WINE

Introduction

Wine may be defined as the usually fermented juice of a plant product (as a fruit) used as a beverage. In many countries wines have from time immemorial been produced from all manner of plant materials and not only from fruits.

The conversion of raw food materials into finished fermented products is often considered to be one of the best examples of “value added” processing. If this is the case, then perhaps no other process or product exemplifies this more than the fermentation of grapes into wine. Wine making rely heavily on modern microbiology and biochemistry, traditional techniques and tried and true manufacturing practices are still important, and in many cases, necessary to produce high quality products. Thus, wine making serves not only as an example of value-added processing, but also as an example of an ancient technology that has adopted twenty-first century science.

History

The history of wine is nearly as old as the history of human civilization. The earliest writings discovered on the walls of ancient caves and in buried artifacts contain images of wine and wine-making instruments. Wine is mentioned more than 100 times in both the Hebrew and Christian bibles and many of the most well know passages involve wine. The very first vines, for example, were planted by Noah, who presumably was the first wine maker; later Jesus performed the miracle of turning water into wine. Wine also was an important part of Greek and Roman mythology and is described in the writings of Homer and Hippocrates. For thousands of years, even through the present day, wine has had great ritual significance in many of the world’s major religions and cultures, and it is an important part of the world economy and commerce.

Grape cultivation (viticulture) and wine making appears to have begun in the Zagros Mountains and Caucasus region of Asia (north of Iran, east of Turkey). Domestication of grapes dates back to 6000 B.C.E., and large-scale production, based on archaeological evidence, appears to have been established by 5400 B.C.E. A fermented wine-like beverage made from honey and fruit appears to have been produced in China around 7000 B.C.E., and rice-based wines, similar to modern day sake, were produced in Asia a few thousand years later. Wines were imported into France, Italy, and other Mediterranean countries by seafaring traders sometime around 1000 B.C.E., and vines and viticulture techniques were likely introduced into those regions several centuries later.

Wine is also one of the oldest of all fermented products that has been commercialized, mass produced, and studied. In fact, many of the early microbiologists and chemists were concerned with wine making and wine science. Less than 150 years ago, when the very existence of microorganisms was still being debated, Pasteur showed that not only did microorganisms exist, but that they were responsible for both production and spoilage of wine. Of course, wine preservation has been important since ancient days, when early Egyptian and Roman wine makers began using sulfur dioxide (in the form of burned sulfur fumes) as perhaps the first application of a true antimicrobial agent.

Wine Manufacture Principles

Making wine, as far as the actual steps are concerned, looks to be a rather simple and straightforward process (Figure 1). Grapes are harvested and crushed, the crushed material or juice is fermented by yeasts and bacteria, the organisms and insoluble materials are removed, and the wine is aged and bottled. In reality, the process is far from easy, and each of these pre-fermentation, fermentation, and post-fermentation steps must be carefully executed if high-quality wine is to be consistently produced.

