be added, however, in the techniques of chaptalization and amelioration. Sucrose is enzymatically split into its component monosaccharides, glucose and fructose, by one of several invertases. Hydrolysis usually occurs external to the cell membrane by an invertase located between the cell wall and plasma membrane (periplasm). Most other grape sugars are not fermented by *Sacch. cerevisiae* but may be used by several spoilage yeasts and bacteria.

At maturity, the sugar concentration of most wine grapes ranges between 20 and 25%. At this concentration, the osmotic effect of sugar can delay the onset of fermentation. Yeast cells may be partially plasmolyzed, inducing a noticeable lag period (Nishino *et al.*, 1985). Cell viability may be decreased, cell division limited, and sensitivity to alcohol toxicity increased. At sugar concentrations about 25 to 30%, the likelihood of fermentation terminating prematurely increases considerably. Strains of *Sacch. cerevisiae* differ greatly in sensitivity to sugar concentration.

The nature for the remarkable tolerance of wine yeasts to the plasmolytic action of sugar is unclear, but the property appears related to increased synthesis of, or reduced permeability of the cell membrane to, glycerol (see Brewster *et al.*, 1993). These responses to an in-

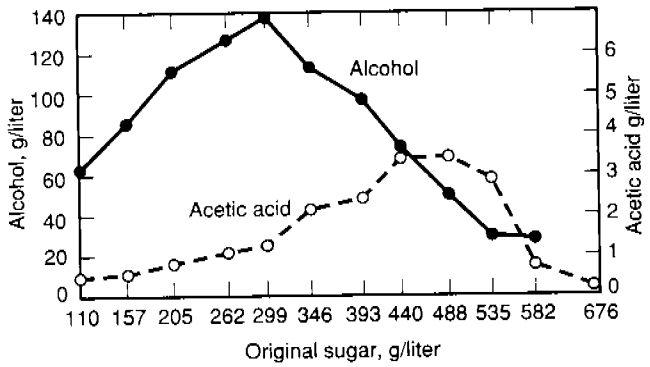


Figure 7.20 Effect of sugar concentration on alcohol and volatile acid production. (After unpublished data of C. von der Heide from Schanderl, 1959, reproduced by permission.) A rough approximation of degrees Brix can be obtained by dividing the sugar concentration by 10.

creased osmolarity of the environment permit glycerol to act as an osmoticum, equilibrating the osmotic potential of the cytoplasm with the surrounding juice.

Sugar content affects the synthesis of several important aromatic compounds. High sugar concentrations increase the production of acetic acid (Fig. 7.20) and its esters. However, as indicated in Fig. 7.21, the effect of total soluble solids on esterification is not solely due to sugar content. For example, synthesis of isoamyl and 2-phenethyl acetate rises with increasing maturity (°Brix) but decreases in juice from immature grapes augmented with sugar to achieve the same degrees Brix.

Over a wide range of sugar concentrations, ethanol production is directly related to juice sugar content. However, above about 30% sugar, ethanol production per gram sugar begins to decline (Fig. 7.20). In some strains of *Saccharomyces cerevisiae* sugar content also

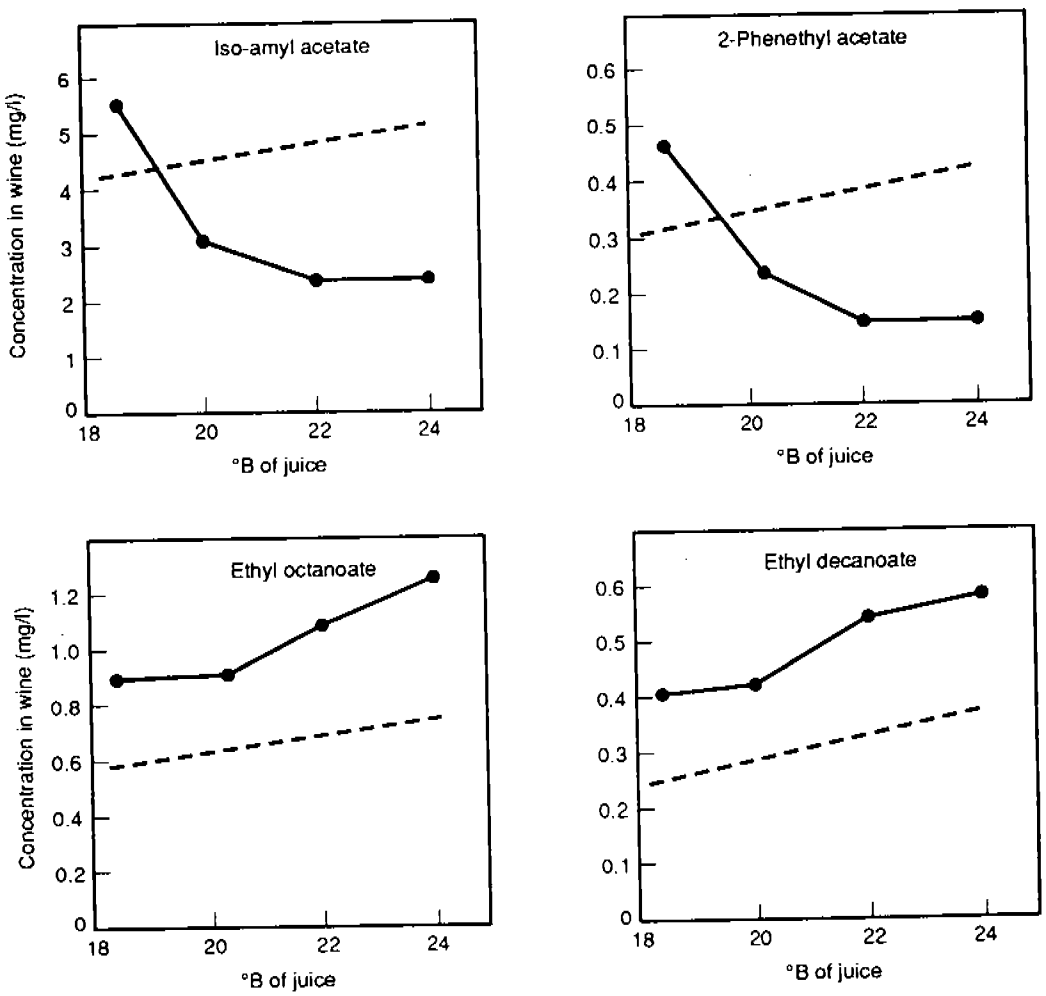


Figure 7.21 Ester concentration of wines made from grapes of varying maturity and with chaptalized must: wines for musts of four degrees of maturity (—); average slope from wines produced from chaptalized must (– – –). (From Houtman *et al.*, 1980, reproduced by permission.)

directly affects the synthesis of acetic acid (Henschke and Dixon, 1990).

ALCOHOLS

All alcohols are toxic to varying degrees. Because *Sacch. cerevisiae* shows considerable ethanol tolerance, much effort has been spent attempting to understand the nature of this tolerance, and why it breaks down at high concentrations. Although alcohol buildup eventually inhibits fermentation, it begins to affect yeast action at much lower concentrations. For example, suppression of sugar uptake can begin at about 2% ethanol (Dittrich, 1977), and rise quickly with increasing temperature. Disruption of the translocation of ammonium and several amino acids also occurs as the alcohol content increases (see Casey and Ingledew, 1986). Although higher (fusel) alcohols are more inhibitory than ethanol, their occurrence at substantially lower concentrations limits their toxicity.

Although most strains of *Sacch. cerevisiae* can ferment in the presence of up to 13 to 15% ethanol, there is wide variation in this ability. Cessation of growth routinely occurs at concentrations well below those that inhibit fermentation. It is generally believed that one of the major toxic effects of alcohol is disruption of the semifluid nature of the cell membrane. This destroys the ability of the yeast to control cell functions and can lead to nutrient loss and cell death.

Ethanol, usually in the form of brandy, may be used to arrest microbial activity. This property is used selectively in sherry and port production. In port, ethanol is added early in fermentation to retain much of the sugar content of the must. This, correspondingly, retains the concentration of most volatile compounds found in the fermenting must. Early cessation of fermentation probably leaves the wine higher in acetic acid, acetaldehyde, and acetoin content, but lower in glycerol, fixed acids, and higher alcohols (Figs. 7.13 and 7.14). In sherry production, the addition of wine spirits at the end of fermentation inhibits the growth of acetic acid bacteria (>15% ethanol) and flor yeasts (>18% ethanol).

The accumulation of alcohol during fermentation has a very important dissolving action on phenolic compounds. Most of the distinctive taste and color of red wines depends on the extraction of flavanols and anthocyanins by ethanol during vinification. Extraction is further enhanced in the presence of sulfur dioxide (Oszmianski *et al.*, 1988).

NITROGENOUS COMPOUNDS

Under most circumstances, juice and must contain nitrogen sufficient for several fermentations. However, there are several conditions in which nitrogen can become limiting. For example, prefermentative clarification diminishes juice nitrogen content; if the reduction is sufficiently marked, it can slow fermentation and cause it to become stuck. This may result from the irreversible inactivation of sugar transport by ammonia starvation (Lagunas, 1986). In addition, juice nitrogen content may be reduced by 33 to 80% in grapes infected by *Botrytis cinerea* (Rapp and Reuther, 1971). In sparkling wine production, nitrogen deficiency caused by the initial fermentation and clarification of the *cuveé* wines is usually counteracted by the addition of ammonium salts such as ammonium phosphate.

The juice of some grape varieties is more likely to show nitrogen limitation than others, for example, 'Chardonnay' and 'Colombard.' This is especially true when the juice has been given undue centrifugation or filtration.

Nitrogen demand appears to be greatest during the exponential phase of growth during fermentation, and nitrogen is incorporated most rapidly during that period (Fig. 7.22). The inorganic nitrogen source preferentially incorporated is ammonia. The oxidized state of ammo-

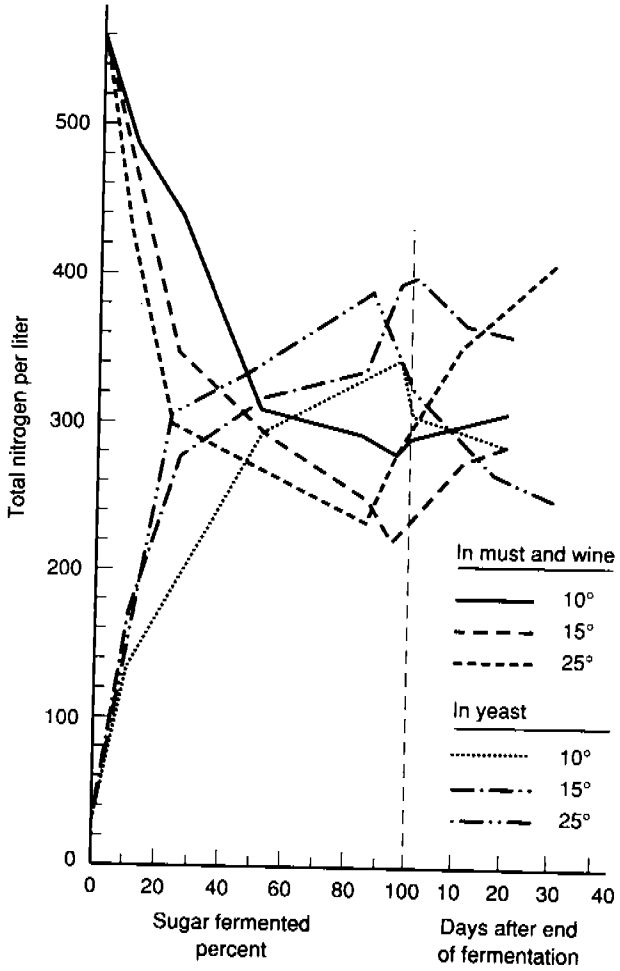


Figure 7.22 Changes in total nitrogen during and after fermentation in musts and in yeasts fermenting them. (After Nilov and Valuiko, 1958, in Amerine *et al.*, 1980, reproduced by permission.)

nia permits its direct incorporation into organic compounds. Although ammonia is potentially capable of repressing the uptake of amino acids, its concentration in the juice is insufficient for that to occur. *Saccharomyces cerevisiae* has several amino acid transport systems (Cartwright *et al.*, 1989). One is nonspecific and directs the uptake of all amino acids except proline. The other systems are more selective, transporting only particular groups of amino acids. Certain amino acids are preferentially incorporated, such as phenylalanine, leucine, isoleucine, and tryptophan, whereas others, such as alanine, arginine, and proline, are poorly assimilated (Ough *et al.*, 1991). Urea can be readily incorporated into yeast cells, but it is no longer recommended as a form of nitrogen supplement. Urea has been implicated in the production of the carcinogen ethyl carbamate (Ough *et al.*, 1990). Amines and peptides also may be incorporated as nitrogen sources, but protein nitrogen is unavailable. Wine yeasts are capable of neither transporting proteins across the cell membrane nor enzymatically degrading them to amino acids outside the cell.

Nitrogen is required for the synthesis of proteins, pyrimidine nucleotides, and nucleic acids. Yeast cells generally synthesize their amino acid and nucleotide requirements from inorganic nitrogen and sugar. Consequently, most yeast strains do not require these metabolites in the must, but they can be assimilated from the medium when available. Assimilation of metabolites avoids the diversion of metabolic intermediates and energy to amino acid biosynthesis.

Nitrogen content can influence the synthesis of aromatic compounds during fermentation. Most noticeable is the reduction in fusel alcohol content by ammonia and urea. This effect can be reversed by the assimilation of certain amino acids from the juice or must. These opposing effects appear to result from the use of fusel alcohols in the biosynthesis of amino acids from ammonia and urea, and their release on deamination of amino acids assimilated from the must. The sensory impact of these changes, if any, is unknown.

During fermentation, and especially after, there is a slow release of nitrogen compounds back into the wine, probably owing to autolysis of dead yeast cells (Fig. 7.22). This release may activate subsequent microbial activity. For this reason, the first racking often occurs shortly after fermentation. When malolactic fermentation is desired, however, racking is delayed until the bacterial conversion of malic to lactic acid is complete. Racking also may be delayed if lees contact with the wine is desired.

LIPIDS

Lipids function as the basic constituents of cellular membranes (phospholipids and sterols), in energy storage (oils), as pigments (carotenoids), and as regulator molecules complexed with proteins (lipoproteins) and carbohydrates (glycolipids).

Yeasts synthesize their own lipid requirements when grown aerobically, but they are unable to generate long-chain unsaturated fatty acids and sterols under anaerobic conditions. This is less significant in red wine vinification as adequate supplies of precursors may be obtained during fermentation on the skins. Anaerobic inhibition of lipid synthesis, however, can result in sluggish fermentation of highly clarified white juice. Clarification can remove more than 90% of the fatty acid content. This is particularly marked with the unsaturated fatty acids—oleic, linoleic, and linolenic acids (Bertrand and Miele, 1984). In addition, sterols such as ergosterol and oleanolic acid are probably removed from the juice. Nevertheless, yeasts typically possess sufficient reserves of these vital compounds to initiate fermentation and complete several cell divisions. Eventually, though, the accumulated deficit in sterols and unsaturated fatty acids can enhance the ethanol-induced reduction in glucose uptake and result in a stuck fermentation.

Wine yeasts are particularly sensitive to the toxicity of mid-chain carboxylic acids such as octanoic and decanoic acid. As by-products of yeast metabolism, they accumulate during fermentation. They increase the ethanol-induced leakage of nutrients such as amino acids from the cells (Sá Correia *et al.*, 1989). The toxic effects are limited or reversed by the addition of ergosterol and long-chain unsaturated fatty acids (Fig. 7.23) or by the addition of various absorptive substances, such as activated charcoal, bentonite, silica gel, or yeast hulls ("ghosts"). The latter consist primarily of cell wall remnants. By removing octanoic and decanoic acids via absorption from the juice, their potential to disrupt yeast membranes is reduced. In addition, yeast hulls can be sources of sterols and unsaturated fatty acids (see Munoz and Ingledew, 1990).

Another possible solution to problems associated with low sterol and unsaturated fatty acid content is to use yeasts high in sterol content. Sterol synthesis is commonly suppressed in the presence of glucose, but some strains do not show repression. Growing yeasts under highly aerobic conditions, as in active dry yeast production, generates cells high in unsaturated fatty acids, and with up to three times the sterol content of cells grown semiaerobically (Tyagi, 1984). Musts inoculated with strains possessing high sterol contents frequently ferment more sugar than strains possessing low sterol contents.

PHENOLS

The phenolic content of must can have various effects on the course of fermentation. For example, the phenols in red grapes, notably anthocyanins, can stimulate fermentation, while the procyanidins of white grapes can be slightly inhibitory (Cantarelli, 1989). Phenolic com-

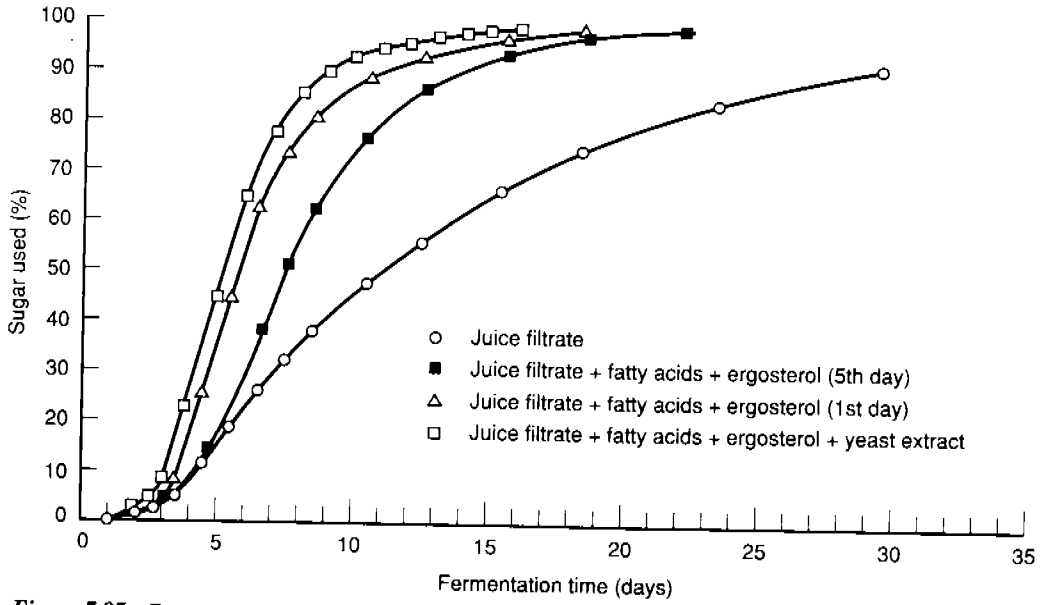


Figure 7.23 Fermentation curves of juice filtrates with additions of yeast extract, unsaturated fatty acids, and ergosterol at inoculation and on the fifth day thereafter. (From Houtman and du Plessis, 1986, reproduced by permission.)

pounds also are a determining factor in the activation of film formation important in *fino* sherry production (Cantarelli, 1989). Certain phenols, notably the esters of gallic acid, are toxic, but others such as chlorogenic and isochlorogenic acids may stimulate fermentation. The primary situation where phenols commonly suppress yeast metabolism is during the second fermentation in sparkling wine production. This is one of the reasons why few red sparkling wines are produced using traditional techniques.

In addition to affecting fermentation, phenols also may be modified by yeast action. For example, ferulic and *p*-coumaric acids are decarboxylated to aromatic vinyl phenols (4-vinyl guaiacol and 4-vinyl phenol, respectively) by some strains of *Sacch. cerevisiae* (Chatonnet *et al.*, 1989). Other phenolic constituents in the must may influence the conversion.

SULFUR DIOXIDE

In most cases, the primary reason for adding sulfur dioxide is to restrict or prevent the growth of undesirable microbes. Sulfur dioxide has a distinct advantage over most other antimicrobial agents, because of the relative insensitivity of wine yeasts to its action. In contrast, sulfur dioxide is toxic, or inhibitory, to most bacteria and other yeasts at low concentrations. Actively growing yeasts are even more tolerant than their dormant counterparts. The toxicity of sulfur dioxide also is aided by the low pH of grape must (Fig. 7.24).

At currently used concentrations (usually less than 50 ppm in healthy grapes), sulfur dioxide is unlikely to affect the rate of alcoholic fermentation. However, sulfur dioxide can slow the onset of fermentation. The presence of 15 to 20 ppm can reduce the viability of a yeast inoculum from 10^6 to 10^4 cells/ml or less (Lehmann, 1987). Sulfur dioxide also can significantly influence yeast metabolism. Sulfur dioxide readily binds with several carbonyl compounds produced by yeasts, notably acetaldehyde, pyruvic acid, and α-ketoglutaric acid. Binding with sulfur dioxide increases the production and subsequent release of carbonyl compounds into the juice or must. Thus, their concentration in the finished wine correlates well with the concentration of sulfur dioxide initially added to the must. Sulfur dioxide also favors glycerol synthesis, while acetic acid production tends to decline. Fixed acidity generally does not change, par-

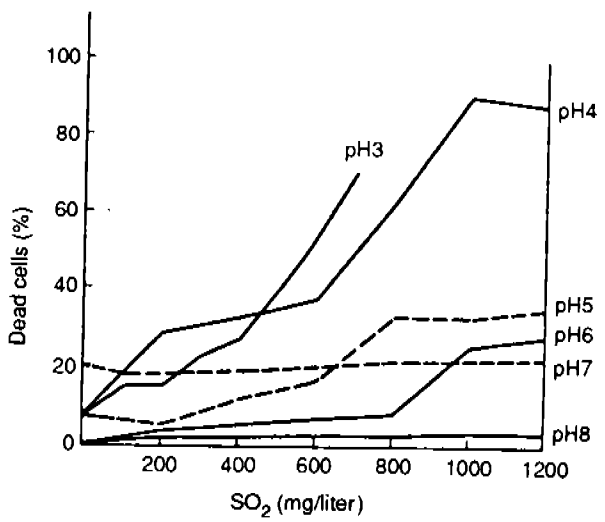


Figure 7.24 Effect of pH on the lethal dose of SO_2 for wine yeasts. (From Farkaš, 1988, reproduced by permission.)

tially because sulfur dioxide suppresses the metabolism of both lactic acid and acetic acid bacteria.

The binding of sulfur dioxide to carbonyl compounds inadvertently increases the amount of sulfur dioxide needed to suppress the action of spoilage organisms. Bound sulfur dioxide is much less antimicrobial than molecular SO_2.

Although sulfur dioxide is the best wine antimicrobial agent currently available, it does not control certain spoilage yeasts. For example, many strains of the yeasts *Saccharomycodes ludwigii, Zygosaccharomyces bailii,* and *Brettanomyces* spp. are particularly tolerant to sulfur dioxide (Hammond and Carr, 1976).

When present, elemental sulfur can be assimilated and used in the synthesis of sulfur-containing amino acids and coenzymes. It also may be oxidized to sulfate and sulfur dioxide, or reduced to hydrogen sulfide. Reduction of sulfur to hydrogen sulfide may be a means, albeit aromatically unpleasant, of maintaining a favorable redox balance in yeast cells under anaerobic conditions.

OXYGEN AND AERATION

The process of fermentation itself requires no oxygen. Even in the presence of ample oxygen, *Sacch. cerevisiae* preferentially ferments sugars. Nevertheless, trace amounts of oxygen favor fermentation by permitting the direct oxidation of precursors in the biosynthesis of sterols and long-chain unsaturated fatty acids. Production and proper functioning of cell membranes in wine yeasts require sterols (ergosterol and lanosterol) as well as C_{16} and C_{18} fatty acids. Some of the latter, such as linoleic and linolenic acids, are unsaturated. Molecular oxygen also is required for the synthesis of the vitamin nicotinic acid.

Stemming and crushing usually induce sufficient oxidation of the juice for yeast growth. The juice quickly becomes saturated with oxygen during the processes. The capacity of juice to absorb oxygen depends partially on the duration of skin contact (maceration). With the increase in extraction of phenols, oxygen consumption is increased. In addition, this speeds the removal of free oxygen, and the shift from oxidative to reductive conditions. Aeration beyond that which occurs coincidental to stemming and crushing (**hyperoxidation**) is variously viewed as being potentially beneficial (Cheynier *et al.,* 1991) or detrimental (Dubourdieu and Lavigne, 1990). The initial browning commonly associated with crushing is permissible since the color compounds commonly precipitate during fermentation or clarification. It also gives white wine a degree of resistance to subsequent oxidative browning by removing readily oxidizable phenols early in vinification.

During the fermentation of red wine, additional oxygen may be absorbed during pumping over. The resultant incorporaton of about 10 mg O_2 per liter often speeds the process of fermentation. This is more marked when aeration occurs at the end of the exponential phase of yeast growth (Sablayrolles and Barre, 1986). The yeast population is increased and average cell viability is extended. Aeration also increases the production of acetaldehyde, thus favoring color stability by assisting the early formation of polymers of anthocyanins and tannins.

With white juice fermentations, winemakers typically try to avoid oxidation as it increases the synthesis of fusel alcohols and acetaldehyde. The higher concentrations of acetic acid noted may be due to the activation of acetic acid bacteria in the juice. In addition, semiaerobic conditions can depress the synthesis of esters (Nykänen, 1986). However, the effects may be reversed with short aerations at the beginning or a few days after the commencement of fermentation (Bertrand and Torres-Alegre, 1984). The effect of aeration on hydrogen sulfide production is another example of the complexity of the effects of introducing oxygen. Aeration may remove/oxidize H_2S but may also enhance its synthesis (Houtman and de Plessis, 1981). The timing and extent of aeration also can influence urea accumulation and, thereby, the potential to produce ethyl carbamate (Henschke and Ough, 1991).

Reactivation of fermentation in stuck wines usually requires aeration. *Cuvée* wines also may be aerated slightly prior to the second fermentation in sparkling wine production.

Following fermentation, limited aeration (~40 mg O_2/liter, or four saturations) may benefit the maturation of red wines. In contrast, most white wines are painstakingly protected from air contact following fermentation. The exception to this practice occurs when the wines are given extended contact with the lees (occasionally up to 10 months). In that case, limited oxidation occurs when the wine is stirred with the lees.

Oxygen absorption is influenced by many factors, including clarification, skin contact, phenol concentration, sulfur dioxide content, presence of polyphenol oxidases, sugar proportion, temperature, pumping over, rate of fermentation, and, of course, protection from air before, during, and after fermentation.

CARBON DIOXIDE AND PRESSURE

During fermentation, large volumes of carbon dioxide gas are produced, about 260 ml/gram glucose. This equates to over 50 times the volume of the juice fermented. The escape of carbon dioxide is estimated to remove about 20% of the heat generated during fermentation. Some of that heat discharge is probably due to the evaporative heat loss connected with the accompanying escape of water vapor.

Carried off with the carbon dioxide are various volatile compounds. Ethanol loss is estimated to be about 1

to 1.5% of the ethanol produced (Williams and Boulton, 1983) but varies with temperature and sugar utilization. Higher alcohols and monoterpenes are lost to about the same degree (~1%). In contrast, significant losses of both ethyl and acetate esters can occur. Depending on the grape variety, and especially the fermentation temperature, upward of 25% of these aromatically important compounds may be lost (Miller *et al.*, 1987). On average, more acetate esters are lost than ethyl esters. This loss could diminish significantly the fruity character of the resulting wine. Figure 7.25 illustrates the loss of some of these compounds.

Loss of volatiles from fermenting juice is a function of both the relative rates of synthesis and destruction and the relative solubility of compounds in the increasingly alcoholic juice. Loss is further affected by fermentor size and shape; for example, the increased surface area and low liquid pressures of small fermentors favor volatility. In addition, while the reduction of vapor pressure at low temperatures tends to diminish loss, the slower release of carbon dioxide could partially offset this by favoring the absorption and liberation of volatile compounds.

The action of carbon dioxide production causes the development of strong convection currents within the juice. This helps to equilibrate the nutrient and temperature status throughout the juice. However, the presence of a floating or submerged cap, as with red wines, disrupts equilibration (Fig. 7.28).

In vats, and in most tanks, the carbon dioxide produced during fermentation escapes into the surrounding air. When the gas is trapped, the pressure rapidly rises. At pressures above 700 kPa (~7 atm), yeast growth ceases. Low pH and high alcohol contents increase yeast sensitivity to high pressures (Kunkee and Ough, 1966). This has a significant influence in the production of sparkling wine, where the finished product may develop pressures upward of 600 kPa. Nevertheless, yeast fermentation ability may not be inhibited completely until about 3000 kPa. The major inhibitory effects of carbon dioxide buildup seem to result from changes in yeast membrane composition and permeability. In addition, carbon dioxide buildup may affect the balance between carboxylation and decarboxylation reactions (Table 7.1). The effect of pressure on the synthesis of aromatic compounds during vinification seems not to have been investigated.

The pressure created by trapping the carbon dioxide produced during fermentation has occasionally been used to encourage a more constant rate of fermentation. It also has been used to induce the premature termination of fermentation, to give the wine a sweet finish. However, care must be used with this technique as spoilage yeasts, such as *Torulopsis* and *Kloeckera,* are less sensitive to high pressures than is *Sacch. cerevisiae*. The production of acetic acid by spoilage yeasts can give a vinegary taint. Caution also needs to be taken as lactic acid bacteria (*Lactobacillus*) are little affected by pressures that restrict the growth of wine yeasts (Dittrich, 1977).

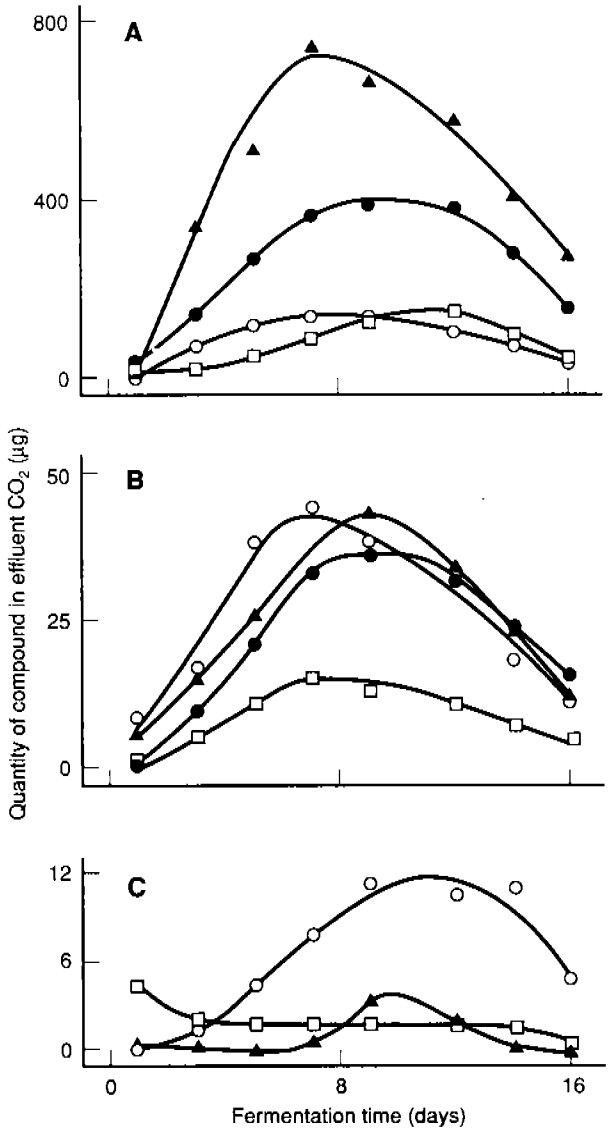


Figure 7.25 Yeast aromatics released with CO_2 during fermentation at 15°C. (A) ▲, Isoamyl acetate; ○, ethyl *n*-hexanoate; ■, ethyl *n*-octanoate; ●, isoamyl alcohol. (B) □, Isobutyl acetate; ○, hexyl acetate; ●, ethyl *n*-butanoate; △, isobutanol. (C) ○, Ethyl *n*-decanoate; □, 1-hexanol; ▲, 2-phenylethanol. (From Miller *et al.*, 1987, reproduced by permission.)

pH

The pH range normally found in juice or must has little effect on the rate of fermentation, or on the synthesis and release of aromatic compounds by yeasts. Only at abnormally low pH values (<3.0) is fermentation somewhat inhibited. However, low pH may assist the uptake of some amino acids by providing protons required for transport across the cell membrane (Cartwright *et al.*,

Table 7.1 Possible Effects of Carbon Dioxide on Key Enzymes of *Saccharomyces cerevisiae*[a]

Reaction	Comment
Pyruvate → acetaldehyde → ethanol (CO_2)	Reduced production of ethanol
Pyruvate (ATP, ADP, CO_2) → oxaloacetate → amino acids	Stimulation, less available pyruvate for ethanol production
Acetyl-CoA (CO_2) → malonyl-CoA → fatty acids	Stimulation, less available pyruvate for ethanol production
Pyruvate (CO_2) → malate	Stimulation, less available pyruvate but malate enzyme level is not high
Phosphoenolpyruvate (ADP, ATP, CO_2) → oxaloacetate	Stimulation, less available pyruvate but enzyme is repressed by glucose
6-Phosphogluconate ($NADP^+$, NADPH, CO_2) → ribulose 5-phosphate	Reduced production of biosynthetic precursors, thus cell yield will decrease; will reduce rate of production of ethanol

[a] From Jones *et al.* (1981), reproduced by permission.

1989). Although the growth rate of yeasts at a pH of 3.0 may be about half that at pH 4.0, this appears to be of little practical significance in winemaking.

The most important effects of pH on fermentation are indirect, such as noted above concerning the antibiotic action of sulfur dioxide. Low pH also prevents many potentially competitive organisms from growing in must. In addition, pH affects the survival of some fermentation by-products in wine. The most well-known effect concerns the hydrolysis of ethyl and acetate esters, where hydrolytic breakdown occurs more rapidly at low pH values.

VITAMINS

Vitamins play a crucial role in the regulation of yeast metabolism as coenzymes or enzyme precursors (Table 7.2). Although vitamins are not metabolized as energy sources, their concentrations decrease markedly during fermentation (see Amerine and Joslyn, 1970). Nevertheless, yeast requirements typically are satisfied by either biosynthesis or assimilation from the juice. However, certain conditions may reduce the availability or concentration of vitamins in the juice. Fatty acids produced during fermentation can inhibit the uptake of thiamine, oversulfiting degrades thiamine, and infection of the grapes by fungi lowers the vitamin content. Under such conditions, vitamin supplements may improve or be required to reinitiate fermentation.

Adequate concentrations of thiamine reduce the synthesis of carbonyl compounds that bind to sulfur dioxide, thereby diminishing the amount of SO_2 needed to control spoilage organisms adequately. In addition to limiting carbonyl synthesis, thiamine also reduces the concentration and relative proportions of higher alcohols produced during fermentation.

Although seldom a problem, deficiencies in pyridoxine and pantothenic acid can disrupt yeast metabolism, resulting in increased hydrogen sulfide synthesis.

INORGANIC ELEMENTS

Inorganic elements often are essential components in the active sites of many enzymes, and they help in regulating cellular metabolism and maintaining cytoplasmic pH and ionic balance (Table 7.3). Although inorganic elements are normally assumed to be in adequate supply in juice, there is difficulty in assessing accurately yeast requirements and available ion concentrations in grape juice and must. Not only do organic compounds such as

Table 7.2 Role of Vitamins in Yeast Metabolism[a]

Vitamin	Active form	Metabolic role	Optimum conc. (mg/liter)
Biotin	Biotin	All carboxylation and decarboxylation reactions	0.005–0.5
Pantothenate	Coenzyme A	Keto acid oxidation reactions; fatty acid, amino acid, carbohydrate, and choline metabolism	0.2–2.0
Thiamine (B_1)	Thiamine-pyrophosphate	Fermentative decarboxylation of pyruvate; oxo acid oxidation and decarboxylation	0.1–1.0
Pyridoxine	Pyridoxal phosphate	Amino acid metabolism; deamination, decarboxylation, and racemization reactions	0.1–1.0
p-Aminobenzoic acid and folic acid	Tetrahydro-folate	Transamination; ergosterol synthesis; transfer of one-carbon units	0.5–5.0
Niacin (nicotinic acid)	NAD^+, $NADP^+$	Dehydrogenation reactions	0.1–1.0
Riboflavin (B_2)	FMN, FAD	Dehydrogenation reactions and some amino acid oxidations	0.2–0.25

[a] From Jones *et al.* (1981), reproduced by permission.

amino acids sequester elements, thereby reducing their effective concentration, but ions can antagonize uptake of one another. Occasionally, as in the case of potentially toxic aluminum ions, this may be beneficial.

The abundance of potassium ions probably makes K^+ the most significant metallic ion in juice and must. High potassium content may interefere with the efficient uptake of amino acids, such as glycine. Under anaerobic conditions, potassium excretion may be necessary to maintain an acceptable ionic balance, owing to the simultaneous incorporation of protons (H^+) with glycine (Cartwright *et al.*, 1989). High potassium concentrations also can generate tartrate instability, which is associated with high juice and wine pH. High pH can lead to microbial instability, increase the tendency of white wines to brown, and induce color instability in red wines.

TEMPERATURE

Temperature is one of the most influential factors affecting fermentation. Not only does temperature both directly and indirectly influence yeast metabolism, but it is one of the factors over which the winemaker has the greatest control.

At the upper and lower limits, temperature can cause cell death. However, inhibitory effects are experienced well within the extremes. The inhibitory effects of high

Table 7.3 Major Inorganic Elements Required for Yeast Growth and Metabolism[a]

Ion	Role	Concentration (μM)[b]
K^+	Enhances tolerance to toxic ions; involved in control of intercellular pH; K^+ excretion is used to counterbalance uptake of essential ions, e.g., Zn^{2+}, Co^{2+}; K^+ stabilizes optimum pH for fermentation	20×10^3
Mg^{2+}	Levels regulated by divalent cation transport system; Mg^{2+} seems to buffer cell against adverse environmental effects and is involved in activating sugar uptake	5×10^3
Ca^{2+}	Actively taken up by cells during growth and incorporated into cell wall proteins; Ca^{2+} buffers cells against adverse environments; Ca^{2+} counteracts Mg^{2+} inhibition and stimulates effect of suboptimal concentrations of Mg^{2+}	1.5×10^3
Zn^{2+}	Essential for glycolysis and for synthesis of some vitamins; uptake is reduced below pH 5, and two K^+ ions are excreted for each Zn^{2+} taken up	50
Mn^{2+}	Implicated in regulating the effects of Zn^{2+}; Mn^{2+} stimulates synthesis of proteins	15
Fe^{2+}, Fe^{3+}	Present in active site of many yeast proteins	10
Na^+	Passively diffuses into cells; stimulates uptake of some sugars	0.25
Cl^-	Acts as counterion to movement of some positive ions	0.1
Mo^{2+}, Co^{2+}, B^{2+}	Stimulates growth at low concentrations	0.5

[b] For growth of 25 g cells/liter.

temperatures are increased by other growth-limiting factors, such as the presence of ethanol and certain C_8 to C_{10} carboxylic acids. At low temperatures, yeasts tend to be less sensitive to the toxic effects of high alcohol concentrations. This may be due to the higher proportion of unsaturated fatty acid residues in the plasma membrane (Rose, 1989). This influence may help to explain the higher maximum viable cell count of fermentations conducted at cooler temperatures (Ough, 1966a). It also may explain the increased yeast viability associated with periodic aeration of barrels of wine left on the lees. Oxygen could permit the desaturation of precursors in the production of needed unsaturated fatty acids.

The growth rate of yeast cells is also strongly affected by the fermentation temperature. This is particularly marked during the exponential growth phase. For example, cell division may occur about every 12 hr at 10°C, every 5 hr at 20°C, and every 3 hr at 30°C (Ough, 1966a). The rate of cell division also is affected by pH and °Brix value of the juice.

At warm temperatures (>20°C), yeast cells experience a rapid decline in viability at the end of fermentation. At cooler temperatures, cell growth is retarded, but viability is enhanced. Cool temperatures also exaggerate the lag phase of fermentation. For this reason, winemakers may warm white juice to 20°C before adding the yeast inoculum. Once fermentation has commenced, the juice may subsequently be cooled to a more desirable fermentation temperature, commonly between 10° and 15°C.

The temperature at which active dry yeast is rehydrated prior to inoculation can affect the onset of fermentation. Temperatures between 38° and 40°C appear optimum for rehydration (Kraus *et al.*, 1981). Active dry yeast often contains about 20 to 30 $\times 10^9$ cells/g. A rapid onset of fermentation limits oxidation and the growth of spoilage organisms.

In addition to the above-mentioned effects on yeast growth and survival, temperature has many subtle, and not so subtle, effects on yeast metabolism. One of the most marked is the influence of temperature on the rate of fermentation. Cool temperatures dramatically slow the rate of fermentation and may cause premature termination. Excessively high temperatures may disrupt enzyme and membrane function and also result in stuck fermentation. Although quick onset and completion of fermentation have advantages, the preferred temperature for vinification is often less than the optimum for ethanol production. Because yeast strains differ in response to temperature, the optimum temperature for vinification can vary widely.

The modern preference in most wine producing regions is to conduct white wine fermentations between 8° and 15°C. Some European wineries still prefer to ferment between 20° and 25°C. Most New World winemakers favor cool temperatures because they give fresher more fruity wines. Fruitiness in wine is a highly valued characteristic throughout much of the world. Important in this regard is the increased synthesis of fruit esters, such as isoamyl, isobutyl, and hexyl acetates. The esters are both synthesized and retained to a greater degree at cool temperatures (Fig. 7.26A). Other esters, such as ethyl octanoate and ethyl decanoate, are produced optimally at 15°C, whereas 2-phenethyl acetate achieves its highest concentration at 20°C (Fig. 7.26B). Greater production of ethanol and higher alcohols also may be observed at cool fermentation temperatures. In addition, cooler fermentation temperatures reduce the liberation of yeast colloids and thereby facilitate rapid clarification.

Red wines are typically fermented at temperatures higher than those for white wines. Temperatures between 24° and 27°C are commonly considered optimal. However, such temperatures are not universally preferred. For example, wines from 'Pinotage' are reported as being better when fermented at 15°C (du Plessis, 1983). The warmer temperatures preferred for red wine vinification probably are related more to the effect on phenol extraction than on fermentation rate. Temperature and alcohol are the major factors influencing pigment and tannin extraction from seeds and skins. Both groups of compounds dominate the characteristics of young red wines. The potentially undesirable consequences of higher fermentation temperatures, such as the production of increased amounts of acetic acid, acetaldehyde, and acetoin and lower concentrations of some esters, probably are less noticeable against the more intense fragrance of red wine. The greater synthesis of glycerol at higher temperatures is often considered to give red wines a smoother mouth-feel. Data from Noble and Bursick (1984) are in conflict with this common view.

Other important influences arise from factors not directly related to the effect of temperature on fermentation. For example, temperature affects the rate of ethanol loss during vinification (Williams and Boulton, 1983). Nevertheless, losses of hydrophobic low molecular weight compounds such as esters are more marked, and such losses have a greater potential impact on the sensory quality of the wine produced.

During fermentation, much of the chemical energy stored in grape sugars is released as heat. It is estimated that the release is equivalent to about 23.5 kcal/mol glucose (see Williams, 1982). This is sufficient for juice with a reading of 23° Brix to increase in temperature by about 30°C during the course of fermentation. If this were to occur, the yeast cells would die before completing fermentation. In practice, such temperature increases are not realized. Because the heat is liberated over several days to weeks, some of the heat is lost with escaping

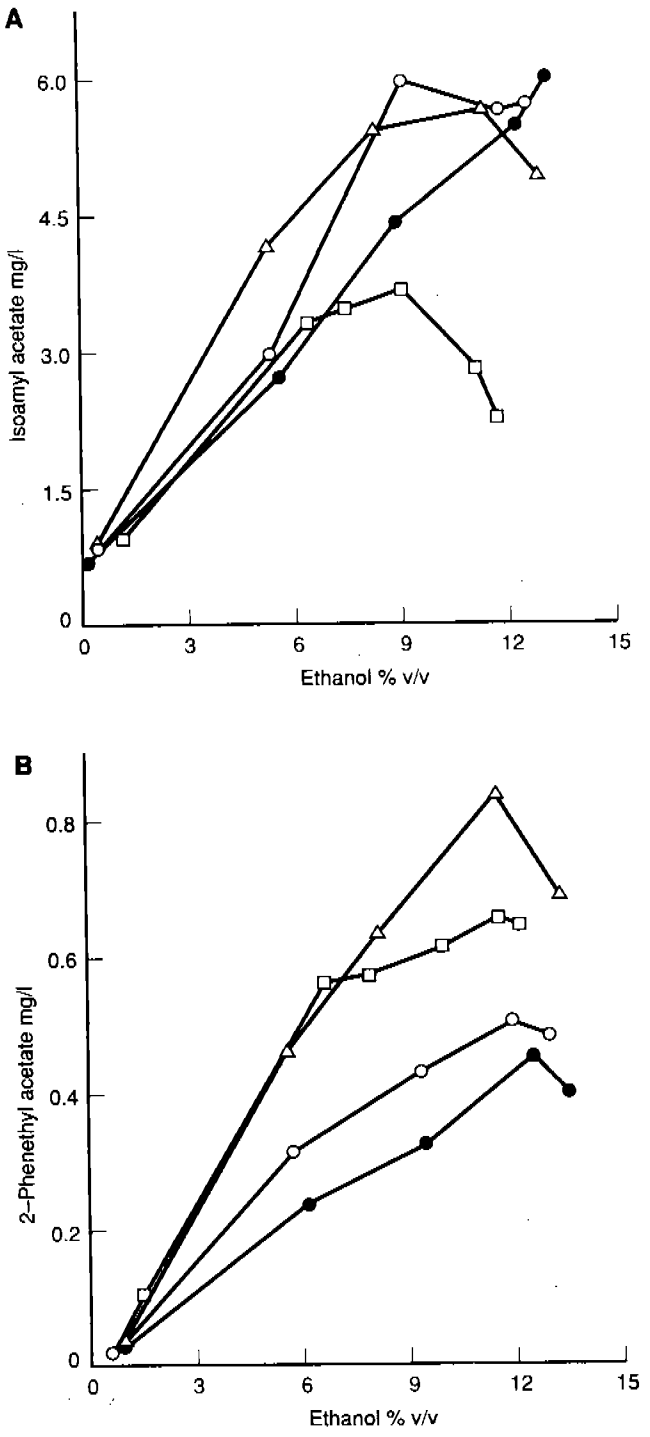


Figure 7.26 Effect of temperature and progress of fermentation on isoamyl acetate (A) and 2-phenethyl acetate (B) content. ●, 10°C; ○, 15°C; △, 20°C; and □, 30°C. (From Killian and Ough, 1979, reproduced by permission.)

carbon dioxide and water vapor. Heat also radiates through the surfaces of the fermentor into the cellar environment. Nevertheless, the rise in temperature can easily reach levels critical to yeast survival if temperature control measures are not implemented in large fermentors.

Important in heat buildup is the initial juice or must temperature. This sets the rate at which the temperature rises. The higher the potential juice temperature, the greater the initial rate of fermentation and heat release, and the sooner a lethal temperature may be reached. Thus, a cool temperature at the beginning of fermentation can limit the degree of temperature control required.

Also important to temperature control is the size and shape of the fermentor, and the presence or absence of a cap. The rate of heat lost is directly related to the surface area to volume ratio of the fermentor. By retaining heat, the volume of juice fermented can significantly affect the rate of fermentation: the larger the fermentor, the greater the retention of heat and the subsequent likelihood of overheating. This feature is illustrated in Fig. 7.27.

The tumultuous release of carbon dioxide during fermentation may be sufficient to maintain a uniform temperature throughout the vat or tank. This is usual for fermenting white and rosé juice, where vertical and lateral variation in temperature is seldom more than 1°C. At cold fermentation temperatures, however, turbulence may be insufficient to equilibrate the temperature throughout the fermentor, and temperature strata may develop.

With red wines, cap formation can disrupt effective circulation and mixing of the must. The maximum cap-

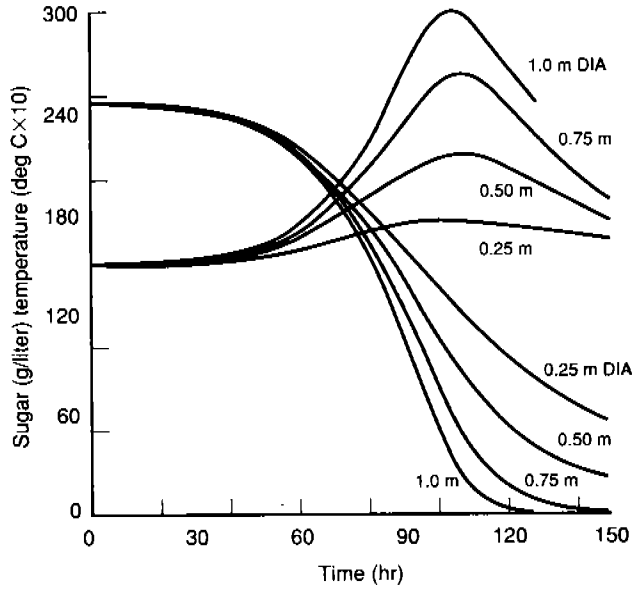


Figure 7.27 Effect of barrel diameter on fermentation rate and temperature rise during fermentation. (From Boulton, 1979, by permission.) Although the data are not presented in terms of cooperage capacity, barrels possessing maximum diameters of 0.5, 0.75, and 1.0 m, respectively, could have capacities ranging from 75 to 150, 225 to 500, and 500 to 1200 liters depending on barrel height and stave length.

to-liquid temperature difference is often about 10°C (Fig. 7.28). Punching down induces only a transitory temperature equilibration between the cap and the juice. In contrast, little temperature variation exists within the main volume of the must. Because high cap temperatures are a common feature of red wine fermentations, it has been suggested that red wine vinification consists of two simultaneous but different phases, namely, a liquid phase, where the temperature is cooler and readily controlled, and a largely uncontrolled high-temperature phase in the cap (Vannobel, 1986). Because the rate of fermentation is much more rapid in the cap, the alcohol content rises quickly to above 10%. The higher temperatures found in the cap, plus the association of alcohol, probably increase the speed and efficiency of phenol extraction from the skins trapped in the cap.

Temperature regulation is achieved by a variety of techniques. Appropriate timing of the harvest can provide fruit at a desired temperature for the initiation of fermentation. Relatively small fermentation cooperage and vinification in cool cellars have been used for centuries to achieve a degree of temperature control. Carbonic maceration (see Chapter 9) slows the rate of fermentation and correspondingly diminishes the peak temperature reached during fermentation. However, maintenance of fermentation temperatures within a narrow range requires direct cooling in all but small barrels (~225 liters).

Where heat transfer through the fermentor wall is sufficiently rapid, cooling the fermentor surface with water, or by passing a coolant through an insulating jacket, can be effective. Where thermal conductance is insufficient, fermenting must may be pumped through external heat exchangers, or cooling coils may be inserted directly into the fermenting must. In special fermentors, carbon dioxide is trapped and the pressure buildup used to slow fermentation and heat accumulation.

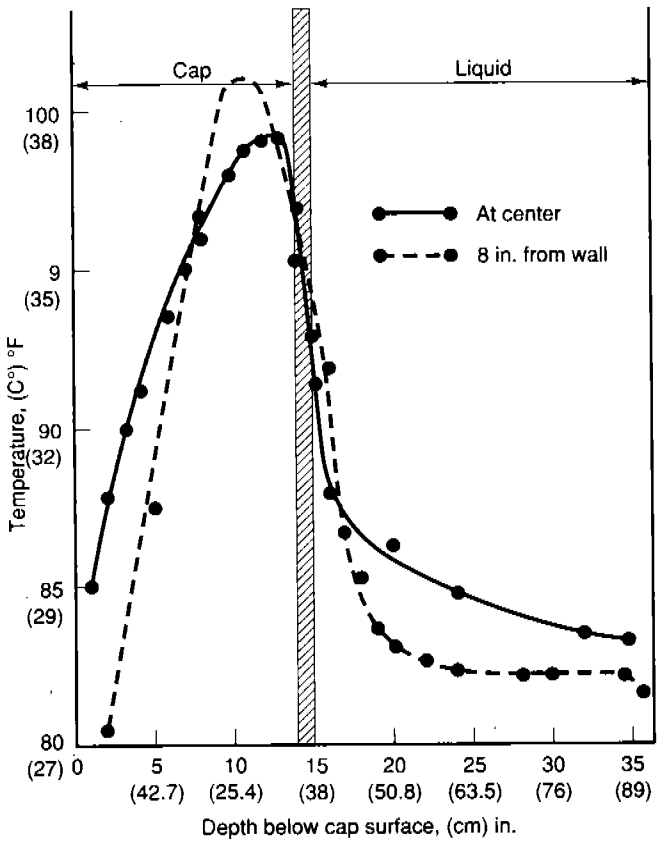


Figure 7.28 Vertical temperature profile through cap and liquid at 40 hr. Cross-hatching indicates that the boundary between the cap and liquid is not sharply defined. (From Guymon and Crowell, 1977, reproduced by permission.)

PESTICIDE RESIDUES

Under most situations, no more than trace amounts of pesticide residues are found in juice or must. At such concentrations, they have little or no perceptible effect on fermentation, or on the sensory qualities of the wine. Used properly, pesticides help the fruit reach maximum quality. When used in excess or applied just before harvest, however, pesticides may negatively affect winemaking.

Various factors influence the pesticide content on or within fruit. For example, heavy rains or sprinkler irrigation may wash contact pesticides off the fruit. Rains have less of an effect on systemic pesticides that are absorbed into plant tissues. Ultraviolet radiation in sunlight can degrade some pesticides and decrease the residual levels. Microbial decomposition is also likely.

Crushing, and especially maceration, can influence the incorporation of crop protection chemicals into must. The long maceration used in red wine production can increase the extraction of contact fungicides. Maceration generally has little effect on the content of systemic pesticides as they are already present in the juices before crushing.

Clarification, either by cold settling or by centrifugation, significantly reduces the concentration of contact fungicides, such as elemental sulfur, but has less effect on systemic pesticide residues (Fig. 7.29). The persistence of pesticide residues, once dissolved, depends largely on their stability under the physicochemical conditions found in juice and wine. For example, more than 70% of dichlofluanid (Euparen) residues may be degraded under the acidic conditions of juice and wine (Wenzel *et al.*, 1980).

Of pesticide residues, fungicides not surprisingly have the greatest effect on the growth and fermentability of yeasts. Newer fungicides, such as metalaxyl (Ridomil) and cymoxanil (Curzate), do not appear to affect fermentation. In contrast, triadimefon (Bayleton) can depress fermentation, presumably by disrupting sterol metabolism. The older, broad-spectrum fungicides such as

resulted in undue juice clarification. The resultant loss in sterols, unsaturated fatty acids, and nitrogenous nutrients can increase yeast sensitivity to the combined toxicity of ethanol and saturated mid-chain carboxylic acids, notably octanoic and decanoic acids and their esters. Crushing, pressing, and other prefermentative activities scrupulously conducted in the absence of oxygen heighten these effects. Molecular oxygen is required by yeasts for the biosynthesis of sterols and long-chain unsaturated fatty acids essential for cell membrane synthesis and function.

Juice from overmature or botrytized grapes generally has very high sugar contents. The osmotic effect of high sugar concentrations can partially plasmolyze yeast cells, resulting in slow and/or incomplete fermentation. Botrytized juice also contains lower than usual concentrations of available nitrogen as well as vitamins, owing to assimilation by *Botrytis cinerea*. Nutrient depletion adds to the combined inhibitory effects of high sugar content and the toxicity of ethanol and C_8 and C_{10} saturated carboxylic acids. In addition, polysaccharides synthesized by *B. cinerea* may have inhibitory effects on yeast fermentation (Ribéreau-Gayon *et al.*, 1979). Further, the toxicity of acetic acid typically found in botrytized grapes may contribute to poor fermentability. The presence of 10^5 to 10^6 acetic acid bacteria/ml can be lethal to *Sacch. cerevisiae* (Grossman and Becker, 1984). Depending on the style of wine desired, the retention of significant concentrations of residual sugar may or may not be desirable.

For the production of low alcohol wines possessing high residual sugar contents, a stuck fermentation may be purposely induced by chilling and clarification to remove the yeasts.

"Killer" yeasts can generate off-flavors and disrupt fermentation. To counter the effects of killer strains, several workers have incorporated one or both primary killer traits into commercial wine yeast strains (Boone *et al.*, 1990; Sulo *et al.*, 1992). Killer properties have been found in several naturally occurring *Sacch. cerevisiae* strains, as well as species of *Hansenula, Pichia, Torulopsis, Candida,* and *Kluyveromyces.*

Expression of the killer property occurs variously in different yeasts. A cytoplasmic double-stranded RNA virus encodes the property in *Sacch. cerevisiae,* whereas two linear DNA molecules control the property in *Kluyveromyces lactis.* In other genera, chromosomal genes may be involved. The toxic principle is associated with the production and release of a protein or glycoprotein. The toxin attaches to the wall, and subsequently the membrane, of sensitive yeast cells. The attachment creates pores in the membrane, destroying the ability of the cell to control ion flow and resulting in cell death.

Most killer strains of *Sacch. cerevisiae* produce one of two types of killer proteins (K_1 and K_2), though others are suspected. Killer cells are immune to the effects of their own toxin but may be sensitive to those produced by other strains. Eleven different killer (K) factors have been identified, most of which do not affect *Sacch. cerevisiae.* Killer toxins commonly affect only related yeast strains.

The killer proteins produced by *Sacch. cerevisiae* act optimally at a pH of 4 to 5, a range above that normally found in wine. However, both toxins are stable within wine pH values. Therefore, the toxins appear to be at least partially active during fermentation. Cells appear to be most sensitive to the toxin during the exponential phase of cell growth. The importance of killer toxins in winemaking probably will depend on juice pH, the addition of protein-binding substances such as bentonite or yeast hulls, the ability of killer strains to ferment, and the degree to which the yeast population multiplies during fermentation.

Control of stuck or sluggish fermentation in any particular case will depend on knowing the precise cause (Munoz and Ingledew, 1990). Temperature regulation has eliminated overheating as the major cause of stuck fermentations. Use of commercial strains carrying both K_1 and K_2 factors and addition of materials to reduce the active concentration of killer toxins or fungicides are additional solutions to particular problems of stuck or sluggish fermentations. The likelihood of stuck fermentation also may be minimized by reduced prefermentative clarification, limited oxidation during crushing and pressing, the addition of ergosterol and/or long-chain unsaturated fatty acids (i.e., oleic, linoleic, or linolenic acids), the addition of ammonium salts, and/or the addtoin of yeast ghosts or other absorptive materials. The addition of nutrients and absorptive substances appears to have optimal effects when applied midway in or near the end of the exponential phase of yeast growth.

Malolactic Fermentation

It is unlikely that the value of any winemaking process other than malolactic fermentation is associated with such diversity of opinion. The diversity is not surprising since malolactic fermentation to varying degrees can improve or reduce wine quality. In addition, conditions where its occurrence would be beneficial discourage the processs. Conversely, situations where malolactic fermentation is either unnecessary or undesirable tend to promote its development.

The principal effect of malolactic fermentation is a reduction in acidity. In wines of excessive acidity, the reduction is desirable. Thus, winemakers in most cool wine-producing regions view malolactic fermentation positively, especially for red wines. In contrast, wines

produced in warm regions may be low in acidity or high in pH. Malolactic fermentation can aggravate a difficult situation, leaving the wine "flat" tasting and microbially unstable. Consequently, the issue of malolactic fermentation elicits markedly diverse responses from winemakers in different parts of the world.

Lactic Acid Bacteria

Lactic acid bacteria are characterized by a unique set of properties. Their name refers to one of these, the production of large amounts of lactic acid. Depending on the genus or species, lactic acid bacteria may ferment sugars solely to lactic acid or to lactic acid, ethanol, and carbon dioxide. The former mechanism is called **homofermentation,** and the latter is termed **heterofermentation.** Bacteria capable of both types of fermentation grow in wine. Homofermentation potentially yields two ATPs per glucose (similar to yeast fermentation), while heterofermentation yields but one.

The most beneficial number of the group is one of the heterofermentative species, *Leuconostoc oenos.* It is probably the most frequently occurring species of lactic acid bacteria in wine and commonly is the only species inducing malolactic fermentation in wines of low pH ($\leq$3.5). Spoilage forms are generally members of the genera *Lactobacillus* and *Pediococcus. Lactobacillus* contains both homo- and heterofermentative species, whereas *Pediococcus* is strictly homofermentative.

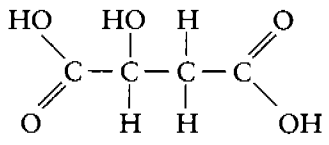
Although the bacteria are classified primarily on the basis of sugar metabolism, whether sugar metabolism is important to their growth in wine is unclear. Even dry wines possess sufficient residual sugars (mostly pentoses) to support considerable bacterial growth. However, sugars are not necessarily metabolized in wine. Occasionally, the concentrations of glucose and fructose even increase during malolactic fermentation (Davis *et al.,* 1986). This increase apparently is unrelated to malolactic fermentation, as it also occurs in its absence.

Lactic acid bacteria are further distinguished by their limited biosynthetic abilities. They require a complex series of nutrients, including B vitamins, purine and pyrimidine bases, and several amino acids. Indicative of the limited synthetic capabilities is the inability of lactic acid bacteria to produce heme proteins. As a consequence, they produce neither cytochromes nor the enzyme catalase. Without cytochromes, they cannot respire. Consequently, energy metabolism is strictly fermentative.

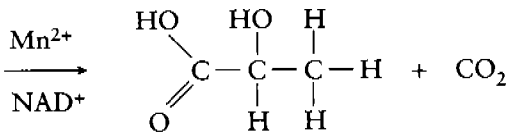
Most bacteria incapable of synthesizing heme molecules are strict anaerobes, that is, are unable to grow in the presence of oxygen. Oxygen reacts with certain cytoplasmic components, notably flavoproteins, to produce the toxic oxygen radicals **superoxide** and **peroxide.** In aerobic organisms, superoxide dismutase and catalase rapidly inactivate these toxic radicals. Lactic acid bacteria produce neither enzyme. They escape the toxicity of the oxygen radicals by accumulating large quantities of Mn^{2+} ions, or by producing a peroxidase. Manganese detoxifies superoxide by converting it to oxygen. The rapid action of manganese also prevents the synthesis of hydrogen peroxide from superoxide. Peroxidase reduces organic compounds in the presence of peroxide, oxidizing the peroxide to water. Lactic acid bacteria are the only bacterial group that are both strictly fermentative and aerotolerant.

Although fermentative metabolism is inefficient in terms of energy production, the generation of large amounts of acidic wastes quickly lowers the pH of most substrates. The resulting low pH inhibits the growth of most other bacteria. Lactic acid bacteria are one of the few bacterial groups capable of growing below a pH of 5. This property has preadapted lactic acid bacteria to grow in acidic environments such as wine.

Although lactic acid bacteria grow under acidic conditions, growth is poor in must and wine. For example, species of *Lactobacillus* and *Pediococcus* commonly cease growing below pH 3.5. Even *Leuconostoc oenos,* the primary malolactic bacterium, is inhibited below pH 3.0 to 2.9. *Leuconostic oenos* grows optimally within a pH range of 4.5 to 5.5. Thus, the major benefit of malolactic fermentation for the bacteria surprisingly may be acid reduction. By metabolizing a dicarboxylic acid (malic acid) to a monocarboxylic acid (lactic acid), acidity is reduced and pH increased. The increased pH provides conditions more favorable to bacterial growth.



L-malic acid



L-lactic acid

The enzyme involved in malolactic fermentation by lactic acid bacteria, the **malolactic enzyme,** is unique. Unlike other conversions of malic to lactic acid, the enzymatic reaction directly decarboxylates L-malic acid to L-lactic acid. Because the reaction is not associated directly with energy capture, the conversion can only dubiously be called a fermentation. However, some ATP is generated through the joint export of lactic acid and hydrogen ions (protons) from the cell (Cox and Henick-

Kling, 1990). The pH differential produced across the cell membrane is sufficient to drive the oxidative phosphorylation of ADP to ATP. Release of a small amount of reducing energy also may occur via the oxidation of malic acid to pyruvic acid, some of which is subsequently reduced to lactic acid.

The primary energy source for the growth of lactic acid bacteria in wine is still unclear. The situation is complicated by the marked influence pH has on the abilities of the bacteria to ferment sugars and by the considerable variability between strains. *Leuconostoc oenos* appears to show little ability to ferment sugars, at least below pH 3.5 (Davis *et al.*, 1986). However, a more recent report suggests that fructose and glucose may be the energy source for *Leuco. oenos* (Arnick *et al.*, 1992). Species of *Lactobacillus* and *Pediococcus*,, which generally grow only above pH 3.5, appear to ferment hexoses and pentoses in wine. Fumaric and citric acids are metabolized by several strains of *Leuco. oenos*, but not by most lactobacilli and pediococci. The metabolism of citric acid is apparently associated with the accumulation of acetoin and diacetyl (Shimazu *et al.*, 1985). Amino acids, notably arginine, also may act as energy sources (Feuillat *et al.*, 1985).

All lactic acid bacteria growing in wine assimilate acetaldehyde and other carbonyl compounds. The metabolism of carbonyl compounds may retard malolactic fermentation by liberating sulfur dioxide bound to carbonyl compounds.

As noted with anaerobic yeast metabolism, fermentation can result in the generation of an excess of reduced NAD^+ (NADH). To maintain an acceptable redox balance, the bacteria must regenerate NAD^+. How lactic acid bacteria accomplish this in wine is unclear. Some species reduce fructose to mannitol, presumably for this purpose. This may explain the common occurrence of mannitol in wine associated with malolactic fermentation. Some strains also regenerate NAD^+ with flavoproteins and oxygen. This reaction might explain the reported improvement in malolactic fermentation in the presence of small amounts of oxygen.

Besides the important physiological differences, anatomical features distinguish the various genera of lactic acid bacteria (Fig. 7.30). *Leuconostoc* usually consists of spherical to lens-shaped cells, commonly occurring in pairs or chains but occasionally singly. *Pediococcus* species usually occur as packets of four spherical cells. *Lactobacillus* produces long, slender, occasionally bent, rod-shaped cells commonly occurring in chains. Some of the lactic acid bacteria that may occur in wine are listed in Table 7.4.

Effects of Malolactic Fermentation

Malolactic fermentation has three distinct, but interrelated, effects on wine quality. It reduces acidity, influences microbial stability, and may affect the sensory characteristics of the wine.

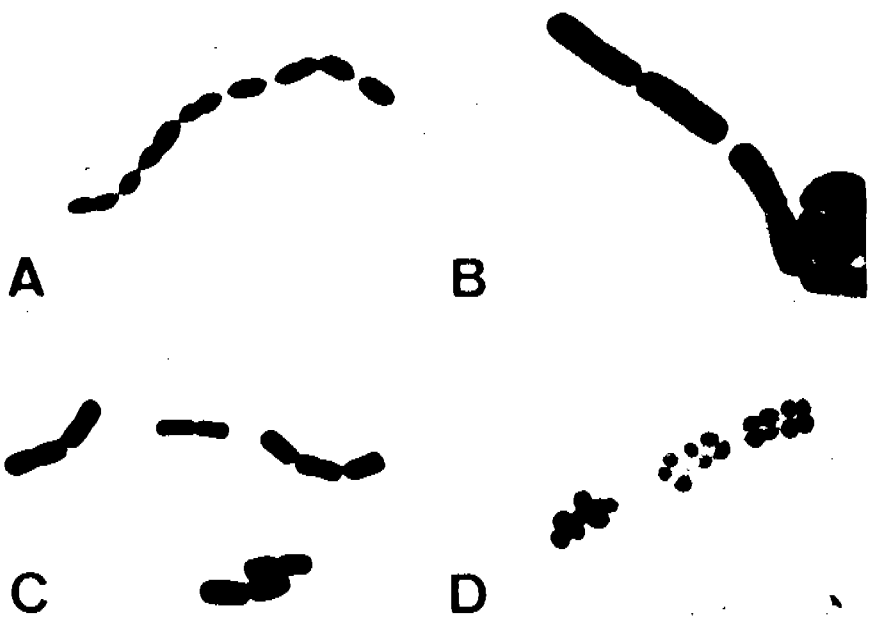
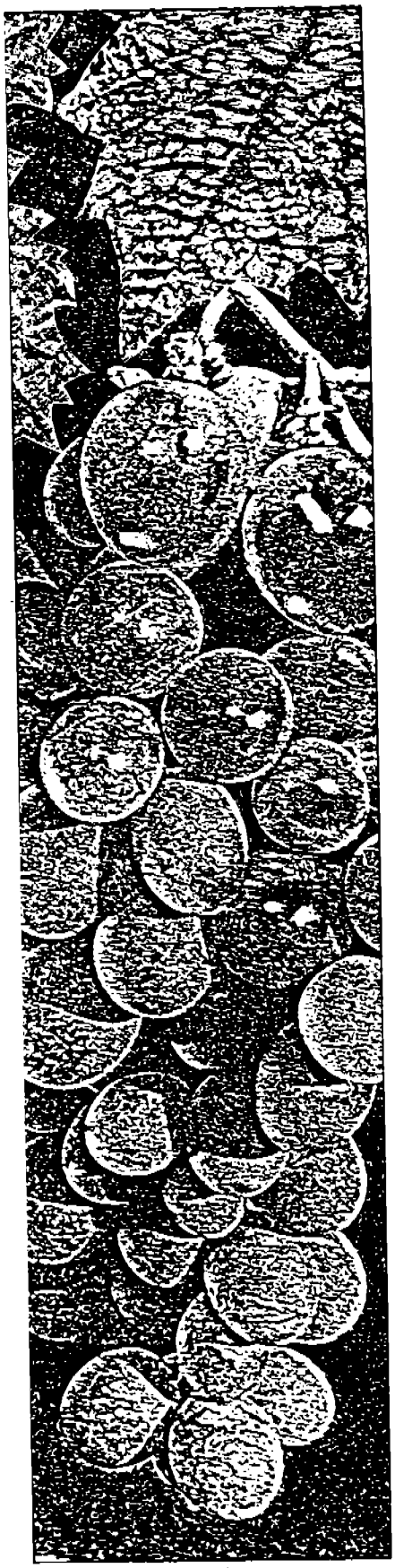


Figure 7.30 Micrographs of important members of the Lactobacillaceae found in wine. (A) *Leuconostoc oenos* (×6000). (B) *Lactobacillus casei* (×8500). (C) *Lactobacillus brevis* (×5500). (D) *Pediococcus cerevisiae* (×5000). (From Radler, 1972, reproduced by permission.) Cell shape and grouping may depend on the medium in which the bacteria grow.



8

Postfermentation Treatments and Related Topics

All wines undergo a period of adjustment (maturation) before bottling. Maturation involves the precipitation of particulate and colloidal material from the wine as well as a complex range of physical, chemical, and biological changes that tend to maintain and/or improve the sensory characteristics of the wine. These processes occur spontaneously, but can be facilitated by the winemaker. Although wines usually benefit from the judicious intervention of the winemaker, caution is required to avoid unnecessarily modifying of the character of the wine.

Wine Adjustments

Adjustments attempt to correct deficiencies found in the grapes and sensory imbalances that develop during fermentation. In certain jurisdictions, acidity and sweetness adjustments are permitted only before fermentation. This is regrettable, as it is impossible to predict precisely the course of fermentation. Judicious adjustment after vinification can improve the finished wine, without compromising regional or varietal characteristics.

Acidity and pH Adjustment

Theoretically, acidity and pH adjustment could occur at almost any stage during vinification. Nevertheless, postfermentative correction is probably optimal. During fermentation, deacidification often occurs spontaneously owing to acid precipitation and yeast or bacterial metabolism. In addition, some strains of *Saccharomyces cerevisiae* synthesize significant amounts of malic acid during fermentation (Farris *et al.*, 1989). Thus, the extent and type of deacidification needed are difficult to assess before the end of fermentation. However, if the juice is above pH 3.4, some lowering of the pH before fermentation may be advisable to favorably influence fermentation and avoid large adjustments following fermentation.

No precise recommendations for optimal acidity are possible as they reflect stylistic and regional preferences. More fundamentally, acidity and pH are complexly interrelated. The major fixed acids in grapes (tartaric and malic) occur in a dynamic equilibrium of various ionized and nonionized states (Fig. 8.1). Both tartaric and malic acids may exist as undissociated (nonionized) acids, in half-ionized forms (one ionized carboxyl group), in fully ionized states (both carboxyl groups ionized), as half-salts (one carboxyl group associated with a cation), as full salts (both carboxyl groups bound to cations), or as double salts with other acid molecules and cations. The proportion of interconvertible states depends largely on the concentration of the acids and potassium ions. Because of the complexity of the equilibria, it is presently impossible to predict precisely the consequences of changing any one of the factors affecting acidity. A range between 0.55 and 0.85% total acidity is generally considered desirable. Red wines are customarily preferred at the lower end of the range, while white wines are preferred at the upper end.

Another important aspect of acidity is pH. It represents the proportion of H^+ to OH^- ions in aqueous solutions. The higher the proportion of H^+, the lower the pH; conversely, the higher the proportion of OH^-, the higher the pH. Wines vary considerably in pH, with values below 3.1 being sensed as sour while those above 3.7 taste "flat." White wines are commonly preferred at the lower end of the pH range, while red wines are frequently favored in the mid range.

Relatively low pH values are preferred for many reasons. They give wines a fresh taste, improve microbial stability, reduce browning, diminish the need for SO_2 addition, and enhance the production and stability of "fruit" esters. Concentrations of monoterpenes also may be affected. For example, the concentration of geraniol, citronellol, and nerol may rise at low pH, while those of linalool, α-terpineol, and hotrienol fall. In red wines, color intensity and hue are better at lower pH values.

Because of the importance of pH, choice of acidity correction procedure is influenced considerably by how it affects pH. Because tartaric acid is more highly ionized than malic acid within the usual range of wine pH values, adjusting the concentration of tartaric acid has a greater effect on pH than an equivalent change in the concentration of malic acid (Fig. 8.2). Thus, where correction in pH is desired, adjusting the concentration of tartaric acid is preferable. Where changes in pH should be minimized, adjustment in the malic acid concentration is favored. Acidification before fermentation is discussed in Chapter 7.

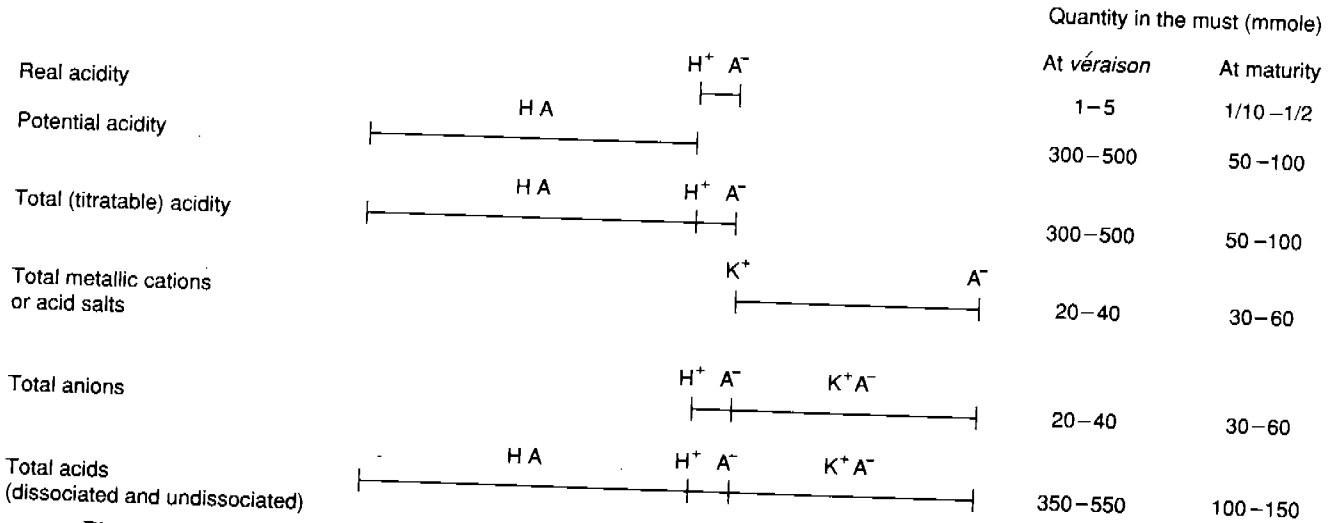


Figure 8.1 Different notions characterizing the acid–base equilibrium of a solution. The values given correspond to those found in must. A^-, Acid anion; H^+, hydrogen ion; HA, undissociated acid; K^+, potassium ion; K^+A^-, undissociated potassium salt. (After Champagnol, 1986, reproduced by permission.)

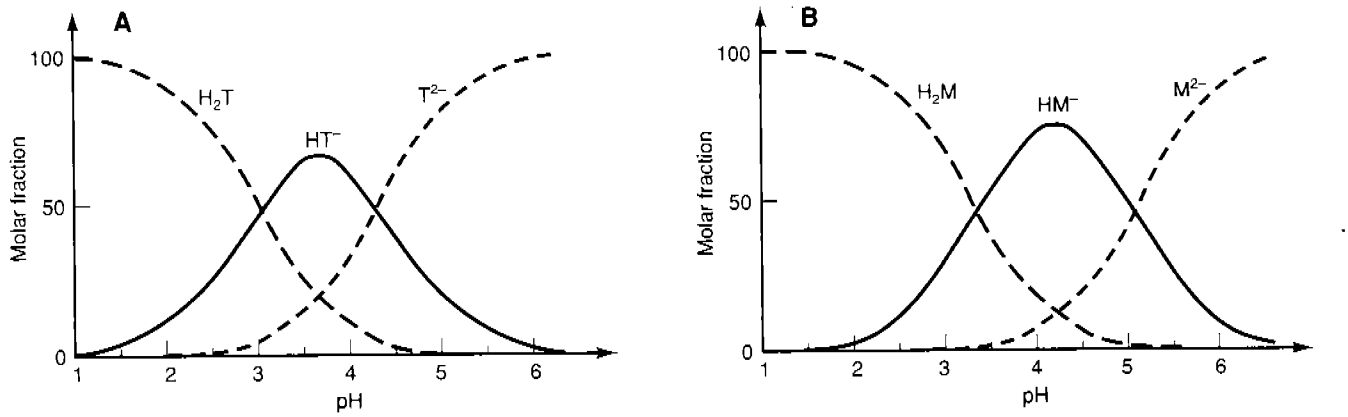


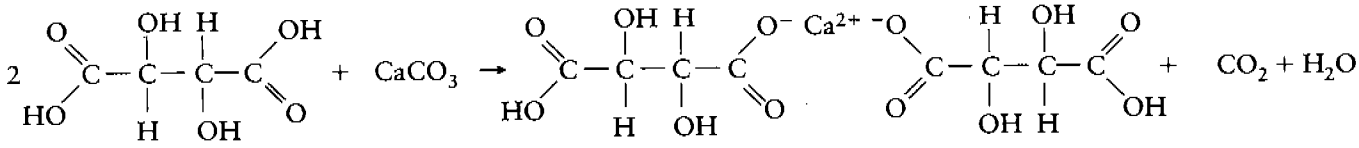
Figure 8.2 Relative concentrations of the three main forms of (A) tartaric and (B) malic acids as a function of pH. H_2, Full acid (unionized); H, half-acid (half-ionized); $^{2-}$, fully ionized acid. (After Champagnol, 1986, reproduced by permission.)

DEACIDIFICATION

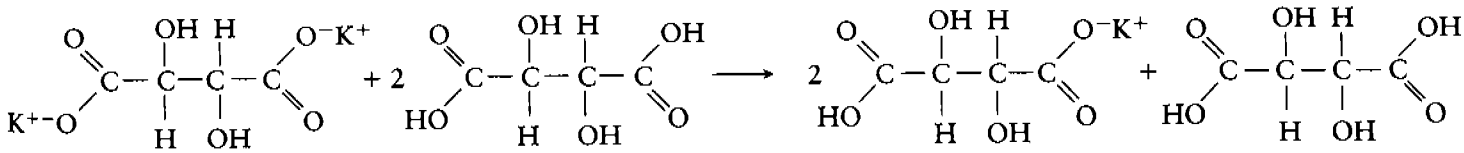
Wine may be deacidified by either physicochemical or biological means. Physicochemical deacidification involves either acid precipitation or column ion-exchange. Biological deacidification usually involves malolactic fermentation (see Chapter 7).

Precipitation Precipitation primarily entails the neutralization of tartaric acid; malic acid is less involved owing to the higher solubility of its salts in wine. Neutralization can result as cations (positively charged ions) of an added inorganic salt exchange with hydrogen ion(s) of the organic acid. Salt formation can reduce the solubility of an acid, inducing crystallization and precipitation. Removal of the precipitated salt by racking, filtration, or centrifugation makes the reaction nonreversible.

To induce neutralization and precipitation of tartaric acid, finely ground calcium carbonate may be added to the wine. The reaction is as follows:



Neutralization also can result from the formation of insoluble half-salts. Deacidification with potassium tartrate acts in this manner:



Deacidification with calcium carbonate is probably the most common procedure, as use of potassium carbonate is prohibited in several countries and potassium tartrate tends to be more expensive. Although widely used, calcium carbonate has a number of disadvantages. Its primary drawback is the slow rate at which calcium tartrate precipitates. In addition, formation of the soluble salt of calcium malate may produce a salty taste. Furthermore, if tartrate removal is excessive, the resultant increase in pH may leave the wine tasting "flat" and susceptible to microbial spoilage.

Some of the disadvantages of calcium carbonate addition may be avoided by the use of "double-salt" deacidification. The name refers to the belief that the technique functioned primarily by the formation of an insoluble double salt between malic acid, tartaric acid, and calcium. It now appears that very little of the hypothesized double salt forms. Instead, the procedure seems to both speed the precipitation of calcium tartrate and facilitate

the partial precipitation of calcium malate (Cole and Boulton, 1989).

The major difference between the single- and double-salt procedures is that the latter involves the addition of calcium carbonate to only a small proportion (~10%) of the wine to be deacidified. Sufficient calcium carbonate is added to raise the pH above 5.1 This assures adequate dissociation of both malic and tartaric acids (see Fig. 8.2) and induces the rapid formation and precipitation of the salts. A patented modification of the double-salt procedure (Acidex) incorporates 1% calcium malate–tartrate with the calcium carbonate. The double salt possibly acts as seed crystals, promoting the rapid growth of salt crystals.

In double-salt procedures, the remainder of the wine is slowly blended into the treated portion with vigorous stirring. Subsequent crystal removal occurs by filtration, centrifugation, or settling. Stabilization of the treated wine may take 3 months, during which residual salts are allowed to precipitate before bottling.

Although precipitation works well with wines of medium to high total acidity (6–9 g/ml) and medium to low pH (<3.5), it can result in an excessive pH rise in wines showing both high acidity (>9 g/ml) and high pH (>3.5). This situation is most common in cool climatic regions, where malic acid constitutes the major acid and the potassium content is high. In this situation, column ion-exchange may be used (Bonorden *et al.*, 1986) or tartaric acid added to the wine before addition of calcium carbonate in double-salt deacidification (Nagel *et al.*, 1988). Precipitation following neutralization removes the excess potassium with acid salts, and the additional tartaric acid lowers the pH to an acceptable value.

Because "protective" colloids can significantly affect the precipitation of acid salts, it is important to conduct deacidification trials on small samples of the wine to be treated. This will establish the amount of calcium carbonate or Acidex required for the desired degree of deacidification.

Column Ion-Exchange Ion exchange involves passing the wine through a resin-containing column. During passage, ions in the wine exchange with those in the column. The types of ions exchanged can be adjusted by the type of resin used and the ions present for exchange on the resin.

For deacidification, the column is packed with an anion-exchange resin. Tartrate ions are commonly exchanged with hydroxyl ions (OH^-), thus removing tartrate from the wine. The hydroxyl ions released from the resin associate with hydrogen ions, forming water. Alternately, malate may be removed by exchange with a tartrate-charged resin. The excess tartaric acid may be subsequently removed by neutralization and precipitation.

Currently, the major limiting factor in the use of ion exchange other than legal restrictions and cost, is its tendency to remove flavorants and color from the wine, reducing wine quality.

Biological Deacidification Biological deacidification, via malolactic fermentation, is possibly the most common means by which acidity correction occurs in wine. As malolactic fermentation can occur before, during, and after alcoholic fermentation, it was discussed previously in Chapter 7. The yeast *Schizosaccharomyces pombe* also effectively decarboxylates malic acid, but the strong tendency of the yeast to generate hydrogen sulfide and other off-odors makes its use generally ill-advised (see Beelman and Gallander, 1979). However, as some strains of *Saccharomyces cerevisiae* can degrade nearly 50% of the malic acid content in juice (Rankine, 1966), their use in fermentation could achieve partial deacidification.

ACIDIFICATION

When wines are too low in acidity or pH, tartaric acid is commonly added to correct the fault. The advantages of tartaric acid as an acidulant include its high microbial stability and a dissociation constant (K_a, commonly expressed as the negative logarithm pK_a) that allows it to have a marked effect in lowering the pH. The main disadvantage is crystal formation if the wine has a high potassium content. Citric acid addition does not have this problem and can assist in preventing ferric casse. However, the ease with which citric acid can be metabolized means that it is microbially unstable. Alternately, ion exchange may be used to lower pH by exchanging H^+ for the Ca^{2+} or K^+ of tartrate and malate salts.

Sweetening

In the past, stable naturally sweet wines were rare. Most of the sweet wines of antiquity probably contained boiled-down must or honey. Stabilization by the addition of distilled alcohol is a comparatively recent development. The stable naturally sweet wines of the past few centuries seem to have been produced from highly botrytized grapes (see Chapter 9). In contrast, modern technology can produce a wide range of sweet wines without recourse to botrytization, baking, or fortification.

Wines may be sweetened with sugar, for example, sparkling wines. However, most still wines possessing a sweet finish are sweetened by the addition of partially fermented or unfermented grape juice, termed **sweet reserve** (*süssreserve*). The base wine is typically fermented dry and sweetened just before bottling. To avoid microbial contamination, both wine and sweet reserve are sterilized by filtration, or pasteurized, and the blend is bottled under aseptic conditions using sterile bottles and corks.

Various techniques are used in preparing and preserving sweet reserve. One procedure involves separating a small portion of the juice to produce the sweet reserve with the same varietal, vintage, and geographical origin as the wine it sweetens. If the sweet reserve is partially fermented, yeast activity may be terminated prematurely by chilling, filtration, or centrifugation, or by trapping the carbon dioxide released during fermentation. The pressure buildup stops fermentation. If the sweet reserve is stored as unfermented juice, microbial activity is restricted after clarification by cooling to −2°C, applying CO_2 pressure, pasteurizing, or sulfiting to above 100 ppm of free SO_2. In the latter case, desulfiting before use is conducted by flash heating and sparging with nitrogen gas. If desired, reverse osmosis or cryoextraction can concentrate the juice. Heat and vacuum concentration are additional possibilities but are likely to result in greater flavor modification and fragrance loss.

Dealcoholization

In recent years, there has been an increasing market for low alcohol and dealcoholized wines. Techniques have been available for the production of such wines since the early 1900s. Until comparatively recently, the process required heating and the evaporation of 50 to 70% of the wine to reduce the alcohol content to 4 g/liter. With the advent of vacuum distillation, the temperature required could be reduced, thus avoiding the baked or cooked character associated with heating. Nevertheless, important volatiles were lost with the alcohol. Most can be retrieved and added back to the wine, but the final product was still lacking in some of the original character. Modern strip column distillation techniques apparently require a loss of only about 20 to 30% of the wine to reach 9 g ethanol/liter (Ireton, 1990; Duerr and Cuénat, 1988). The use of dialysis apparently results in little flavor loss (Wucherpfennig *et al.*, 1986). Nevertheless, the most widely used dealcoholization technique currently involves reverse osmosis.

Reverse osmosis derives its name from the reversal of the normal flow of water in osmosis. Osmosis is the diffusion of water across a differentially permeable membrane, from a region of higher to lower concentration. If sufficient pressure is exerted on the more concentrated solution, diffusion of water and other permeable substances is reversed, with net movement occurring across the membrane into the dilute solution. The technique is widely used in the selective concentration or elimination of low molecular weight compounds from juices. The limitation of its use in viniculture to dealcoholization probably results from the current unavailability of membranes with appropriate permeability characteristics.

With the use of a proper support system, and sufficient pressure, reverse osmosis can reduce the alcohol content of wine to almost any degree desired. However, as water is removed along with the ethanol, water must be added back to the concentrated wine or added to the wine before use of reverse osmosis. This creates legal problems where the addition of water to wine is prohibited. Depending on the particular membrane used, compounds such as esters, aldehydes, and organic acids may be lost. This can result in significant fragrance loss in the dealcoholized wine. The isolation of the compounds and their addition to the wine generally has not proved satisfactory. However, addition of grape juice or concentrate may provide some of the former fragrance of the wine. Addition of organic acids lost during reverse osmosis may further enhance the flavor of the wine.

The problem of adding water has been circumvented by an ingenious system involving double reverse osmosis (Bui *et al.*, 1986). It produces alcohol-reduced and alcohol-enriched wines simultaneously. By interconnecting both systems, no water needs to be added to the alcohol-reduced concentrate, nor is there the legal problem of producing an alcohol "distillate." The system cannot produce completely dealcoholized wines, however.

Where the addition of water is permissible in the production of low alcohol beverages, dilution is the simplest means of dealcoholization. Flavor enhancement, as with wine coolers, offsets flavor dilution.

Color Adjustment

Heating newly fermented wine to 35 to 40°C for 24 to 48 hr before pressing also has been advocated where immature or slightly molded grapes are involved (Ribéreau-Gayon and Ribéreau-Gayon, 1980). The process is reported to improve flavor, color, and tannic structure. However, excessive heating can induce the loss of color, make the wine aggressively tannic, and require inoculation to achieve malolactic fermentation.

Both red and white wines may be partially or completely decolored by the membrane technique called **ultrafiltration.** Depending on the permeability characteristics of the membrane, ultrafiltration selectively retains macromolecules based on molecular size. With membranes of lower cutoff values (~500 daltons), ultrafiltration also can remove the "pinking" produced by small procyanidin molecules. Use of filters with even lower cutoff values can produce blush or white wines from red or rosé wines. The major factor limiting the more widespread use of ultrafiltration in enology is its potential to remove important flavorants along with macromolecules.

An alternative technique for removing brown and pink pigments involves the addition of polyvinylpolypyrrolidone (PVPP). By binding tannins into large macro-

molecular complexes, PVPP greatly facilitates their removal by filtration or centrifugation.

Other means of color removal have involved the addition of casein or special preparations of activated charcoal. With activated charcoal, the simultaneous removal of aromatic compounds and the occasional donation of off-odors have limited its use.

Blending

Blending of wine from different varieties is a long established procedure in many wine regions. The mixed varietal planting of the past had a similar result but precluded the selective blending of individual varietal wines after fermentation. Separating lots of wine based on varietal, vineyard, or maturity differences is more difficult and expensive, but it permits blending based on the wishes of the cellarmaster.

The art of the blender is especially important in the production of fortified and sparkling wines. Without blending, the creation and maintenance of the brand name products that characterize fortified and sparkling wines would be impossible. Computer-aided systems have been proposed to facilitate this important activity (Datta and Nakai, 1992).

The production of standard-quality table wines also is largely dependent on blending. Consistency of character typically is more important than the vintage, varietal, or vineyard origin of the wine. The skill of the blender is often amazing, given the diversity of wines often involved in the formation of each blend.

Blending also is used in the production of many premium table wines. In this case, however, the wines usually come from the same geographical region and may be from a single vineyard and/or vintage. Limitations on blending are usually precisely articulated in the Appellation Control laws affecting the region concerned; frequently the more prestigious the region, the more restrictive the legislation.

Currently, there is little to guide blenders other than past experience. Few studies have investigated the scientific basis of the blender's art. Nevertheless, several studies have proposed methods of predicting color based on the pigmentation characteristics of potential base wines. Color is very important because of its strong biasing influence on quality perception. Blending diagrams may be developed using colorimeter readings (Little and Liaw, 1974). The diagrams are based on the reflectance of the wines in the red, green, and blue portions of the visible spectrum. Based on the results, the selection and proportions of each lot required to achieve a desired color may be established. A more complex system based on a Scheffé design has been proposed by Negueruela *et al.* (1990).

One of the advantages of blending is an improvement in fragrance and flavor. In a classic study by Singleton and Ough (1962), pairs of wines ranked similarly, but noted to be distinctly different, were reassessed along with a 50:50 blend of both wines. In no case was the blend ranked more poorly than the lower ranked of the two source wines. More importantly, about 20% of the blends were ranked higher than the unblended source wines (Singleton and Ough, 1962).

Although the origin of the improved sensory quality of blended wines is unknown, it probably relates to the increased flavor subtlety of the blend. Human taste and odor perception commonly respond in a nonlinear manner to increasing or decreasing concentrations of flavorants. Therefore, dilution of a flavor ingredient during blending need not necessarily diminish perception. Thus, a blend may express the flavors of all the wines used. In addition, dilution may reduce the concentration of undesirable odors to below the detection threshold. Finally, the qualitative perception of some off-odors improves with dilution (see Chapter 11).

When blending should occur depends largely on the type and style of wine involved. In sherry production, blending occurs periodically throughout the long maturation period. With sparkling wines, blending is commonly done in the spring following the harvest. At this point the unique features of the wines are beginning to be expressed. Red wines also are typically blended in the spring following the vintage. Important in deliberation is not only the proportional amounts of each wine to be blended, but also the amount of pressings to use. Wines from poor vintages customarily benefit more from the addition of extra press wine than do wines from good vintages. Press fractions contain a higher proportion of pigment and tannins than the free-run. After blending, the wine is often aged for several weeks, months, or years before bottling. The addition of pressings also can provide extra "body" and color to white wines.

Wines may not be blended for a number of reasons. Wines produced from grapes of especially high quality are usually kept and bottled separately to retain the distinctive characteristics undiluted. For wines produced from famous vineyard sites, blending with wine from other sites, regardless of quality, would prohibit the owner from using the name of the site. This could markedly reduce the market value of the wine. With famous sites, uniqueness of origin can be more important than perceptible quality.

Stabilization and Clarification

Stabilization and clarification involve procedures designed to produce a permanently clear wine with no flavor faults. Because the procedures can themselves create problems, it is essential that they be used judi-

ciously and only to the degree necessary to solve specific problems.

Stabilization

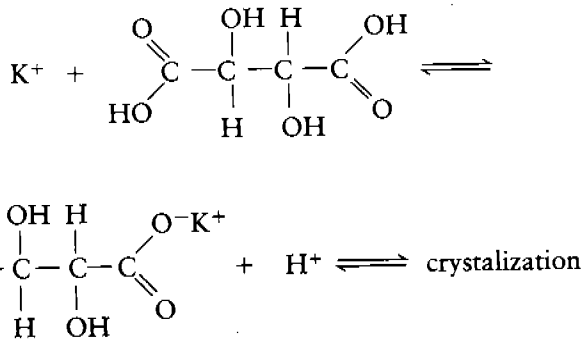
TARTRATE AND OTHER CRYSTALLINE SALTS

Tartrate stabilization is one of the facets of wine technology most influenced by consumer perception. The presence of even a few tartrate crystals is inordinately feared, or at least misinterpreted, by many wine consumers. As a consequence, considerable effort and expense are expended in avoiding the formation of crystalline deposits in bottled wine. Stabilization is normally achieved by enhancing crystallization, followed by removal. However, it also may be attained by delaying or inhibiting crystallization.

Potassium Bitartrate Instability Juice is typically supersaturated with potassium bitartrate at crushing. As the alcohol content rises during fermentation, the solubility of bitartrate decreases. This induces the slow precipitation of potassiumn bitartrate (cream of tartar). Given sufficient time, the salt crystals usually precipitate spontaneously. In northern regions, the low temperatures found in unheated cellars may produce adequately rapid precipitation, but spontaneous precipitation is seldom satisfactory in warmer areas. Early bottling aggravates the problem. Where spontaneous precipitation is inadequate, refrigeration often achieves rapid and satisfactory bitartrate stability.

Because the rate of bitartrate crystallization is directly dependent on the degree of supersaturation, wines only mildly unstable may be insufficiently stabilized by cold treatment. In addition, protective colloids may retard crystallization such as mannoproteins (Lubbers *et al.*, 1993). If the colloids precipitate after bottling, the released bitartrates are free to crystallize. Because of the incomplete understanding of tartrate crystallization in wine, and the importance of the process, it is still under active investigation.

At the simplest, potassium bitartrate exists in a dynamic equilibrium between various ionized and salt states:



Under supersaturated conditions, crystals form and eventually reach a critical mass that induces precipitation. Crystallization continues until an equilibrium develops. If sufficient crystallization and removal occur before bottling, bitartrate stability is achieved. Because chilling decreases solubility it speeds crystallization.

Theoretically, chilling should establish bitartrate stability. However, charged particles in wine can interfere with crystal initiation and growth. For example, positively charged bitartrate crystals attract negatively charged colloids onto their surfaces, blocking growth. The charge on the crystals is created by the tendency of more potassium than bitartrate ions to associate with the crystals early in growth (Rodriguez-Clemente and Correa-Gorospe, 1988). Crystal growth also may be delayed by the binding of bitartrate ions to positively charged proteins. This reduces the amount of free bitartrate and, thereby, the rate of crystallization. Because both bitartrate and potassium ions may bind with tannins, crystallization tends to be delayed more in red than in white wines. The binding of potassium with sulfites is another source of delayed bitartrate stabilization.

For cold stabilization, table wines are routinely chilled to near the freezing point of the wine. Five days is usually sufficient at −5.5°C, but 2 weeks may be necessary at −3.9°C. Fortified wines are customarily chilled to between −7.2° and −9.4°C, depending on the alcoholic strength. The stabilization temperature can be estimated using the formula empirically established by Perin (1977):

$$\text{Temperature } (-°\text{C}) = (\%\ \text{alcohol} \div 2) - 1$$

Direct seeding with potassium bitartrate crystals is occasionally employed to stimulate crystal growth and deposition. Another technique involves filters incorporating seed crystals. The chilled wine is agitated and then passed through the filter, where crystal growth is encouraged by the dense concentration of crystal nuclei. The filter acts as a support medium for the seed nuclei.

At the end of conventional chilling, the wine is filtered or centrifuged to remove the crystals. Crystal removal is performed before the wine warms to ambient temperatures.

Because of the expense of refrigeration, various procedures have been developed to determine the need for cold stabilization. At present, none of the techniques appears to be sufficiently adequate. Potassium conductivity, while valuable, is too complex for regular use in most wineries. Thus, emperical freeze tests are still the most commonly used means of assessing bitartrate stability. For details on the various tests, the reader is directed to Goswell (1981) or Zoecklein *et al.* (1990).

Reverse osmosis is an alternative technique to chilling, agitation, and the addition of nucleation crystals. With the removal of water, the increased bitartrate concentration augments crystallization and precipitation. After crystal removal, the water is added back to reestablish the original balance of the wine. Electrodialysis is another membrane technique occasionally used for bitartrate stabilization.

Another technique particularly useful with wines having high potassium contents is column ion-exchange. Passing the wine through a column packed with sodium-containing resin exchanges sodium for potassium. Sodium bitartrate is more soluble than the potassium salt and is therefore much less likely to precipitate. Although effective, ion exchange is not the method of choice. Not only is it prohibited in certain jurisdictions, for example, EEC countries, but it also increases the sodium content of the wine. The high potassium, low sodium content of wine is one of its healthful properties.

If the wine is expected to be consumed shortly after bottling, treatment with metatartaric acid is an inexpensive means of establishing short-term tartrate stability. Metatartaric acid is produced by the formation of ester bonds between hydroxyl and acid groups of tartaric acid. The polymer is generated during prolonged heating of tartaric acid at 170°C. When added to wine, metatartaric acid restricts potassium bitartrate crystallization and interferes with the growth of calcium tartrate crystals. As metatartaric acid slowly hydrolyzes back to tartaric acid, the effect is temporary. At storage temperatures between 12° and 18°C, it may be effective for about 1 year. Because hydrolysis is temperature dependent, the stabilizing action of metatartaric acid quickly disappears above 20°C. The metatartaric acid is added just before bottling.

Calcium Tartrate Instability Instability caused by calcium tartrate is more difficult to control than that induced by potassium bitartrate. Fortunately, it is less common. Calcium-induced problems usually arise from the excessive use of calcium carbonate in deacidification, but they also may come from cement cooperage, filter pads, and fining agents.

Calcium tartrate stabilization is more difficult because precipitation is not activated by chilling. It may take many months for stability to develop spontaneously. Seeding with calcium tartrate crystals, simultaneously with calcium carbonate in deacidification, greatly enhances precipitation (Neradt, 1984). Because the formation of crystal nuclei requires more free energy than crystal growth, seeding circumvents the major limiting factor in the development of stability. A racemic mixture of calcium tartrate seed nuclei, containing both L and D isomers of tartaric acid, is preferred. The racemic mixture is about one-eighth as soluble as the naturally occurring L-tartrate salt. The slow conversion of the L-form to the D-form during aging is one of the major causes of the calcium tartrate instability in bottled wine. Because premature clarification removes "seed" crystals that promote crystallization, filtration of wines deacidified with calcium carbonate should be delayed until stability has been achieved. Crystal growth and precipitation occur optimally between 5° and 10°C. Protective colloids such as soluble proteins and tannins can restrict crystal nucleation, but they do not inhibit crystal growth (Postel, 1983).

Calcium content may be directly reduced through ion exchange. Because of the efficiency of ion removal, typically only part of the wine needs to be treated. The treated sample is then blended back into the main volume of the wine. Treating only a small portion of the wine minimizes the flavor loss often associated with the ion-exchange technique.

Other treatments showing promise are the addition of pectic and alginic acid colloids to restrict crystallization and keep calcium tartrate in solution (Wucherpfennig *et al.*, 1984).

Other Calcium Salt Instabilities Occasionally, crystals of calcium oxalate form in wine. The development occurs late, commonly after bottling. The redox potential of most wines stabilizes the complex formed between oxalic acid and metal ions such as iron. However, as the redox potential of the wine may eventually rise during aging, ferrous oxalate changes into the unstable ferric form. On dissociation, oxalic acid may bond with calcium, forming calcium oxalate crystals.

Oxalic acid is commonly derived from grape must, but small amounts may form from iron-induced structural changes in tartaric acid. Oxalic acid can be removed by blue fining early in maturation (Amerine *et al.*, 1980), but avoiding the development of high calcium levels in the wine is preferable.

Other potential troublesome sources of crystals are saccharic and mucic acids. Both are produced by the fungus *Botrytis cinerea* during grape infection and form insoluble calcium salts. The addition of calcium carbonate for bitartrate stability can induce their crystallization, precipitation, and separation before bottling.

PROTEIN STABILIZATION

Although less commonly a cause of wine rejection than crystal formation, protein haze can cause considerable economic loss in bottle returns. Protein haze results from the clumping of dissolved proteins into light-dispersing particles. Heating accelerates the process, as does reaction with tannins and heavy metals.

The majority of proteins suspended in wine have an

isoelectric point (p*I*) above the pH range of wine. The isoelectric point is the pH at which a protein is electrically neutral. Consequently, most soluble proteins in wine possess a net positive charge, generated by ionization of the amino groups of proteins to $-NH_3^+$ groups. The similar charge on proteins slows clumping, while Brownian movement and association (hydration) with water delay settling. However, adsorption, denaturation, or neutralization by cation exchange with fining agents can induce proteins to coalesce and produce a haze.

The issue of which proteins are primarily involved in haze production is still poorly understood. In addition, protein instability is only weakly correlated with total protein content. Recent studies suggest that proteins ranging between 12,000 and 30,000 daltons, and having a low p*I* values (4.0 to 6.0), may constitute the most significant unstable protein fraction (Hsu and Heatherbell, 1987). Some glycoproteins also appear to be involved. Smaller proteins may become involved by reacting with tannins, pectins, and metal ions.

A number of procedures have been developed to achieve protein stability. The most common involves the addition of bentonite (Fig. 8.3). Because of the abundance of soluble cations associated with bentonite, extensive exchange of ions can occur with ionized protein amino groups. By weakening the association with water, cations make the proteins more liable to coalesce and precipitate. Flocculation and precipitation are further enhanced by adsorption onto the negatively charged plates of bentonite. Sodium bentonite is preferred as it separates more readily into individual silicate plates in wine. This gives it the largest surface area of any clay and, thereby, the greatest potential for cation exchange and protein adsorption.

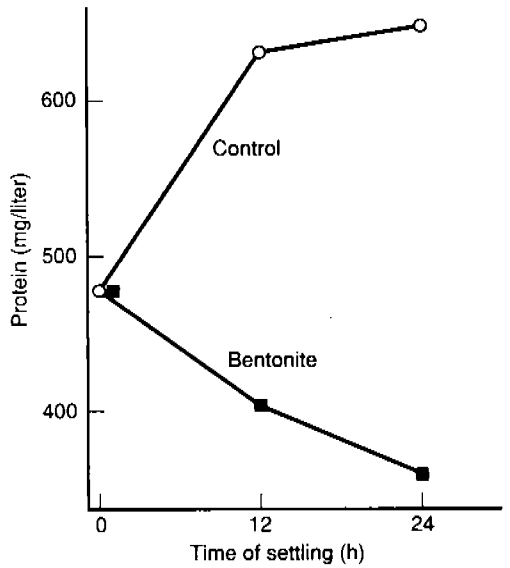


Figure 8.3 Total soluble protein in 'Gewürztraminer' must settled at 20°C for 24 hr in the presence or absence of 0.5 g/liter bentonite. (From Tyson *et al.*, 1982, reproduced by permission.)

Although negatively charged proteins bind less effectively to bentonite, they also less commonly induce haze formation. They generally adhere only to the edges of the clay plates, where positive charges tend to occur. All proteins, including those with a neutral charge, may associate with bentonite through weak hydrogen bonds.

Other fining agents, such as tannins, are occasionally used in lieu of bentonite. Addition of tannins is ill-advised with white wine, as it can leave an off-odor and generate an astringent mouth-feel. However, when immobilized in porous silicon dioxide, tannic acid causes minimal flavor modification or wine loss (Weetal *et al.*, 1984). Kieselsol, a colloidal suspension of silicon dioxide, has occasionally been used to remove proteins. Wines also may be protein stabilized through heat treatment, followed by filtration or centrifugation.

In a recent comparison of various protein stabilization tests, Dubourdieu *et al.* (1988) recommend exposure of wine samples to 80°C for 30 min. On cooling, the sample is observed for signs of haziness.

Recently, ultrafiltration has been investigated as an alternative to bentonite or other types of fining (Hsu *et al.*, 1987). It has the advantage of minimizing wine loss caused by sediment formation. Ultrafiltration also eliminates the need for a final "polishing," centrifugation, or filtration. Although generally applicable for white wines, ultrafiltration results in excessive color and flavor loss in red wines.

POLYSACCHARIDE REMOVAL AND STABILITY

Pectinaceous and other mucilaginous polysaccharides can cause difficulty during filtration, as well as induce haze formation. The polysaccharides act as "protective" colloids by binding with other colloidal materials, slowing or preventing their precipitation. Multiple hydrogen bonds formed between water molecules and the polysaccharides help them remain in suspension.

Pectin levels can be reduced by the addition of pectinase. Pectinase is an mixture of enzymes that breaks the pectin polymer down into simpler, noncolloidal, galacturonic acid subunits. Other grape-derived polysaccharides, such as arabinans, galactans, and arabinogalactans, have little effect on haziness or filtration and do not require specific removal. The same is true for the mannans produced by yeasts. In contrast, β-glucans present in botrytized juice can cause serious filtration problems even at low concentrations (Villettaz *et al.*, 1984). This is especially serious in highly alcoholic wines, where ethanol induces aggregation of the glucans. A Kieselsol/gelatin mixture is apparently effective in re-

moving these mucilaginous polymers. Alternately, the wine may be treated with a formulation of β-glucanases (Villettaz *et al.,* 1984). The enzymes hydrolzye the polymer, destroying both its protective colloidal action and filter plugging property.

TANNIN REMOVAL AND OXIDATIVE CASSE

Tannins may be directly and indirectly involved in haze development. On exposure to oxygen, tannins oxidize and polymerize into brown, light-diffracting colloids, causing **oxidative casse.** Polyphenol oxidases released from crushed grape cells speed the reactions, but slower nonenzymatic autooxidation reactions continue after enzyme inactivation (Fig. 6.8). Depending on the timing and degree of oxidation, tannin oxidation can result in a loss in color intensity or shift in hue, and it may enhance long-term color stability. Addition of sulfur dioxide limits oxidation through its joint antioxidant and antienzymatic properties. Moldy fruit contaminated with fungal polyphenol oxidases (laccases) are particularly susceptible to oxidative casse. Because laccases are poorly inactivated by sulfur dioxide, pasteurization may be the only convenient means of protecting the juice from excessive oxidation. Grapes free of fungal infection rarely develop oxidative casse. As the casse usually develops early during maturation and precipitates before bottling, it does not cause in-bottle clouding.

Chilling wine to achieve bitartrate stability may induce a protein/tannin haze. Filtration must occur while the wine is still cold, as the association between protein and tannin dissociates on warming. The removal of protein–tannin complexes enhances both tannin and protein stability.

Tannin stability is normally achieved by adding fining agents such as gelatin, egg albumin, and casein. The positive charge of the proteins attracts tannins, which are negatively charged. The interaction produces large protein–tannin complexes that settle out quickly and can be eliminated effectively by racking. The complexes may be removed earlier by filtration or centrifugation if required. The removal of excess tannins diminishes a major source of astringency, generates a smoother mouth-feel, reduces the likelihood of oxidative casse, and limits the formation of sediment following bottling.

With white wines, addition of PVPP (polyvinylpolypyrrolidone) is a particularly effective means of removing tannins. Ultrafiltration also may be used to remove undesired tannins and other polyphenolic compounds from white wine. Ultrafiltration is seldom used with red wines, as the filter simultaneously may remove important flavorants and anthocyanins.

Additional, though infrequent, sources of phenolic instability include oak chips or shavings used to rapidly develop oak character (Pocock *et al.,* 1984) and the accidental incorporation of excessive amounts of leaf material in the grape crush (Somers and Ziemelis, 1985). Both can generate in-bottle precipitation if the wine is bottled early, but they can be avoided by permitting sufficient time for spontaneous precipitation during maturation. The instability associated with oak chip use results from the overextraction of ellagic acid. The **phenolic deposit** produced consists of a fine precipitate composed of off-white to fawn-colored ellagic acid crystals. The **flavonol haze** associated with the presence of leaf material during the crushing of white grapes is produced by the formation of fine yellow quercetin crystals.

Many premium-quality red wines develop a **tannin sediment** during prolonged bottle aging. This potential source of haziness is typically not viewed as a fault by wine connoisseurs. Individuals who customarily consume aged wines know its origin and often consider it a sign of quality.

METAL CASSE STABILIZATION

A number of heavy metals form insoluble salts and induce additional forms of haziness (*casse*). Although occurring much less frequently than in the past, metal casse is still of concern to winemakers.

The most important metal ions involved in casse formation are iron (Fe^{3+}, Fe^{2+}) and copper (Cu^{2+}, Cu^{+}). They may be derived from grapes, soil contamination, fungicidal residues, or winery equipment. Most metal ions so derived are lost during fermentation by coprecipitation with yeast cells. Troublesome concentrations of metal contaminants usually are associated with pickup subsequent to vinification.

Corroded stainless steel, improperly soldered joints, unprotected copper or bronze piping, and tap fixtures are the prime sources of contamination. Additional sources may be fining and decoloring agents, such as gelatin, isinglass, and activated charcoal, as well as cement cooperage.

Ferric Casse Two forms of iron casse are known, white and blue (Fig. 8.4). **White casse** is most frequent in white wine and forms when soluble ferrous phosphate, $Fe_3(PO_4)_2$, is oxidized to insoluble ferric phosphate, $FePO_4$. The white haziness that results may be due to solely ferric phosphate or to a complex between it and soluble proteins. In red wines, the oxidation of ferrous ions (Fe^{2+}) to the ferric state (Fe^{3+}) can result in the formation of **blue casse.** Ferric ions form insoluble particles with anthocyanins and tannins. The oxidation of ferrous to ferric ions usually occurs when the wine is exposed to air. Sufficient oxygen may be absorbed during bottling to induce clouding in an unstable wine.

Ferric casse development is dependent on both the metallic content of the wine and its redox potential. Its

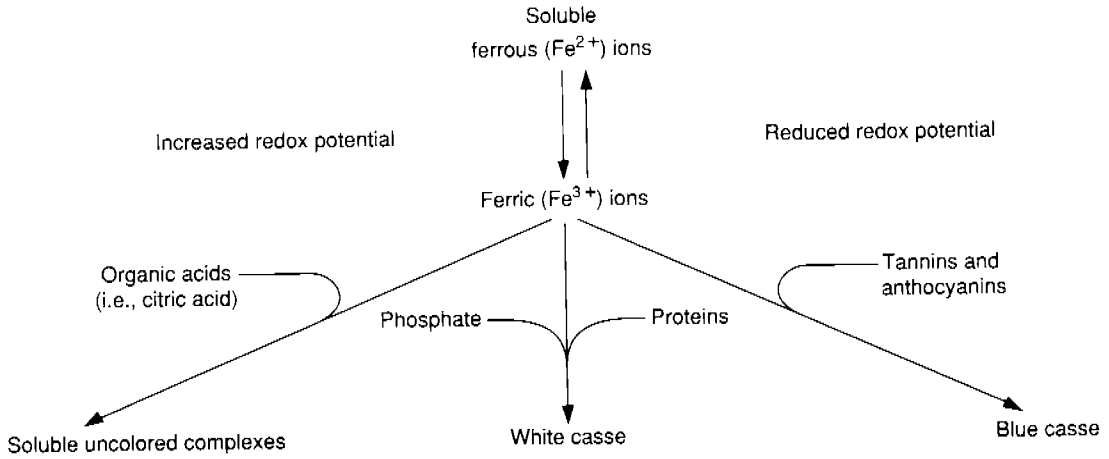


Figure 8.4 Iron-induced casse formation.

occurrence also is affected by pH, temperature, and the level of certain acids. White casse forms only below pH 3.6, and is generally suppressed at cool temperatures, whereas blue casse is accentuated at cold temperatures. The frequency of white casse increases sharply as the iron concentration rises above 15 to 20 mg/liter. Critical iron concentrations for blue casse production are more difficult to predict, as its occurrence is influenced markedly by the phosphate content of the wine and traces of copper (1 mg/liter). In addition, citric acid can chelate ferric and ferrous ions and reduce the effective (free) concentration in wine. Citric acid may be added to wine (~120 mg/liter) to limit the occurrence of ferric casse (Amerine and Joslyn, 1970).

Wines may be directly stabilized against ferric casse by iron removal. For example, the addition of phytates such as calcium phytate selectively removes iron ions. EDTA (ethylenediaminetetraacetic acid), pectinic acid, and alginic acid can be used to remove both iron and copper ions. Removal with ferrocyanide is probably the most efficient method, as it precipitates most metal ions, including iron, copper, lead, zinc, and magnesium. Addition as potassium ferrocyanide is known as **blue fining.** Blue fining is prohibied in many countries and is strictly controlled where it is permitted. As a buffered complex with bentonite, potassium ferrocyanide is sold as a proprietary formulation called Cufex. The Cufex formulation reduces the likelihood of toxic cyanide residues remaining in the wine after treatment. In either case, insoluble metal ferrocyanide complexes are removed by filtration.

Ferric casse may be equally controlled by the addition of agents that limit the flocculation of insoluble ferric complexes. Gum arabic acts in this manner. It functions as a protective colloid, restricting haze formation. Because gum arabic limits the clarification of colloidal material, it can only be safely applied after the wine has received all other stabilization procedures.

Copper Casse While iron casse forms as a result of exposure to oxygen, copper casse forms only in the absence of oxygen. It develops only after bottling and is associated with the decrease in redox potential that accompanies aging. Exposure to light speeds the reduction of copper, critical in casse development. Sulfur dioxide is important, if not essential, as a sulfur source in copper casse formation. In a series of incompletely understood reactions, involving the generation of hydrogen sulfide, cupric and cuprous sulfides may form. The sulfides produce a fine, reddish brown deposit, or they flocculate with proteins to form a reddish haze. Copper casse is particularly a problem in white wines, but it also can cause haziness in rosé wines. Wines with copper contents greater than 0.5 mg/liter are particularly susceptible to copper casse (Langhans and Schlotter, 1985).

MASQUE

Occasionally, a deposit called *masque* forms on the inner surface of bottles of sparkling wine. It results from the deposition of material formed by the interaction of albumin, used as a fining agent, and fatty acids (Maujean *et al.*, 1978). Riddling and disgorging used to remove yeast sediment does not remove *masque*. *Masque* affects only traditionally produced (*méthode champenoise*) sparkling wines. With this technique, the bottle used for the second fermentation is the same as that in which the wine is sold.

MICROBIAL STABILIZATION

Microbial stability is not necessarily synonymous with microbial sterility. At bottling, wines may contain a considerable number of viable, but dormant, microor-

ganisms. Under most situations, they cause no stability or sensory problems.

The simplest procedure conferring limited microbial stability is racking. Racking removes cells that have fallen out of the wine by flocculation or coprecipitation with tannins and proteins. The sediment includes both viable and nonviable microorganisms. The latter slowly undergo autolysis and release nutrients back into the wine. Cold temperatures help to maintain microbial viability but retard or prevent growth.

For long-term microbial stability, especially with sweet wines, the addition of antimicrobial compounds or sterilization is required. The antimicrobial agent most frequently used is sulfur dioxide. It may be added at various times during wine production, but almost always after fermentation. Concentrations of 0.8 to 1.5 mg/liter molecular sulfur dioxide inhibit the growth of most yeasts and bacteria. The total sulfur dioxide content required to maintain a desirable level of molecular SO_2 depends on the pH of the wine and the number of sulfur-binding compounds (see Chapter 6).

At present, no other permitted wine additive possesses the wide-spectrum antimicrobial activity of sulfur dioxide. Sorbic acid is an effective inhibitor of several yeasts but not others, such as *Zygosaccharomyces bailii* (Rankine and Pilone, 1973). In addition, sorbic acid can be metabolized by lactic acid bacteria, generating a strong geranium-like odor. Benzoic acid and sodium benzoate were once employed as yeast inhibitors, but their general ineffectiveness and taste modification have essentially eliminated their use. If used just before bottling, dimethyl dicarbonate (DMDC) can effectively sterilize the wine without producing sensory defects or leaving a residue. DMDC decomposes rapidly to carbon dioxide and methanol (Calisto, 1990). In the absence of sulfur dioxide or DMDC, bottled wines can be securely stabilized against microbial growth only by physical means, namely, pasteurization and filter sterilization.

Pasteurization is the older of the two techniques. It has the advantage of inactivating polyphenol oxidases (laccases), which are little affected by the concentrations of sulfur dioxide commonly used. It also facilitates protein and copper casse stabilization by denaturing and precipitating colloidal proteins. Although pasteurization may generate increased amounts of "protective" colloids, cause slight decolorization, and modify wine fragrance, it does not influence the aging process of phenols in wine (Somers and Evans, 1986).

Because the low pH and ethanol content of wine markedly depress the thermal resistance of yeasts and bacteria, heating for shorter periods or at lower temperatures than typical is recommended by Barillère *et al.* (1983). Barillère *et al.* indicate that about 3 min at 60°C should be sufficient for a wine at 11% ethanol. Flash pasteurization at 80°C usually requires only a few seconds. Sulfur dioxide reduces still further the need for heating, as high temperatures markedly increase the proportion of free SO_2 in wine. Although pasteurization destroys most microbes, it does not inactivate the endospores of *Bacillus* species that occasionally cause wine spoilage.

Partially because of the complexities of establishing the most appropriate time and temperature conditions for pasteurization, membrane filters have replaced pasteurization in most situations. Filters also result in few physical or chemical disruptions to the sensory characteristics of wine. Membrane filters with a pore size of 0.45 μm or less are adequate for juice and wine filtration.

Wine sterilization requires the simultaneous use of measures to avoid recontamination. This involves sterilizing all parts of the bottling line and the use of sterile bottles and corks.

Sulfur dioxide is commonly added before wines are pasteurized or sterile filtered to confer protection against oxidation.

Fining

Fining is commonly used to accelerate the spontaneous precipitation of suspended material in wine. Fining agents bind to or adsorb particulate matter. The aggregates formed are generally sufficiently large to precipitate quickly. If not, removal can be achieved by centrifugation or filtration. In addition to facilitating clarification, fining may help stabilize wines against haze formation by removing compounds involved in haze production.

Because fining is an aid to, not a replacement for, spontaneous stabilization, it should be used only to the degree necessary. It is important to avoid sensory disruption by minimizing changes to the chemical and physical balance of the wine. Figure 8.5 illustrates the potential of fining agents to produce aroma changes through the selective removal of aromatic compounds. Fining also should be conducted as quickly as possible to avoid oxidation. Tests to determine the need for fining are discussed in most enological references, for example, Zoecklein *et al.* (1990). A brief description of the primary fining agents is given below.

ACTIVATED CHARCOAL (CARBON)

Activated charcoal is used primarily to decolorize wine and remove off-odors. Different preparations may be required for each application. The large surface area (between 500 and 1500 m^2/g) and electrical charge allow activated charcoal to adsorb a wide range of compounds effectively. Although this is useful in removing mercaptan off-odors, it simultaneously removes desir-

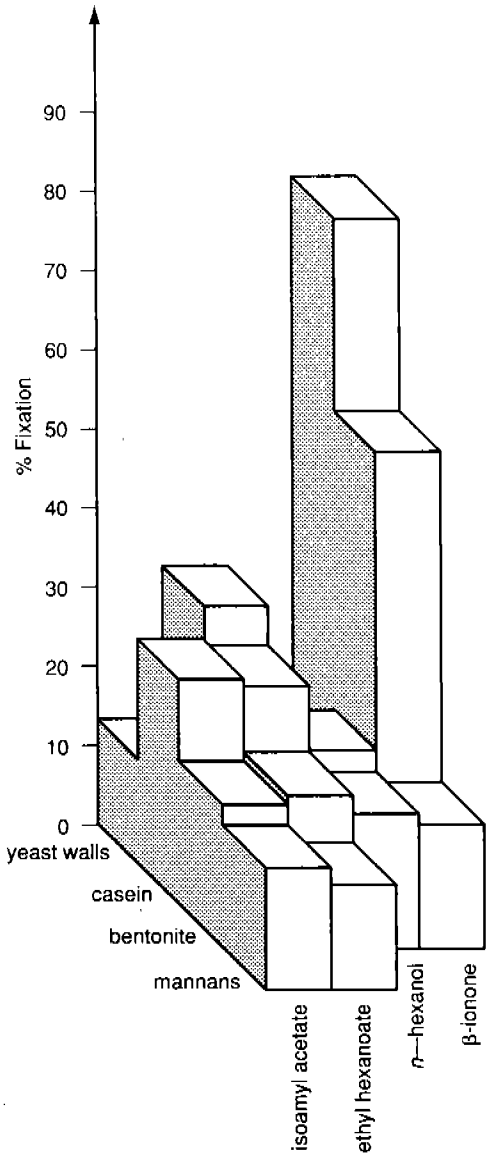


Figure 8.5 Percent removal of several aromatic compounds during fining with bentonite or 1% casein, mannans, or yeast cell walls. (Reprinted with permission from Voilley *et al.*, 1990. Copyright 1990 American Chemical Society.)

able flavor compounds. It also may give the treated wine an atypical odor. Furthermore, activated charcoal has an oxidizing property. As a consequence, ascorbic acid may be incorporated along with the activated charcoal. Although valuable in some situations, activated charcoal must be used with caution.

ALBUMIN

Egg white has long been used in fining wines. The active ingredient is the protein albumin. As with other protein fining agents, albumin is primarily employed to remove excess tannins. Peptide linkages of the albumin form hydrogen bonds with hydroxyl groups on tannins. The opposing charges on the molecules favor the formation of large protein–tannin aggregates.

Egg albumin is presently available in pure form. Use of pure albumin avoids the necessity of adding sodium chloride to whipped egg whites to solubilize the albumins.

BENTONITE

Bentonite is a form of montmorillonite clay widely used as a fining agent. It is used in clarifying juice and wines, in removing heat-unstable proteins, and in deterring the development of copper casse. Depending on the objectives of the winemaker, the ability of bentonite to induce partial decolorization and remove nutrients such as amino acids is either an advantage or disadvantage. Together with other fining agents, such as tannins and casein, bentonite can speed the settling of particulate matter. It also can correct for the addition of excessive amounts of proteinaceous fining agents, by inducing their precipitation. Because bentonite settles out well and is easily filtered, it is one of the few fining agents that does not itself create a stability or clarification problem. Bentonite also has comparatively little effect on the sensory properties of the treated wine. The major drawbacks are promotion of color loss from red wines and a tendency to produce voluminous sediment. The latter can cause considerable wine loss during racking.

The bentonite often preferred in the United States is Wyoming bentonite. Because the predominant cation is monovalent (sodium), the particles swell readily in water and separate into separate sheets of aluminum silicate. The sheets are about 1 nm thick and 500 nm wide. The separation of the sheets provides an immense surface area over which cation exchange, adsorption, and hydrogen bonding can occur. When fully expanded, sodium bentonite has a surface area of about 700 to 800 m^2/g. Calcium bentonite is less commonly used as it tends to clump on swelling and provides less surface area for fining. Nevertheless, it has the advantage of producing a heavier sediment that is easier to remove and does not liberate sodium ions into the wine.

The net negative charge of bentonite attracts positively charged proteins. The latter are neutralized by cation exchange with the clay, and adsorption to the clay results in flocculation and settling as clay–protein complexes.

CASEIN

Casein is the major protein found in milk. In association with sodium or potassium ions, it forms a soluble caseinate salt that easily dissolves in wine. In wine, the salt dissociates and insoluble caseinate is released. The

caseinate adsorbs and removes negatively charged particles as it settles out. Casein finds its primary use as a decolorant in white wines. It also has some deodorizing properties.

GELATIN

Gelatin is a soluble albuminlike protein derived from the tissues of animals after prolonged boiling. As a result the product loses some of its gelling properties but becomes a more effective fining agent.

Gelatin is employed primarily to remove excess tannins from wines. When gelatin is added to white wine, there is a risk of leaving a gelatin-derived haze. This may be avoided by the simultaneous addition of flavorless tannins, Kieselsol, or other protein-binding agents. These materials assist in the formation of the fine meshwork of gelatin fibers that removes tannins and other negatively charged particles. Excessive fining with gelatin can result in undesirable color removal in red wine.

KIESELSOL

Kieselsol is an aqueous suspension of silicon dioxide. Because it is available in both positively and negatively charged forms, Kieselsol can be formulated to adsorb and remove both positively and negatively charged colloidal material. It is commonly used to remove bitter polyphenolic compounds from white wine. Combined with gelatin, it is effective in clarifying wines containing mucilaginous protective colloids. Kieselsol tends to produce a less voluminous sediment than bentonite and removes little color from red wines.

ISINGLASS

Isinglass is derived from proteins extracted from the air bladder of fish, notably sturgeons. Similar to most other proteinaceous fining agents, isinglass is primarily used to remove tannins. Because it is less subject to overfining, isinglass requires less added tannin than gelatin to function in fining white wine. Regrettably, it produces a voluminous sediment that tends to plug filters.

POLYVINYLPOLYPYRROLIDONE

Polyvinylpolypyrrolidone (PVPP) is a resinous polymer that acts analogously to proteins in binding tannins. It is very efficient in removing brown pigments from white wines. It functions well at cool temperatures and precipitates spontaneously. PVPP can be isolated from the sediment, purified, and reused.

TANNIN

Insect galls on oak leaves are the typical source of tannins used in fining. Tannins are commonly used in combination with gelatin. The tannin/gelatin mixture forms a delicate meshwork that sweeps colloidal proteins out of wine. Tannins in the mesh join with soluble proteins in the wine to form both weak and strong chemical bonds. Nonionized carboxyl and hydroxyl groups of the tannins establish weak hydrogen bonds with peptide linkages of the proteins, while quinone groups form covalent bonds with the amino and sulfur groups of proteins. The latter produce strong, stable links between soluble proteins and the tannin/protein meshwork.

Clarification

In contrast to fining, clarification involves only physical means of removing suspended particulate matter. As such, it usually follows fining, though an initial clarification often occurs before fermenting white juice. Juice clarification often improves flavor development in white wine, and it helps to prevent microbial spoilage following fermentation. After fermentation, preliminary clarification by racking removes sedimented material. Subsequently, finer material may be removed by centrifugation or filtration.

RACKING

Until the twentieth century, racking and fining were essentially the only methods available to facilitate wine clarification. Presently, racking can vary from manually decanting wine from barrel to barrel to highly sophisticated, automated, tank-to-tank transfers. In all cases, separation from the sediment (**lees**) occurs with minimal agitation to avoid resuspending the particulate matter. Decanting stops when unavoidable turbulence makes the wine cloudy. The residue may be filtered to retrieve wine otherwise lost with the lees. Racking is generally more effective in clarifying wine matured in small cooperage than in large tanks.

The first racking is conventionally done several weeks after alcoholic fermentation. Racking may be delayed if malolactic fermentation is desired until the process has come to completion. Racking also may be delayed to permit prolonged lees contact, which is considered important to flavor development in some wines. The delay permits yeast autolysis to occur and favors the release of cell-bound compounds, such as ethyl octanoate, ethyl decanoate, amino acids, and cell wall mannoproteins. Prolonged lees contact is often associated with periodic manual stirring. This may provide sufficient aeration to limit the production of reduced-sulfur off-odors.

By the first racking, most of the yeast, bacterial, and grape cell fragments have settled out of the wine. Subsequent rackings remove most of the residual microbial population, along with precipitated tannins, pigments, and crystalline material. Later rackings also remove sediment induced by fining. If sufficient time is provided,

racking and fining can produce stable, crystal clear wines. However, the trend to early bottling, within a few weeks or months of fermentation, provides insufficient time for racking and spontaneous precipitation to generate a stable, clear wine. Consequently, centrifugation and filtration are used to achieve the required level of clarity.

In addition to aiding clarification, racking plays several additional valuable roles in wine maturation. By removing microbial cells and other sources of nutrients, racking enhances microbial stability. The transfer process also disrupts stratification that may develop within the wine. This is particularly important in large storage tanks, where stratification can lead to variation in redox potential and rates of aging throughout the wine. Racking also removes the primary source of such reduced-sulfur taints as hydrogen sulfide and mercaptans, which may form under low redox potentials that develop in thick layers of lees.

Aeration and liberation of carbon dioxide result as a consequence of racking. Modest oxygen uptake during racking assists color stability in red wine, but its value in white wine maturation is more controversial. As noted earlier, slight aeration appears to be beneficial in white wines matured on the lees but is avoided otherwise. Oxygen exposure can be minimized with modern automatic pumping systems using carbon dioxide or nitrogen sparging. The turbulence generated during pumping and filling helps to release carbon dioxide found in its supersaturated state following fermentation. The escape is essential for the wine to lose its slight *pétillance* before bottling. If necessary, adjustment with additional sulfur dioxide occurs during racking.

The number of rackings recommended varies considerably from region to region, depending on empirically established norms. The size of the cooperage also is a determining factor. The larger the storage vessel, the more frequent the required racking. This is necessary to avoid the development of a thick layer that is conducive to off-odor production.

The method of racking depends largely on the size of the cooperage and the economics of manual versus mechanical transfer. Manual draining by gravity, or with a simple hand pump, is adequate where volumes are small and labor costs low. For most large wineries, however, manual racking would be prohibitively expensive, in terms of both labor and time. Mechanical pumping is the only reasonable option. Also, if aeration and sulfiting are deemed desirable, they can be controlled more precisely through mechanical rather than manual racking.

CENTRIFUGATION

Centrifugation employs rotation at high speeds to expedite settling. It is equivalent to spontaneous sedimentation, but occurs within minutes rather than months. It often replaces racking when early bottling is desired. Centrifugation also is useful when the wine is heavily laden with particulate matter. Centrifugation is much more efficient at removing large amounts of particulates compared to plate filters. Highly turbid wines are prone to off-odor development if permitted to clarify spontaneously.

Blanketing the wine with inert gases has minimized a former liability of centrifugation, namely, oxidation. Automation combined with continuous centrifugation has improved the efficiency and economy of the process to such an extent that centrifugation is often the preferred clarification technique.

FILTRATION

Filtration involves the physical retention of material on, or within, a fibrous or porous material. Depending on the pore size, filtration removes coarse particles with diameters larger than 100 μm down to molecules and ions with diameters less than 10^{-3} μm. However, the greater the retentive property of the filter, the greater is the likelihood of rapid plugging. As a consequence, filtration typically follows preliminary clarification by fining, centrifugation, or racking. This is especially important when using membrane sterilization or ultrafiltration.

With the development of new filters and support systems, filtration is currently classified into four categories. Conventional filtration employs depth-type fibrous filters that remove particles down to about 1 μm in diameter. Other filtration techniques involve membranes containing crevices, channels, or pores that cross the membrane. Depending on the size range of the membrane perforations, the sieving action is termed microfiltration, ultrafiltration, or reverse osmosis/dialysis. Ultrafiltration and microfiltration usually are differentiated on the basis of nominal pore size (0.2–0.05 and 1.0–0.1 μm, respectively). Microfiltration is used primarily to remove fine particles or in sterilization. Ultrafiltration may be used to remove macromolecules and colloidal materials. Reverse osmosis and dialysis are utilized to remove or concentrate low molecular weight molecules or ions. Dialysis involves the same principle (diffusion) as reverse osmosis discussed earlier in the chapter, but it does not use pressure to reverse the direction of diffusion. Electrodialysis uses an electrical differential across the membrane to influence the diffusion of charged particles.

Filtration primarily acts by blocking the passage of material larger than the maximum pore size of the filter (Fig. 8.6). However, as material smaller than the smallest perforations are often retained by a filter, other principles are involved. Surface adsorption by electrical at-

size or molecular weight. Such **cartridge filters** contain some of the nonplugging features of depth filters and offer the control of particle size retention of conventional membrane filters. These polypropylene filters are resistant to most chemical reagents. This allows them to be cleansed and used repeatedly. Such new developments may reduce, if not eliminate, the need to conduct filterability tests prior to sterile filtration. Filterability tests are fully discussed by Peleg *et al.* (1979) and de la Garza and Boulden (1984).

Microfiltration is extensively used to sterilize wines. Microfiltration avoids possible flavor modification occasionally associated with pasteurization.

Ultrafiltration has been used to a limited extent in protein stabilization in wine. While effectively removing most colloidal material from wine, ultrafiltration also can remove important pigments and tannins from red wines (Fig. 8.10). Its use with white wines appears not to produce unacceptable flavor loss (Flores *et al.*, 1991). The benefits and liabilities of ultrafiltration are still under active investigation.

Aging

The tendency of wine to improve during aging is one of its most fascinating properties. Regrettably, most wines improve only for a few months to years before showing irreversible loss in quality. In contrast, red wines produced from varieties such as 'Cabernet Sauvignon,' 'Tempranillo,' 'Nebbiolo,' 'Pinot noir,' and 'Syrah' may continue to improve in flavor and subtlety for decades. In addition, white wines produced from varieties such as 'Riesling,' 'Chardonnay,' 'Sauvignon blanc,' and 'Viura' also show excellent aging potential.

Quality loss is commonly explained as a dissipation of the fresh, fruity bouquet, along with any aroma donated by the grape. Wines noted for continued improvement typically show similar aromatic losses, but they gain in **aged bouquet.** Aging is considered desirable when the aged bouquet, subtle flavor, and smooth texture more than compensate for the fading varietal and fruity character of the young wine.

Only since the early 1980s have sufficiently precise

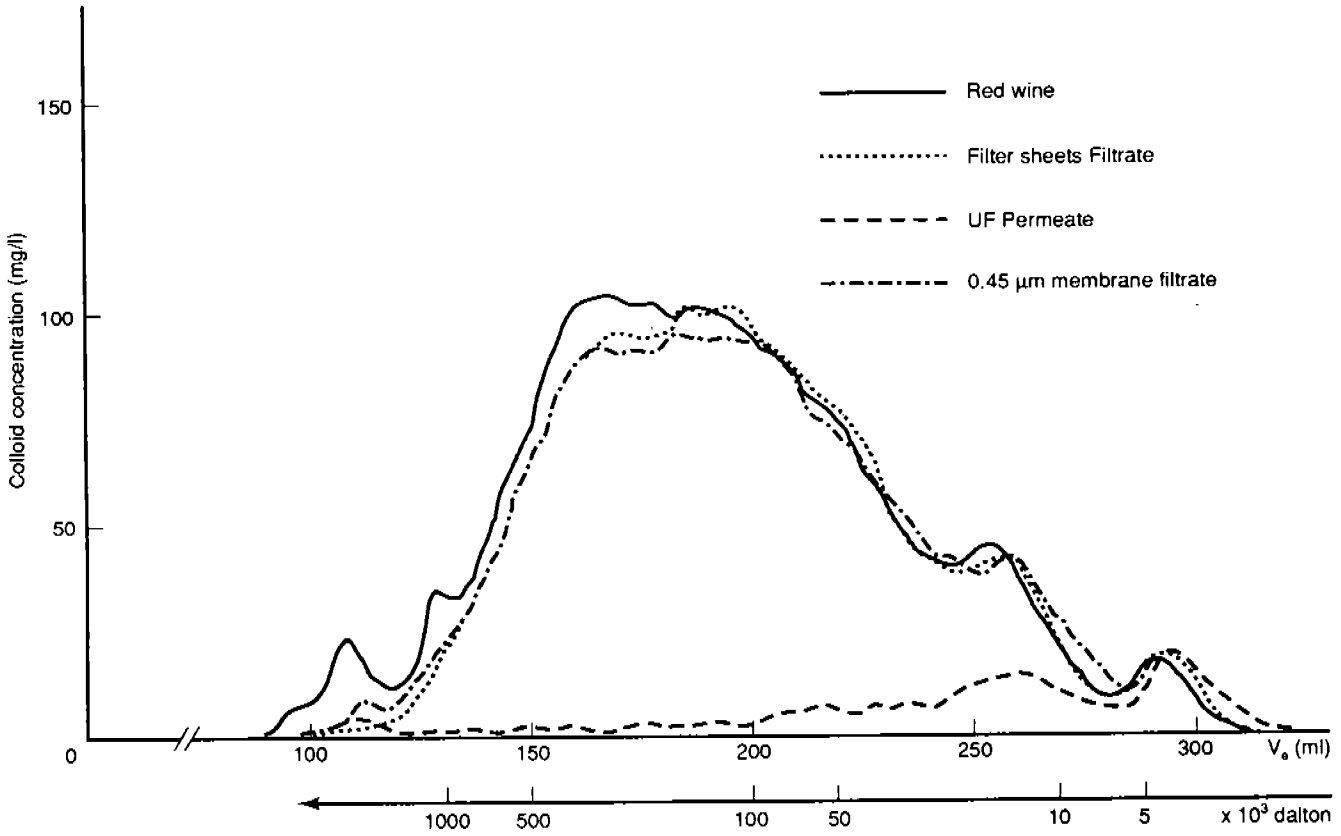


Figure 8.10 Removal of colloids from red wine during different filtration procedures, expressed as elution volumes (V_e, in ml) and molecular mass (daltons) of the various colloidal fractions. UF, Ultrafiltration. (From Cattaruzza *et al.*, 1987, reproduced by permission.)

analytical tools become available to begin unraveling the nature of wine aging. However, as aging has been studied in only a few wines, caution must be used in extrapolating the findings to other wines. Currently, more is known about why most wines decline in quality after several years than why a few wines retain or improve in character for decades.

Knowledge of how wines age, and how the effects of aging might be directed, is important to all involved or interested in wine. At the simplest, quality loss can adversely affect shelf life and the financial return to the producer. At the other end of the spectrum, the prestige connected with long aging potential adds greatly to the desirability and value of a wine to connoisseurs. It also permits consumers to participate through the conditions and duration of aging they permit. Because the factors affecting aging are poorly understood, a mystique is often associated with vineyards and varieties making wines that age well.

Aging is occasionally considered to consist of two phases. The first, called **maturation,** refers to changes that occur between alcoholic fermentation and bottling. Although maturation often lasts from 6 to 24 months, it may continue for decades. During maturation, the wine may undergo malolactic fermentation, be stored in oak cooperage, be racked several times, and be treated to one or more clarification techniques. During racking and clarification, wines may absorb about 40 ml O_2 per year, an amount insufficient to give the wine a noticeable oxidized character. Only in some fortified wines is obvious oxidation an important component of maturation.

The second phase of aging commences with bottling. Because this phase occurs essentially in the absence of oxygen, it has been called **reductive aging.** This contrasts with **oxidative aging,** an alternative term for maturation occasionally used for the aging of some fortified wines.

Effects of Aging

Many age-related changes in wine chemistry have been noted. As with other aspects of wine chemistry, determining the significance of the changes is more difficult than simply detecting them. To establish significance, it is necessary to show that the changes detectably influence sensory perception. Because most chemicals occur at concentrations below the sensory threshold, most changes affect neither wine flavor nor the development of an aged bouquet.

APPEARANCE

One of the most obvious changes to occur during aging is a color shift toward brown. Red wines initially deepen in color after fermentation, but subsequently become lighter and take on a brickish hue. Decreased color intensity and browning are indicated, respectively, by a drop in optical density and a shift in the absorption spectrum toward the blue (Fig. 8.11). Such changes are often measured as a ratio of the optical density measurements at 520 and 420 nm. High 520/420 nm values indicate a red color, while low values indicate a brown color. In contrast, white wines darken in color and take on yellow, gold, and eventually brown shades during aging.

Although little studied in white wine, color change has been extensively investigated in red wines. Nevertheless, no consensus has been reached about the relative importance of the mechanisms involved. While small amounts of acetaldehyde produced during limited aeration enhance the polymerization of anthocyanins and flavonoid tannins (Ribéreau-Gayon and Glories, 1987), polymerization also occurs directly under anaerobic, acid-catalyzed conditions (Somers and Evans, 1986). Because it occurs throughout the wine, direct polymerization may be more significant. Since temperature markedly affects the rate of direct polymerization, mild heating has been recommended as an alternative to aeration for color stabilization (Somers and Pocock, 1990). Aeration has the risk of activating dormant acetic acid bacteria and increasing volatile acidity (acetic acid and its esters).

The origin of the shift in color in white wines is poorly understood. It probably involves structural modification in existing pigments or their formation by one or more of the following processes: the slow oxidation of grape and oak phenols and related compounds, metal ion-induced modifications in galacturonic acid, Maillard reactions between sugars and amino acids, and sugar caramelization.

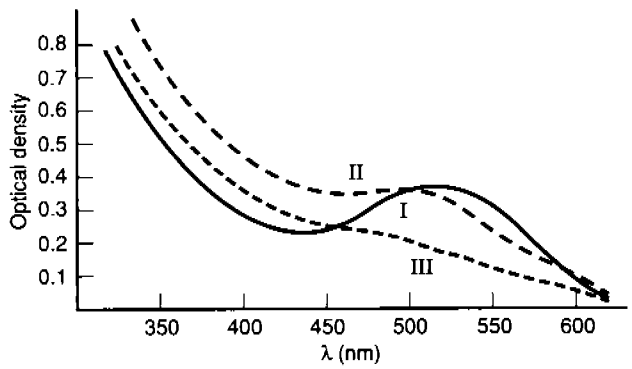


Figure 8.11 Absorption spectra of three red wines of different ages. I, One year old; II, 10 years old; III, 50 years old. (From Ribéreau-Gayon, 1986, reproduced by permission.)

TASTE AND MOUTH-FEEL SENSATIONS

During aging, glucose and fructose may react with other compounds and undergo structural rearrangement. Nevertheless, these reactions do not appear to occur to a degree sufficient to affect perceptible sweetness. In contrast, aging can induce small losses in total acidity. The mellowing of taste that may result probably comes from the formation of esters and the precipitation of acids. Esterification involves the removal of carboxyl groups involved in the sensation of sourness. Slow deacidification also can result from the isomerization of the natural L to the D form of tartaric acid. The racemic mixture so generated is less soluble than the L form, and this is one of the origins of tartrate instability in wine. Isomerization also results in forming racemic mixtures of free L- and D-amino acids (Chaves das Neves *et al.*, 1990). The potential significance of the toxicity of the D-amino acids is unknown.

Important changes also occur in the bitter and astringent sensations of red wines. The best understood of these reactions is the polymerization of tannins with anthocyanins. Their eventual precipitation results in a decline in bitterness and astringency. However, the initial effect may be an increase in astringency. This results from the greater astringency of medium-sized tannin polymers. The reaction of both hydrolyzable and condensed tannins with proteins leads to additional loss in bitter, astringent compounds. The hydrolytic breakdown of hydrolyzable tannins further reduces astringency but may increase bitterness.

FRAGRANCE

Whereas most studies on aging in red wines have concentrated on color stability, most research on aging in white wines has focused on fragrance modification. Flavor loss in most white wines is associated with changes in the ester content. Other sources of reduced fragrance involve structural rearrangements in terpenes.

Loss of Aroma and Fermentation Bouquet Esters produced during fermentation generate much of the fresh, fruity character of young white wines. Esters formed from acetic acid and higher alcohols, such as isoamyl and isobutyl acetates, are particularly important in this regard. Because yeasts produce and release more of these esters than the equilibrium in wine permits, the esters tend to hydrolyze back to the corresponding acids and alcohols. Thus, the fruity aspect donated by the esters fades with time.

Concurrent with the hydrolytic breakdown of acetate esters is the synthesis of certain ethyl esters. These form slowly and nonenzymatically between ethanol and the primary fixed acids of wine. Synthesis of diethyl succinate is particularly marked (Fig. 8.12). However, ethyl

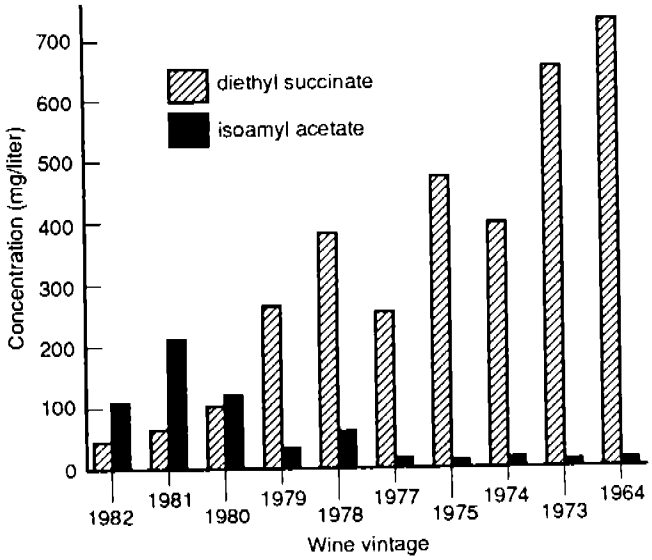


Figure 8.12 Examples of the influence of wine age on the concentration of esters, namely, acetate esters (isoamyl acetate, cross-hatching) and ethanol esters (dithyl succinate, hatching). (Data from Rapp and Güntert, 1986, Elsevier Science Publishers.)

esters probably play little role in bouquet development as they have low volatilities and nondistinctive odors. Their production may be more important in the mellowing wine acidity noted above (Edwards *et al.*, 1985).

A third class of esters is based on ethanol and carboxylic acids. The level of these esters remains stable or increases slightly during aging. Those with shorter hydrocarbon chains, such as butanoate, hexanoate, and octanoate ethyl esters, are somewhat fruity in character. As the hydrocarbon chain becomes longer, the odor shifts to being soapy and finally lardlike. Although important to wine fragrance, the significance of carboxylic acid esters to the development of an aged bouquet is unclear.

Another group of important flavorants that change during aging are the terpenes. The importance of terpenes to the aroma of Muscat and similar varieties has already been mentioned (Chapter 6). As with esters, changes in terpenes affect both the quantitative and qualitative aspects of wine fragrance.

The total concentration of monoterpene alcohols falls markedly during aging. The decline in geraniol, linalool, and citronellol is especially marked and can result in noticeable losses in floral character. For example, the linalool content of 'Riesling' wines can fall by 80% within 3 years. In contrast, the concentrations of linalool oxides, nerol oxide, hotrienol, and α-terpineol increase. Rapp and Güntert (1986) have proposed a reaction scheme for changes in monoterpene alcohols during aging. Most compounds generated have higher perception thresholds than do the terpene progenitors. The oxide

terpene derivatives also have qualitatively different odors. For example, α-terpineol has a musty, pinelike odor, whereas its precursor linalool has a floral aspect. As a group, the four isomeric linalool oxides have an eucalyptus aspect. Terpene-related, heterocyclic oxygen compounds also develop during aging, but the sensory significance of most of these is unknown.

Some of the loss in aromatic terpenes associated with aging may be offset by the slow release of monoterpenes chemically bound in nonaromatic forms. A variable portion of the monoterpene content of wines (and grapes) occurs as nonvolatile glycosides. Terpene glycosides tend to hydrolyze slowly under acidic wine conditions (Gunata *et al.*, 1986). In addition, minor quantities of some monoterpene oxides, such as 2,6,6-trimethyl-2-vinyltetrahydropyran and the 2,2-dimethyl-5-(1-methylpropenyl)tetrahydrofuran isomers, may participate in the cineolelike fragrance of aged 'Riesling' wines (Simpson and Miller, 1983).

Origin of Bottle-Aged Bouquet Presently, three groups of compounds appear to be significantly involved in the generation of a bottle-aged bouquet. These include compounds derived from norisoprenoid precursors and related diterpenes, residual carbohydrates, and reduced-sulfur compounds.

Of isoprenoid degradation products, vitispirane and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) appear to be the most potentially important. The two isomers of vitispirane have qualitatively different odors. However, as they occur in concentrations at or below the detection thresholds, the sensory significance of vitispiranes is doubtful. In contrast, the concentration of TDN seems sufficient to play a meaningful role in bouquet development in wines from varieties such as 'Riesling.' TDN also has been found in some red wines. Because TDN has a kerosenelike odor, accumulation considerably above the threshold value may be undesirable. The presence of glycosides and aglycones, precursors of TDN and vitispirane, has been suggested as an indicator for timing grape harvest to achieve an optimal aging potential (Winterhalter *et al.*, 1990). Other isoprenoid degradation products, such as theaspirane, ionene (a 1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene isomer), and damascenone, appear little involved in the development of bottle bouquet. For example, the concentration of the roselike damascenone declines during aging.

Carbohydrate degradation occurs rapidly during the baking of wines such as Madeira and baked sherries. Equivalent acid-catalyzed dehydration reactions occur much more slowly at cellar temperatures. For example, the caramellike 2-furfural shows a marked increase during aging (Table 8.1). The sensory significance of other degradation products, such as 2-acetylfuran, ethyl 2-furoate, 5-(hydroxymethyl)-2-furaldehyde, 2-formylpyrrole, and levulinic acid, is unknown. The fruity, slightly pungent ethyl ether, 2-(ethoxymethyl) furan, has been found to form on aging of 'Sangiovese' wine (Bertuccioli and Viani, 1976). This suggests that etherification of Maillard-generated alcohols may play a role in development of an aged bouquet.

The concentration of a number of reduced-sulfur compounds may change during aging. Of these, only one occasionally has been correlated with the development of a desirable aged bouquet, namely, dimethyl sulfide. Addition of 20 mg/liter dimethyl sulfide to wines containing 8 to 15 mg/liter dimethyl sulfide enhanced the flavor score of the wines (Spedding and Raut, 1982). Higher concentrations (≥40 mg/liter) were considered detrimental. By itself, dimethyl sulfide has a shrimplike odor.

ADDITIONAL CHANGES

A number of age-related changes develop in sherries that generally do not occur in table wines. Notable are increases in the concentrations of aldehydes and acetals,

Table 8.1 Changes in Aroma Composition from Carbohydrate Decomposition during Aging of a 'Riesling' Wine[a, b]

	Year					
Substance from carbohydrate degradation	1982	1978	1973	1964	1976 (frozen)	1976 (cellar stored)
2-Furfural	4.1	13.9	39.1	44.6	2.2	27.1
2-Acetylfuran	—	—	0.5	0.6	0.1	0.5
Furan-2-carbonic acid ethyl ester	0.4	0.6	2.4	2.8	0.7	2.0
2-Formylpyrrole	—	2.4	7.5	5.2	0.4	1.9
5-Hydroxymethylfurfural (HMF)	—	—	1.0	2.2	—	0.5

[a] From Rappand Güntert (1986), reproduced by permission.
[b] Relative peak height on gas chromatogram (mm).

which develop under the oxidizing conditions prevalent during sherry maturation. In table wines, the aldehyde concentration generally declines after bottling. Correspondingly, aldehyde-derived acetals do not accumulate during aging. An exception involves Tokaji Aszú wines that are often exposed to oxidizing conditions during their maturation (Schreier *et al.*, 1976).

Wines stored in oak slowly amass products extracted from the wood. A discussion of these products, and their significance, is given below.

Structural rearrangements of the main, fixed acids in wine occur during aging. For example, tartaric acid may give rise to oxalic acid and citric acid to citramalic acid via decarboxylation. The sensory significance of such changes is unknown.

During aging, a marked increase occurs in the concentration of abscisic acid. Although abscisic acid is important as a growth regulator in higher plants, its generation in wine is probably purely coincidental. Its production has no known sensory significance.

The concentration of several groups of compounds are little affected by aging, notably higher alcohols and lactones.

Factors Affecting Aging

Several years ago there was active interest in accelerating the aging of wine (Singleton, 1962). Subsequently, interest in the topic has waned. Accelerated aging simulates some of the changes of prolonged aging but generally does not generate the desired subtle complexity of spontaneously bottle-aged wines.

OXYGEN

Some wines are much more sensitive to oxidation than others. Currently, a precise explanation of these differences is not possible. However, variations in the concentration and types of phenols and polyphenol oxidases are undoubtedly important. For example, the most prevalent phenol in white wines is caftaric acid, an ester between caffeic and tartaric acids. On hydrolysis, caffeic acid (an *o*-diphenol) could significantly increase oxidative browning (Cillers and Singleton, 1990). In addition, red grape varieties particularly susceptible to oxidation, such as 'Grenache,' also contain high concentrations of caftaric acid and derivatives. Another important factor affecting the oxidation potential of wine is the pH. As the pH rises, the proportion of phenols in the highly reactive phenolate state increases, enhancing potential oxidation. Finally, differences in anthocyanin composition, the respective reactivity of the various anthocyanins, and their polymerization with tannins greatly affect their susceptibility to oxidation.

Because oxygen prevents the development of a reductive bottle bouquet, considerable effort is expended limiting its access to bottled wine. In addition, oxygen can destroy or mask fruity and varietal odors and generate an oxidized (aldehyde) odor. To assure that bottled wine remains under reductive, anaerobic conditions, good quality closures (corks or pilfer-proof screw caps) should be used and oxygen exposure during bottling minimized.

TEMPERATURE

To avoid loosening the seal between the cork and the bottle, wine needs to be stored under relatively stable temperature conditions. Rapid temperature changes put pressure on the cork/neck seal by generating sudden fluctuations in wine volume, which, if repeated frequently, will loosen the seal between the cork and the glass. This could result in the slow seepage of oxygen into the wine. An even more marked expansion of the wine occurs if the wine freezes. Freezing can produce sufficient volume increase to force the cork out of the bottle.

Temperature also directly influences the rate and direction of wine aging. Because the aging process is primarily physicochemical, heat both speeds and activates the reactions involved. Thus, cool storage ($<10°C$) tends to maintain the fresh, fruity character of most young wines. For example, fragrant acetate esters, such as isoamyl and hexyl acetates, hydrolyze slowly at 0°C but rapidly at 30°C (Marais, 1986). In contrast, formation of less aromatic ethyl esters is rapid at 30°C but negligible at 0°C. Temperature also has a marked effect on age-induced changes in the concentration and types of monoterpene alcohols found in some wines (Rapp and Güntert, 1986).

Heating favors the degradation of carbohydrates into furfurals and pyrroles. Whether a similar activation affects the conversion of norisoprenoid precursors to spiroesters such as vitispirane and theaspirane, or to hydrocarbons such as TDN and ionene, is unknown.

For most wines, prolonged exposure to high temperatures ($\geq 40°C$) rapidly results in quality deterioration. Carbohydrates in the wine undergo Maillard and thermal degradation reactions, turning brown and producing a baked (madeirized) flavor. The wines also tend to develop a heavy sediment. Even temperatures as low as 30°C can produce detectable losses in fragrance within several months, as well as cause the dissipation of a fresh, fruity aroma from 'Colombard' white wines within 2 years (Marais, 1986).

LIGHT

Exposure to sunlight is not known to directly affect the aging process in wine, but it can cause heating that can speed aging reactions. In addition, exposure to near-ultraviolet and blue radiation can activate detrimental oxidative reactions. The best known example of this is the shrimplike/skunky odor of the Champagne fault

called **light-struck** (*goût de la lumière*) (Carpentier and Maujean, 1981). Light activates the synthesis of several sulfur compounds, including dimethyl sulfide, dimethyl disulfide, and methyl mercaptan. Synthesis is considered to involve the activation of riboflavin or its derivatives. Light also facilitates the production of copper casse. As a consequence, wine should be stored in darkness whenever possible. Bottling in glass opaque to ultraviolet and blue radiation also is advisable.

VIBRATION

Wines are normally stored in areas free of vibration. Although vibration is commonly believed to disrupt or accelerate aging, there appears to be no evidence to support this view. Old claims concerning the beneficial aspects of vibration probably refer to facilitated clarification rather than aging.

Rejuvenation of Old Wines

A process has been proposed to rejuvenate wines having lost their fresh character (Cuénat and Kobel, 1987). It involves the dilution of the affected wine with water. The water, along with the chemicals presumably involved in the development of the undesired aged character, is subsequently removed by reverse osmosis. Use of ultrafiltration may be similarly beneficial in rejuvenating old wines.

Oak and Cooperage

Oak has been used in Europe for the transport and maturation of wine for over 2000 years. Many types of wood, other than oak, have been used over this period, but they have generally been limited to the construction of large storage tanks. Oak has been used similarly, but small barrels for fermentation, maturation, and transport have been predominantly constructed from one of the several white oak species. White oak has the anatomical and chemical properties needed for tight cooperage. More recently, bottles have replaced oak as the primary transport container. Consumer preference for light, young, fruity wines also has led to a reduction in the use of wood cooperage. Currently, oak cooperage is reserved primarily for the production of premium white and red wines, and some fortified wines. The flavor and slight oxidation given by barrel maturation donate characteristics usually appreciated in premium wines.

Oak Species and Wood Properties

Not only does white oak possess the properties required for tight cooperage, but its traditional use has led to a habituation to, and appreciation of, its subtle fragrance. Other woods either have undesirable structural or aromatic characteristics or have been studied insufficiently to establish their acceptability.

Quercus alba, Q. robur, and *Q. sessilis* are the species most commonly used. *Quercus alba* and a series of some six related white oak species constitute the oaks employed in the construction of American oak cooperage. *Quercus alba* provides about 45% of the white oak lumber produced in North America. It has the widest distribution of all American white oak species and has the size and structure preferred for select oak lumber.

In Europe, *Q. robur* (*Q. pedunculata*) and *Q. sessilis* (*Q. petraea* or *Q. sessiliflora*) are the primary white oaks employed in cooperage production. Both species grow throughout much of Europe. The proportion of each species in any location depends on the prevailing soil and climatic conditions. *Quercus robur* does better on deep, rich, moist soils, while *Q. sessilis* does well on drier, shallow, hillside soils and accepts more shading and variation in soil pH.

Staves produced from different American white oak species are almost indistinguishable to the naked eye. The same is true for the two important white oak species in Europe. They may be differentiated only with difficulty, and then with certainty solely under the microscope. Both genetic and environmental factors often blur the few anatomical features that distinguish the wood of each species (Fletcher, 1978).

In North America, most of the oak used in barrel construction comes from Kentucky, Missouri, Arkansas, and Michigan, and there has been little tendency to separate or distinguish between oak coming from different states or diverse sites. In contrast, identification of oak origin is common in Europe. Geographical designation may indicate the country, region (i.e., Slovonian, Limousin), political district (i.e., Vosges, Allier), or forest (i.e., Nevers, Tronçais) of the wood (Fig. 8.13).

Conditions affecting growth also affect the anatomy and chemistry of the wood. Slow growth generally results in the development of a less dense heartwood owing to the higher proportion of large-diameter vessels produced in the spring. Subsequently, the large-diameter spring vessels accumulate more phenolics than do smaller summer-produced vessels. Phenol deposition generally occurs 10 to 15 years after vessel formation, when the sapwood differentiates into heartwood. Phenolics not only resist wood rotting, but also contribute to the flavors extracted by wine during in-barrel maturation.

Owing to the higher proportion of large-diameter vessels, slow-grown wood is softer. The lower percentage of cell wall material in the wood makes it more pliable than oak that grew rapidly and has more summer wood. In France, the properties of slowly grown *Q. sessilis,* found in forests such as Nevers and Allier, are commonly pre-

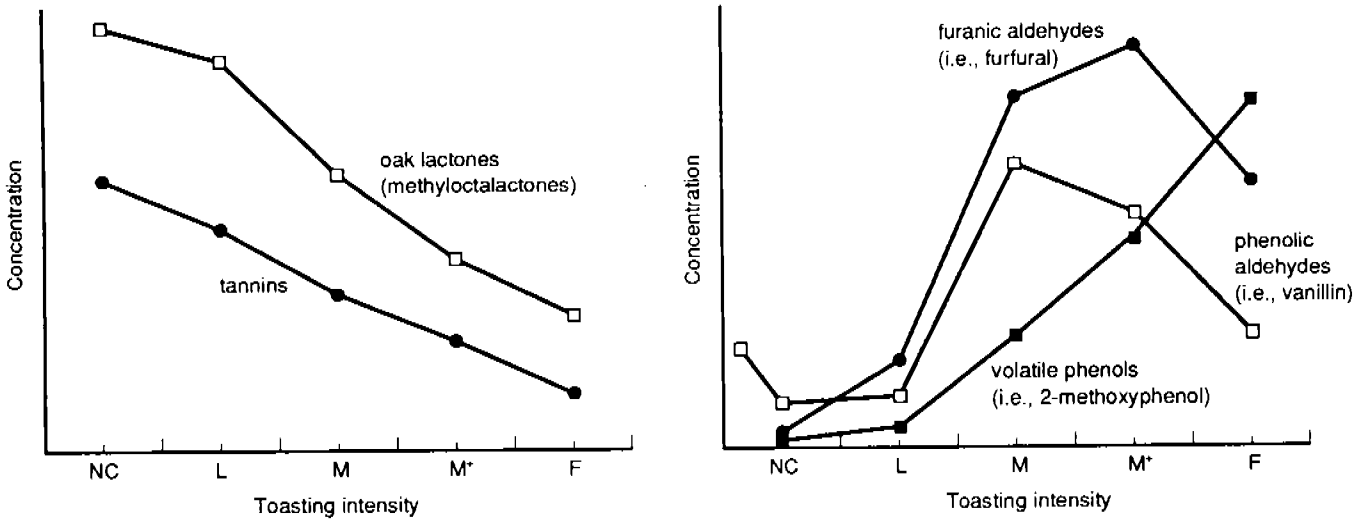


Figure 8.17 Effect of the degree of toasting on the concentration of several compounds extracted from oak barrels constructed from *Quercus sessilis*. NC, Not heated; L, light toast (5 min); M, medium toast (15 min); M+, medium strong toast; F, charing (20 min). (After Chatonnet, 1989, reproduced by permission.)

chime). Shaving the inner edge produces the bevel. Cutting a concave groove slightly below the chime produces the *howel*. A deeper cut into the howel (the *croze*) produces a slot into which the headings fit (Fig. 8.16F).

The outer surface of the barrel is planed to give it a smooth surface, while the inner surface is left rough. The rough inner surface aids wine clarification by providing increased surface area for the deposition of suspended particulate matter.

Next, a bung hole is bored and enlarged with a special auger to receive a tapered wooden peg. A tap hole also may be bored near the end of the middle head stave.

If temporary hoops were employed, they are replaced with permanent hoops. For 225 liter barrels, this usually consists of two chime hoops located just below the heads of the barrel, two bilge hoops positioned one-third in from the ends, and a set of quarter hoops placed about one-fourth of the way in from the heads. So positioned, the hoops limit the wear on the staves during rolling. At this point, the heading pieces are produced.

The head consists of several heading pieces. In contrast to the staves, the joints between the heading are straight, not beveled. Dowels between each heading piece keep them in alignment. Caulking with river rushes, called *flags,* may be used to prevent leakage.

The circular shape of the head is now sawn, in preparation for *cutting the head.* Cutting the head involves shaving two bevels, called *basles,* on the upper and lower surfaces of the head (Fig. 8.16G).

The bottom head is inserted first. Removal of the bottom head hoop allows the head to be forced into the croze. After repositioning the head hoop, the barrel is inverted to permit removal of the opposite head hoop. A heading vice may be screwed into the head and the head lowered sideways into the barrel. The head is pulled up into its groove with the vice. Alternately, a piece of iron forced in a joint between two staves levers the head into position. Positioning the wood grain of the two heads perpendicular to one another limits the pressure, that develops during wood swelling, from acting in the same direction, thus minimizing the likelihood of leakage.

The final task involves hammering the hoops tight. This forces the staves together and closes most cracks. After soaking for a day in water, a well-made barrel becomes leakproof.

COOPERAGE SIZE

Cooperage is produced in a wide range of sizes, depending on the intended use. In the past, large straight-sided tanks and vats were constructed with capacity greater than 5 to 10 hl. They also acted as storage cooperage after fermentation. The large size minimized oxidation and eased cleansing. However, wooden fermentors and storage tanks largely have been replaced by more durable and easily cleaned tanks constucted from stainless steel, epoxy-lined carbon steel, fiberglass, or cement. Currently, wooden cooperage is restricted largely to maturing wine in which an oak character is desired. In addition, small cooperage may be used for in-barrel fermentation.

Many of the benefits of barrel use come from the relatively large surface area of the barrels. Although surface area increases logarithmically with decreasing volume (Singleton, 1974), other factors place practical limits on minimum size. Production economy favors larger size, while ease of movement and earlier maturation of

the wine favors small size. A compromise between the opposing factors has led to the widespread adoption of barrels with a capacity between 200 and 250 liters. Individual regions in Europe often use barrels of a particular capacity. For example, the Bordeaux *barrique* is 225 liters, the Chablis *feuillette,* 132 liters, the Rhine *doppelohm,* 300 liters, the sherry *butt,* 490.7 liters, and the port *pipe,* 522.6 liters. Premium white wines commonly receive about 3 to 6 months of maturation in oak, while red wines often receive between 18 and 24 months of oak maturation before bottling.

While much current literature focuses on the value of maturing wine in small oak cooperage (225 liters), many fine wines are aged in large oak cooperage (>1000 liters). This is especially true in European regions other than France. Well-sealed large tanks permit lower rates of oxidation and donate only small amounts of tannins and oak flavor to wine. In contrast, the Bordeaux *barrique* is estimated to permit the ingress of about 2 to 5 mg O_2/liter/year through the wood (Ribéreau-Gayon *et al.,* 1976). Large oak cooperage also can be used for decades, while small cooperage usually is replaced after several years. In addition, stratification of wine at different redox potentials may develop in large cooperage. Until the sensory effects of maturation in large oak cooperage are better understood, it would be unwise for winemakers automatically to reject this form of maturation; it has been used for centuries with favorable results.

CONDITIONING AND CARE

As with most winemaking practices, opinions differ considerably on how to condition new barrels. Furthermore, the need appears to vary with the source and seasoning of the staves. Minimal treatment usually involves rinsing and presoaking with warm water to swell the wood and seal the joints. In-barrel fermentation is also a well-established conditioning procedure. During the latter, the most readily extractable tannins and phenols dissolve, precipitate, and are lost with the lees. However, as the desirable flavors in oak dissolve more slowly, they are not unduly removed by in-barrel fermentation. Although an effective conditioning procedure, the technique is laborious, and barrels require cleansing before subsequent reuse in wine maturation. Consequently, barrels are conditioned more commonly with a solution of 1% sodium or potassium carbonate. The alkaline solution accelerates both phennol extraction and oxidation. Subsequently, the barrels require a thorough rinsing with a 5% solution of citric acid, and finally a water wash.

In-barrel maturation preferably follows an initial clarification of the wine. This minimizes both the adherence of material to the inner surfaces of the barrel and the excessive accumulation of lees. In addition, barrels commonly are racked several times a year to avoid the buildup of a thick layer of lees. These actions decrese the difficult and unpleasant task of barrel cleaning. In addition, potential contamination of the wood with spoilage microorganisms is minimized. However, tradition or personal preference may reseult in the wine being left on the lees for several months. Some vintners believe that the yeast and tartrate coating that develops slows the release of oak flavors.

After use, barrels require cleansing and disinfection before reuse. Where little precipitate has formed, rinsing with water under high pressure is usually sufficient for cleansing. Where a thick layer of tartrates has built up, barrels may require treatment with 0.1 to 1% sodium or potassium carbonate, followed by a thorough rinsing with water. Burning a sulfur wick in the barrel usually provides sufficient disinfection of the inner surfaces.

Barrels should be refilled with wine as soon as possible after cleansing and disinfection. If left empty for more than a few days, barrels should be thoroughly drained, sulfited, and tightly bunged. Barrels stored empty for more than 2 months should be filled with an acidified sulfur solution at 200 ppm SO_2. The sulfur dioxide inhibits the growth of most microbes, and the water prevents wood shrinkage and cracking. Before barrel reuse, the residual sulfur dioxide is removed with several water rinses.

Treating the outer surfaces of the cooperage with 1% rotenone in boiled linseed oil usually controls oak boring insects, but not fungal growth. Mold growth over the external surface of the barrel may mar the appearance but does not affect barrel strength or influence the sensory properties of the wine contained.

USEFEUL LIFE SPAN

For certain types of wine, legislation specifies the rate at which used barrels must be replaced. In most regions, however, oak use is left to the discretion of the winemaker. Thus, the frequency of reuse depends on economics and intensity of oak character desired. Making these decisions will be facilitated when more is known about the dynamics and sensory impact of flavorant extraction from oak.

Figure 8.18 shows differences in the total and nonflavonoid phenol extraction from American and French oak. Not surprisingly, the differences are most marked during the first fill. The differences are also more striking with French than with American oak. Subsequently, the differences become less marked. As the rate of nonflavonoid extraction does not drop as rapidly as that of total phenolics, the proportional extraction of nonflavonoids increases with each filling. Currently, the sensory significance of this change is unknown.

For aromatic compounds extracted from oak, those

COMPOUNDS EXTRACTED FROM OAK

The solubility of oak constituents, and their degradation by-products, varies widely. Compounds extracted in small amounts may affect only the bouquet, while those extracted in larger amounts may influence all the sensory perceptions of wine. For example, wine dissolves about 30% of the tannins in the innermost few millimeters of the oak staves. This is sufficient to affect the color, taste, mouth-feel, and fragrance of wine. In contrast, wine extracts only about 2% of oak lignins. Lignin breakdown products typically affect only the fragrance. Currently, over 200 volatile compounds have been identified from oak.

Quantitatively, phenolics are the most important group of oak extractives. Of these, about two-thirds are nonflavonoids. The hydrolyzable tannins (ellagitannins) comprise the most significant subgroup of oak nonflavonoid phenols. Lignin degradation products form the second most important group of extracted phenolics. Their extraction depends largely on the alcoholic strength and acidity of the wine. Both factors also are involved in the degradation of tannins and lignins to simpler, more soluble compounds. Toasting eases the extraction of ellagitannins by enhancing the hydrolysis of ellagic acid glycosides.

Oak tannins can add significantly to the astringency and bitter taste of wine. Consequently, white wines are usually matured in oak for shorter periods than red wines, and in barrels conditioned to release fewer extractable tannins. For red wines, the influence of oak on taste depends on the flavor intensity of the wine, with light wines being negatively influenced while full-flavored wines are little influenced. Oak tannins also participate in stabilizing the color of red wines.

Lignin breakdown products add significantly to the development of an oak bouquet. Lignin degradation involves the action of both alcohol and oxygen. It is believed that ethanol reacts with certain lignins, forming ethanol–lignins. As the complexes break down, the lignin monomers (coniferyl and sinapyl alcohols) are released along with the ethanol. The phenolic alcohols slowly oxidize under the acidic conditions of wine to form sinapaldehyde and syringaldehyde, and coniferaldehyde and vanillin, respectively (Puech, 1987). Above threshold values, the oxidized compounds donate woody, vanillalike odors. Toasting, especially at about 200°C, markedly augments their synthesis (Table 8.2). The lower amounts extracted from charred wood probably result from carbonization. Lignin degradation also may generate phenolic acids, such as vanillic and syringic acids, and the coumarin derivatives scopoletin and escutelin. The presence of scopoletin, along with "oak" lactones, is so characteristic that those compounds are considered diagnostic of oak maturation.

Table 8.2 Aromatic Aldehydes Produced by Toasting or Charring Oak Chips[a,b]

	Toasting temperature			
Product (ppm)	100°C	150°C	200°C	Charred
Vanillin	1.1	3.8	13.5	2.8
Propiovanillone	0.6	1.1	1.4	0.9
Syringaldehyde	0.1	3.8	32.0	9.2
Acetosyringone	—	0.025	1.5	0.6
Coniferylaldehyde	Trace	4.3	24.0	4.8
Vanillic acid	—	1.8	6.1	1.1
Sinapaldehyde	Trace	6.5	60.0	9.0

[a] Oak chips (2%, w/v, in ethanol) were toasted at various temperatures. Charring occurs above 250°C.

[b] From Nishimura *et al.* (1983), reproduced by permission.

Oak-derived phenols may be modified further by yeast and bacterial metabolism. The changes can influence both volatility and odor quality. For example, the reduction of furfurals to the corresponding alcohols results in a quality shift from almondlike to hay/verbenalike (Chatonnet, 1991).

Various phenolic and nonphenolic acids have been implicated in the synthesis of esters, acetals, and lactones in wine matured in oak (Nykänen, 1986). The acids can lower wine pH and increase acidity. By increasing the proportion of colored anthocyanins, the acids enhance color intensity. The most prevalent acid is acetic acid. It may be formed during the degradation of hemicelluloses or from the oxidation of acetaldehyde, but the most significant source is probably the metabolism of acetic acid bacteria. Oxygen uptake during cellar activities can activate the growth and metabolism of acetic acid bacteria, which generate acetic acid during the metabolism of ethanol and several sugars.

Lignins, tannins, and inorganic salts also influence the poorly understood phenomenon of ethanol/water interactions. Such interactions are believed to mellow the alcoholic taste of wine and distilled spirits (Nishimura *et al.*, 1983).

Although small amounts of sugars accumulate during the hydrolysis of hemicelluloses, they are insufficient to affect the taste of the wine. The simultaneous pyrolytic conversion of some of the sugars to furfurals appears to be the most significant sensory effect of sugar liberation.

"Oak" lactones occur in many wines matured in oak. Although present in oak, and formed on toasting, oak lactones are extracted slowly by wine. Consequently, it may take more than 1 year for the coconutlike fragrance of oak lactones to affect wine fragrance.

While oak maturation increases the concentration of

many important sensory compounds, it also reduces the concentration of others. For example, the concentration of dimethyl sulfide and dimethyl disulfide decrease during barrel maturation (Nishimura *et al.*, 1983). Methionyl acetate also decreases in quantity, but only in the joint presence of oxygen. The green bean/green chili aspect of some 'Cabernet Sauvignon' wines may dissipate when the wines are matured in oak barrels (Aiken and Noble, 1984).

Aeration

Slight oxidation is commonly viewed as a important consequence of maturation in oak. Wine placed in well-made barrels, bunged tight and rotated so that wine covers the bung, receives oxygen exposure only during cellaring procedures such as racking. Air does not usually diffuse into tight barrels in significant quantities. The water and alcohol lost through the surfaces of the barrel are not replaced. Instead, a partial vacuum develops in the space (*ullage*) vacated by the alcohol and water. As barrels may differ markedly in tightness, the negative pressures observed over barreled wine also may vary considerably (Fig. 8.20). Variation in oxygen penetration may explain the barrel-to-barrel diversity in maturation rates often noted by winemakers.

Evaporative wine loss is more marked in barrels left with the bungs upright, but sampling the wine to check its development is much easier. The bung up position also permits frequent topping to fill the ullage as it develops. Coincidentally, both procedures increase the wine's exposure to oxygen. During normal racking, topping, and sampling, a wine may absorb about 30 to 40 ml O_2/liter per year. It is estimated that about 2 to 5 ml O_2/liter is absorbed through the wood, between 15 to 25 ml O_2/liter during topping, sampling, and through the bung, and up to about 6 ml O_2/liter during each racking (Ribéreau-Gayon *et al.*, 1976). In red wine, the absorbed oxygen is estimated to be consumed within about 6 days at 30°C (Singleton, 1987), which probably is equivalent to about 15 days at 15°C.

The addition of oak chips or shavings to wine has been investigated as an economical alternative to barrel aging. However, the sensory effects appear to differ from those obtained during barrel aging. This may result from the absence of heat-induced hydrolysis, aeration and/or the microbial modification of oak constituents.

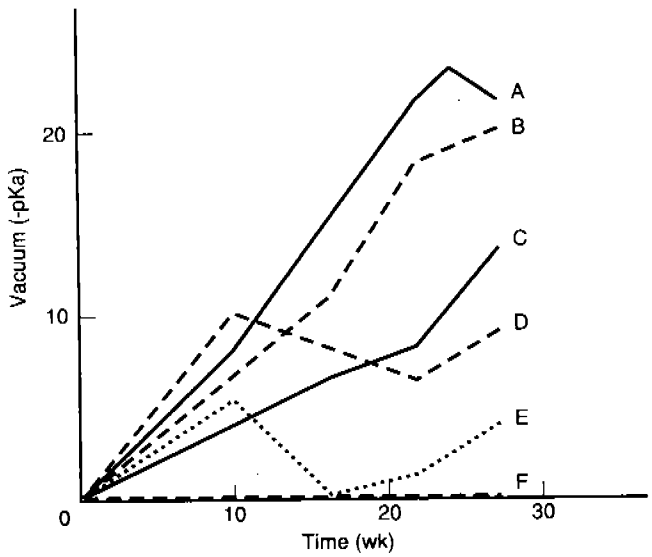


Figure 8.20 Development of a partial vacuum in six wine barrels (A–F) during undisturbed aging of 'Cabernet Sauvignon' wine. (From Peterson, 1976, reproduced by permission.)

In-Barrel Fermentation

Most vinifications take place in large tanks or vats. They permit more uniform fermentation and are easier and more economical to maintain. Fermentation in-barrel, or other small-volume cooperage, is commonly used only with modest quantities of must of unique qualities or origin. Although temperatures during in-barrel fermentation often are higher than those in cooled tank fermentors, the surface area to volume ratio is sufficient to avoid overheating and stuck fermentation. The likelihood of stuck fermentation also may be diminished by the uptake of sterols extracted from the wood (Chen, 1970). Sterols are required for the proper maintenance of yeast membranes during and after fermentation. Sugars released during maturation (Nykänen *et al.*, 1985) also may favor malolactic fermentation, by providing nutrients for the growth of lactic acid bacteria.

In addition to maintaining the individuality of small lots of juice, some winemakers prefer in-barrel fermentation for its effect on wine development. Wine fermented and matured in new oak incorporates less phenolic material than the same wine matured in equivalent barrels after fermentation. This partially results from the coprecipitation of tannins extracted during and shortly after fermentation with yeast cells. The early extraction and oxidation of tannins help consume oxygen, minimizing wine oxidation. Phenols also reduce the accumulation of volatile reduced-sulfur compounds (Nishimura *et al.*, 1983). As the more desirable oak flavors dissolve more slowly than oak tannins, the wine retains proportionally more oak flavor and less harsh tannins. Other differences have been noted, but the sensory significance is uncertain. For example, the reduction of furfural and 5-(hydroxymethyl)-2-furaldehyde to the corresponding alcohols is enhanced (Marsal and Sarre, 1987), as well as the reduction of ferulic and *p*-coumaric acids to 4-vinyl

guaiacol, 4-ethyl phenol (Dubois, 1983). The latter possess spicy, smoky odors. Another significant change is the metabolic conversion of phenolic aldehydes, notably vanillin, to less aromatic phenolic alcohols. In-barrel fermentation increases the level of "oak" lactones (β-methyl-γ-octalactones), nitrogen compounds, and polysaccharides, primarily those derived from yeast mannoproteins. The latter might enhance the smooth mouth-feel of the wine. In contrast, soluble protein levels may decrease.

Disadvantages of Oak Cooperage

For premium wines, fermentation or aging in oak is often worth the effort and expense involved. For most wines, however, exposure to oak is neither cost effective nor necessarily beneficial.

Oak barrels are costly to purchase and expensive to maintain, and new barrels need conditioning before use. The tartrates and tannins that build up on the inside the barrel during wine maturation are both difficult and unpleasant to remove. When not containing wine, barrels must be protected from drying and microbial contamination. Off-flavors produced by bacteria and fungi growing on internal surfaces can taint wine subsequently stored in the cooperage with "corky" off-odors (Amon *et al.*, 1987).

Because the rate of maturation varies from barrel to barrel, frequent and time-consuming barrel sampling is necessary to assess the progress of the wine. Racking is more labor intensive and inefficient than the equivalent in large cooperage. In addition, considerable economic loss can result from the evaporation of wine from the barrel. Up to 2 to 5% of the volume may be lost per year in this way (Swan, 1986). Depending on the relative humidity of the winery, wine may either increase or decrease in alcoholic strength (Guymon and Crowell, 1977). High relative humidity suppresses water evaporatoin but has no effect on alcohol loss. Consequently, the alcoholic strength of wine decreases in humid cellars. Under dry conditions, water evaporates more rapidly than ethanol, increasing the alcoholic strength. In addition to water and ethanol, acetaldehyde, acetal, acetic acid, and ethyl acetate are lost by evaporation from barrel surfaces (Hasuo and Yoshizawa, 1986). Relative humidity also influences the types and amounts of phenols extracted. Low relative humidity decreases total phenolic uptake but increases vanillin synthesis (Hasuo *et al.*, 1983).

Other Cooperage Materials

For both fermentation and storage, cooperage constucted from material other than wood has many advantages. In addition, other materials are less expensive to maintain. Stainless steel often is preferred, but fiberglass and cement also are widely used. Because all are impervious to oxygen, wine oxidation is minimized. This preserves the fresh, fruity character important to most wines designed for early consumption. Stainless steel and fiberglass have the additional benefits of permitting construction in a variety of shapes more difficult to produce with wood or cement. Modern construction materials also facilitate cleaning and dry storage. Gas impermeability permits partial filling, as the ullage can be filled with carbon dioxide or nitrogen to limit oxidation. Furthermore, modern construction materials do not modify the fragrance of wines.

Stainless steel is generally the preferred modern cooperage material because of its strength and inertness. The inertness avoids the need for, and maintenance of, coatings of paraffin wax, glass, or epoxy resin. These are required for cement tanks as excessive amounts of calcium can seep into wine matured in unprotected tanks. In addition, the acidic nature of wine tends to corrode the cement. Stainless steel possesses heat transfer properties permitting comparatively easy temperature control during fermentation. Temperature contol often is obtained with coolant circulated within an insulated, double-lined jacket. Installation is rapid and subsequent movement of the cooperage is possible.

For wine production and storage, stainless steels high in chromium and nickel content are required. Contents of between 17 and 18% chromium by weight provide an adequate surface layer of insoluble chromium oxide. It is the chromium oxide that provides most of the anticorrosive properties of stainless steel. Nickel is present in amounts that may vary between 8 and 14%. It facilitates soldering and further enhances corrosion resistance. When the stainless steel is exposed to wine for short periods, molybdenum may be omitted from the steel. However, for prolonged contact or exposure to sulfited wines, molybdenum is required at a concentration of about 2 to 3%. Titanium may be incorporated as it increases the level of carbon permitted in the finished steel. Titanium also reduces the risk of corrosion next to soldered joints. It often is added at about 0.5%.

With stainless steel, it is important to avoid introducing scratches on the inner surfaces of the tank. Even rinsing with hard water containing minute rust particles can cause damage to the polished surfaces.

Fiberglass tanks also have become common replacements for wooden cooperage. Fiberglass has the advantages of being less expensive and lighter than stainless steel. However, it possesses less strength, is less conductive to heat, is more porous, and tends to be more difficult to clean (has a rougher surface) than stainless steel. In addition, residual styrene may diffuse into the

wine from the polyester resin binding the glass fibers. At concentrations above 100 μg/liter, styrene may taint the wine with a plastic odor (Anonymous, 1991).

Stainless steel, resin-coated regular steels, and fiberglass have permitted the production of an extensive array of cooperage. While the containers may be used for wine maturation, most are designed to facilitate emptying and cleansing after fermentation. Thus, they typically possess a slanted floor and exit ports at or near the base. The position of the port (horizontal or vertical) is largely a function of whether cleaning occurs automatically or manually. Other designs may pivot tanks to facilitate emptying or a fixed helical blade may mix the must during fermentation and aid subsequent discharge.

Cork and Other Bottle Closures

Cork

Cork remains the bottle closure of choice after over 400 years of use in Europe. However, the use of cork in the preservation of wine predates its use as a bottle closure by some 2000 years. The ancient Greeks and Romans frequently used cork to stopper wine amphoras (Tchernia, 1986). The resin-coated stoppers were often covered by a cap of volcanic-tuff cement (*pozzolana*). The oldest known use of cork as a wine seal comes from an Etruscan amphora (sixth century B.C.) unearthed in Tuscany (Joncheray, 1976). The subsequent decline in the use of cork reflected the decline in the use of amphoras following the collapse of the Roman Empire. The major reemergence of cork as a closure for wine containers began about the mid-seventeenth century, coincident with the beginnings of industrial-scale glass bottle manufacture in England. Nevertheless, cork appears to have been used as a bottle closure as far back as the end of the fifteenth century (McKearin, 1973).

Cork is a tissue produced by a special layer of cells, the **cork cambium,** located in the outer bark of plants. The cambium produces cork (**phellem**) to the outside and a thinner layer of cells (**phelloderm**) to the inside. Together, these tissues constitute the outer bark. The inner bark, or **phloem,** consists of cells primarily involved in conducting organic nutrients throughout the plant.

In the majority of woody plants, the cork layer is relatively thin. In only a few species of oak is a deep, relatively uniform layer of cork produced. Of these, only the cork oak, *Quercus suber,* produces cork in commercial quantities. Not only does *Q. suber* produce a thick layer of cork, but the cork can be repeatedly harvested without damaging the tree.

CORK OAK

Quercus suber grows in a narrow region bordering the western Mediterranean (Fig. 8.21), with most commercial stands located in Portugal. Portugal produces about two-thirds of the world's supply (~200 million kg), with the remainder coming from Spain, Algeria, Morocco, Italy, France, and Greece. Dry, upland sites on rocky soils provide the better areas for cork production. Here, the bark is firmer and more resilient. On rich lowland soils, trees produce a thicker but more spongy layer of less valuable cork.

The cork oak grows about 16 m high and has a trunk diameter of 20 to 60 cm at breast height. Typically, the tree begins to branch out about 4 to 5 m above the ground. Thus, the trunk provides large, clear sections of bark. The lower branches of older trees also may yield bark sections of sufficient size to be of commercial value. Trees may live about 500 years, but the most productive period occurs betwen the first and second century of growth.

CULTURE AND HARVEST

Most commercial oak stands are of natural origin. However, selection and planting of superior seedlings are occurring in both existing stands and reforestation areas. Pruning helps to shape the trees for optimal production of quality cork.

When trees reach a diameter of over 4 cm, or are about 20 to 30 years old, the cork is stripped from the trunk for the first time. This stimulates the growth of new cork and the tree in general. The initial or **virgin cork** is not used for the production of bottle closures as its structure is too irregular and porous (Fig. 8.22).

On removal of virgin cork, the exposed tissues turn red, and, by an unknown mechanism, cork production is

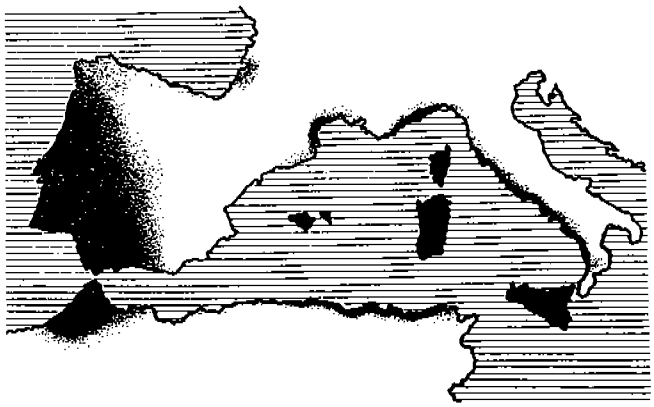


Figure 8.21 Geographic distribution of *Quercus suber,* the cork oak. (From *Subériculture* by J. V. Vieira Natividade, 1956, reproduced by permission of Éditions de l'École nationale du Génie rural des Eaux et des Forêts, Nancy, France.)

after 10 years (Lefebvre, 1981). This explains why fine wines are often recorked about every 25 years.

The slight cone shape of the bore (18.5 → 21 mm), the bulge or indentation in the neck about 1.5 cm below the lip, and the compression of the cork are all important in limiting movement of the cork in the neck. This is especially important if the wine is exposed to temperature extremes, where volume changes can weaken the seal and force the cork out of the bottle.

Leakage Caused by Insertion Problems

During insertion, air tends to be trapped underneath the cork in the neck of the bottle. As cork does not regain its full resilience for several hours, the stopper does not immediately exert its maximum force against the neck. If the bottle is laid on its side or turned upside down shortly after corking, the pressure exerted by the trapped air can force wine out between the cork and the neck of the bottle. To avoid this, wines often are set upright for several hours after corking. During the interval, the entrapped air can escape before the seal becomes firmly established. This is important as air consists of about 78% nitrogen, a gas poorly soluble in wine. If the pressure is not released, it continues to act on the wine and cork when the bottle is placed on its side. Oxygen, the other main atmospheric gas, dissolves quickly in the wine and ceases to exert pressure.

Nitrogen contained in the headspace can augment leakage induced by extremes of temperature. As shown in Fig. 8.27, temperature significantly influences the volume of wine. Temperature–volume changes modify the pressure exerted on the headspace volume and, indirectly, on the cork. Leakage becomes likely at internal pressures above 200 kPa (about twice atmospheric pressure), when the net outward pressure equals that exerted by the cork against the glass.

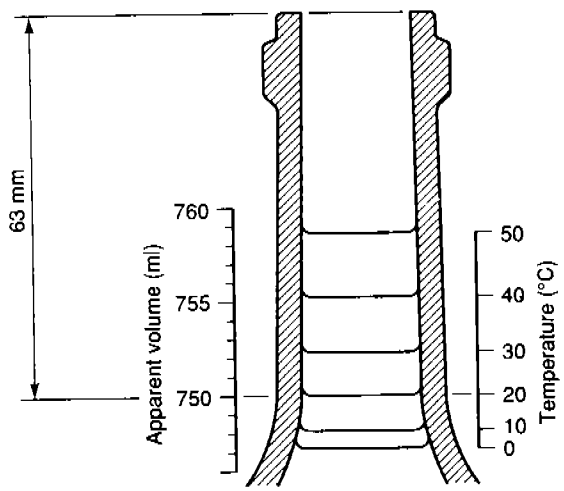


Figure 8.27 Variations in the level of fill as a function of temperature. (From Lefebvre, 1981, reproduced by permission.)

To reduce leakage, most bottles are placed under partial vacuum or flushed with carbon dioxide before corking. The creation of a partial vacuum eliminates the development of a positive headspace pressure during cork insertion. Any vacuum that remains after corking soon dissipates as carbon dioxide escapes from the wine into the headspace. Alternately, flushing the bottle with carbon dioxide displaces the nitrogen and oxygen of air. Because of the high solubility of carbon dioxide in wine, the positive headspace pressure created on cork insertion rapidly dissipates. The uptake of about 20 mg of carbon dioxide in this manner has no sensory effect on the wine.

The use of corking under vacuum or carbon dioxide has benefits beyond reducing the likelihood of leakage. It limits the development of "bottle sickness" by removing the 4 to 5 mg of oxygen otherwise absorbed from the trapped air. Both procedures also avoid lowering the content of free sulfur dioxide by removing oxygen that can react with SO_2. In addition, the procedures permit bottles to be packed and stored in an inverted position directly after corking.

Variation in bottle capacity may be an additional source of leakage problems. If the bottle has a capacity smaller than specified, very little headspace volume may remain after filling. Even with medium length corks (44/45 mm), bottles may possess a headspace volume of as little as 1.5 ml after corking. Since the wine in a 750 ml bottle can expand by about 0.23 ml/°C, a rise in temperature can quickly result in a significant increase in the pressure exerted on the cork. The effect is increased if the headspace gas contains nitrogen or if the wine is sweet or is supersaturated with carbon dioxide (Levreau *et al.*, 1977).

Sugar content can augment leakage by increasing the capillary action between the cork and the glass. Sugar also increases, by about 10%, the rate at which wine volume changes with temperature (Levreau *et al.*, 1977).

Finally, the moisture content of cork can influence the likelihood of leakage. At moisture contents between 5 and 7%, cork has sufficient suppleness not to crumble on compression. It also rebounds sufficiently slowly to allow pressurized headspace gases to escape before a tight seal develops. At lower moisture levels, compression and insertion are likely to rupture or crease the cork. At high moisture levels, more gas is likely to be trapped in the headspace (Levreau *et al.*, 1977).

Bottles and Other Containers

Over the years, wine has been stored and transported in a wide variety of containers. The first extensively used

storage vessels were probably made from animal skins. In most areas, these were replaced by clay amphoras. Use of amphoras in Egypt was well-established at least by 1400 B.C. (Lesko, 1977), and they apparently were being used to store wine in Iran between 3500 and 2900 B.C. (Badler *et al.*, 1990). Amphoras continued to be used in the Middle East up until at least A.D. 625 (Bass, 1971). They fell into disuse throughout much of the western Mediterranean after the decline of the Roman Empire. North of the Alps, wood cooperage became the primary wine storage and transport vessel from the beginning of the Christian era. It held its preeminence in Europe until the twentieth century, when the glass bottle became the primary storage and transport vessel.

Glass bottles were used to a limited extent during Roman times, but only sporadically. This undoubtedly resulted from their fragility and costly production. Developments in glass technology in Italy during the 1500s reintroduced bottles as wine containers. However, because of their fragility, the bottles had to be covered with reeds to protect them from breaking. Further technological developments in England during the 1600s permitted the production of strong glass bottles. Initially, expense limited their widespread adoption, and bottles were used primarily as decanters to transport wine from barrel to table. Use for long distance transport occurred but was limited. Intentional bottle aging of wine appears to have developed with sweet wines, notably *porto* and Tokaji wines. Storage and transport in bottles became necessary only with the development of sparkling wine. Subsequently, bottles slowly began to supplant barrels for the transport and aging of wine.

Bottles are not without disadvantages, however. Because of the variety of shapes, sizes, and colors, collecting for reuse tends to be cost effective only with that portion of the market using standard bottle shapes and colors. Bottles also create a considerable disposal problem if not recycled in some other product. Once a bottle is opened, wine conservation is difficult, and noticeable loss in quality is usually evident within 1 day. Although decanting systems can replace the volume of consumed wine with nitrogen gas, they are practical only in large establishments and are of little value to the average consumer. As a consequence, new containers such as bag-in-box packages have begun to replace the bottle for many standard-quality wines.

Glass Bottles

Glass has many advantages over other materials in bottle manufacture. The chemcial inertness of glass is especially critical in aging premium wines for prolonged periods. Glass also is impermeable to gases and resists all but rough handling. Although trace amounts of sodium, chromium, and nickel may dissolve into wine, the levels involved generally are minuscule. However, changes in glass formulation may increase the amount of chromium extracted from 1 to 4 μg/liter (Médina, 1981). The transparency of glass to near-ultraviolet and blue radiation is a disadvantage but can be countered by the incorporation of several metal oxides. The primary disadvantages of glass are its weight and the energy required in its manufacture.

PRODUCTION

Bottle glass is formed by heating sand (largely silicon dioxide) to about 1500°C in the presence of soda (sodium carbonate), lime (calcium oxide), and small amounts of magnesium and aluminum oxides. The first three materials often contribute about 95% of the mass. Clear glass is typically produced by adding sufficient magnesium to decolorize the iron oxide contaminants that commonly occur in sand. Small amounts iron, manganese, nickel, and chromium oxides may be added to give the glass a desired color. The yellow to green color of most wine bottles comes primarily from ferric and ferrous oxides (Fe_2O_3 and FeO). The specific shade is influenced by the redox potential, degree of hydration, presence of other metals, and the chemical nature of the glass. Amber is often generated by maintaining the reducing action of sulfur during glass fusion, brown by the addition of manganese and nickel, and emerald green with the incorporation of chromic oxide (CrO_3). Adding chromic oxide under oxidizing conditions or vanadium pentoxide (V_2O_5) greatly improves ultraviolet absorption (Harding, 1972).

In bottle manufacture, an appropriate amount of molten glass, the *parison,* is placed in the upper end of a rough (*blank*) bottle mold (Fig. 8.28). Compressed air forces the molten glass down to the bottom of the mold, where the two portions of the neck mold occur. Air pressure, from the center of the neck mold, blows most of the glass back into the configuration of the blank mold and establishes the finished shape of the neck. The blank mold, containing the outer half of the neck mold, is opened and removed. The outer stiff layer of glass, next to the mold, maintains the shape of the bottle during its transfer to the finishing (*blow*) mold. The inner half of the neck mold (*neck ring*) is used to raise and invert the bottle, to position it between the halves of the blow mold. The bottle is released by the neck ring just before closing the halves of the blow mold.

The glass is reheated to bring it back to a moldable temperature. Compressed air, blown in via the neck, drives the glass against the sides of the blow mold. At the same time, a vacuum is created at the base of the mold, removing trapped air. These actions give the bottle its final dimensions. After a short cooling period, to assure

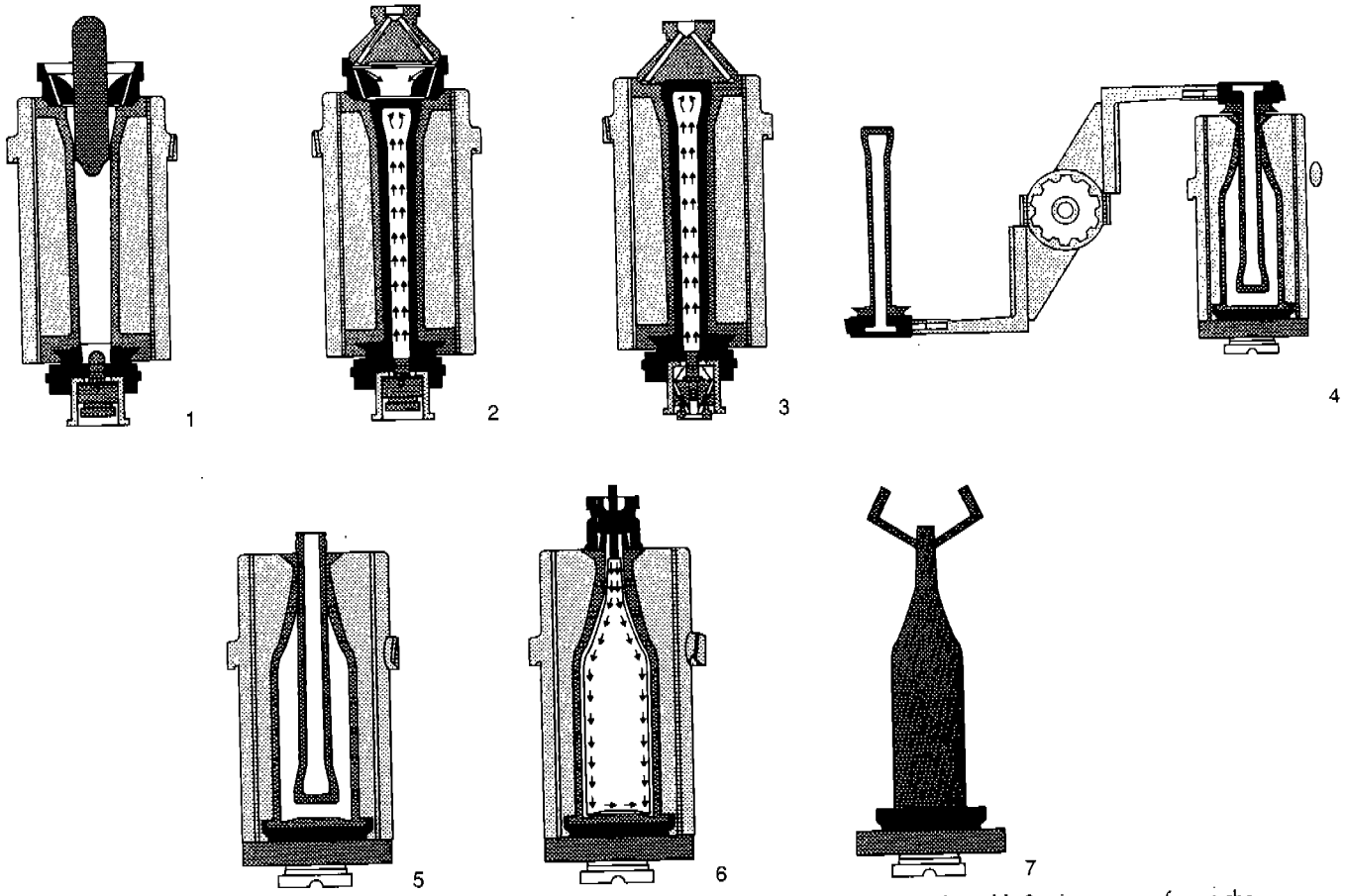


Figure 8.28 Blowing of glass wine bottles. 1, Molten glass (parison) added to the rough mold; 2, air pressure forces the parison to the base of the mold; 3, air pressure from the mold base forces the still molten glass into the rough mold shape; 4, removal of the rough mold and transferral of the bottle into the finishing mold; 5, reheating of the glass; 6, air pressure forces the molten glass into the shape of the finishing mold; 7, removal of the finished bottle. (After Riboulet and Alegoët, 1986, reproduced by permission.)

retention of the final shape, the bottle is removed from the blow mold.

During production, various parts of the bottle cool at different rates. This creates structural heterogeneity that makes the glass fragile. To remove the structural tensions, the bottle is *annealed* by heating to about 550°C. After sufficient annealing, the glass is slowly cooled through the annealing range, then rapidly cooled to ambient temperatures. During annealing, sulfur is typically burnt to produce a thin layer of sodium sulfate on the inner surface of the glass. The associated diffusion of sodium ions to the glass surface increases its chemical durability. Alternately, a thin coating of titanium or other ions may be added. Both procedures harden the surface of the glass and minimize lines of weakness.

SHAPE AND COLOR

Bottles of particular shapes and colors have come to be traditionally associated with wines from several European regions. These often have been adopted in the New World for wines of similar style, or to imply character similarity. Unique bottle shapes and markings also are used in increasing consumer awareness and recognition of particular wines.

In general, bottle shape and color are more important in marketing than to aging, storage, or transport. Tradition and image probably explain why bottles that filter out little ulraviolet radiation are still in common use. White wines are the most susceptible to light-induced damage (Macpherson, 1982), but they are often the least protected, being commonly sold in clear or light-colored bottles. To partially offset light-activated spoilage, white wines often receive higher doses of sulfur dioxide before bottling than red wines.

In contrast, bottle neck design depends primarily on pragmatic issues, such as the type of closure. Still table wines can use plain necks, as they require only a simple cork or RO closure. Sparkling wines, however, require a special lip to which the restraining wire mesh for the stopper is secured.

Bottles of differing filling heights, and permissible capacity variations, are available. Bottles having lower filling heights and smaller volume variation leave more headspace between the wine and the cork. By providing more space for temperature-induced wine expansion, the bottles are less susceptible to leakage.

FILLING

After a hot water rinse, bottles are steam cleaned and allowed to drip dry before filling. Although cleaning is always essential, actual sterilization is necessary only for wines that have been sterile filtered, or when used bottles are being recycled. Because the microbial contamination of new bottles is generally minimal and consists largely of organisms unable to grow in wine, sterilization is usually unnecessary. In addition, the microbial population of the wine may be considerably higher than that in the bottle.

Various types of automatic bottling machines are available. Some operate by siphoning or gravity feed, while others use pressure or vacuum. Siphoning and gravity feeding are the simplest but slowest, while the pressure and vacuum fillers are more appropriate for rapid, automated filling lines.

Regardless of the machine, precautions must be taken against the buildup of microbes within the equipment. Microbial contamination has been known to cause the spoilage of thousands of bottles of wine. Precaution also is taken to minimize oxidation during filling. This is best achieved by flushing the bottles before filling with carbon dioxide gas. Alternately, the headspace may be flushed with carbon dioxide, or filling and cork insertion may occur under vacuum. Bottle sickness, a mild form of oxidation that may follow bottling, is due primarily to oxygen left in the headspace. The quantity contained in headspace air can amount to eight times the oxygen absorbed during filling.

Bag-in-Box Containers

Bag-in-box technology has progressed dramatically from its initial start as a means of marketing battery fluid. Subsequent developments have found wide application in the wine industry. Because the bag collapses as wine is removed, its volume is not replaced with air. This permits wine to be periodically removed over several weeks to months without noticeable loss in quality. This convenience is credited with expanding the wine market in regions without a strong association with wine. In some countries, such as Australia, more than half the wine sold is by the "box" (Anderson, 1987). Bag-in-box packaging also is ideally suited for "house wine" sold in restaurants, especially the larger 10 to 20 liter sizes.

In its modern form, bag-in-box packaging protects wine from oxidation for 9 months or longer. This is usually adequate for wines that require no additional aging and have a high rate of sale.

For protection and ease of stacking and storage, the bag is housed in a corrugated or solid fiber box. A handle permits easy transportation. The large surface area of the box provides ample space for marketing information and attracting consumer attention.

At present, no single membrane possesses all the features necessary for a collapsible wine bag. The solution has been to use a two-layered membrane (Webb, 1987). The inner layer, comprising a low density polyester or ethyl vinyl acetate film, provides the necessary protection against flex cracking. The outer layer, typically a metallized polyester laminated to polyethylene, slows the loss of sulfur dioxide and aromatic compounds and the inward diffusion of oxygen. Most modern plastic films are inert to wine and do not affect the sensory characteristics of the wine. Eliminating amide additives has removed a former source of off-odors.

Taps come in a variety of styles. Each has its own potential problems, such as tendency to leak, relative permeability to oxygen, and expense. Currently, the tap is considered the prime source of oxidized wine in bag-in-box packaging (Armstrong, 1987).

To minimize oxidation, the bags are placed under vacuum before filling, and the headspace is charged with inert gas after filling. To protect the wine against microbial spoilage, and to limit oxidation, the wine is usually adjusted to a final level of 50 mg/liter sulfur dioxide before filling.

Wine Spoilage

Cork-Related Problems

The difficulties associated with off-odor identification are well illustrated with cork-related faults. The origins of cork-related problems are diverse and their chemical nature often unknown. Even experienced tasters have great difficulty in correctly naming most faults. A difficult situation is made more complex if a wine is affected by more than one fault. Wines may be affected by several, distinct, off-odor compounds. Combinations of off-odors may influence both the quantitative and qualitative perception of the faults. Correspondingly, most off-odors can be identified with confidence only with sophisticated analytic equipment. Although much has been learned to date, considerably more needs to be known to limit the occurrence of off-odors.

Although several cork-derived taints have a "musty" or "moldy" odor, others do not. Therefore, cork-related taints are usually grouped relative to presumed origin.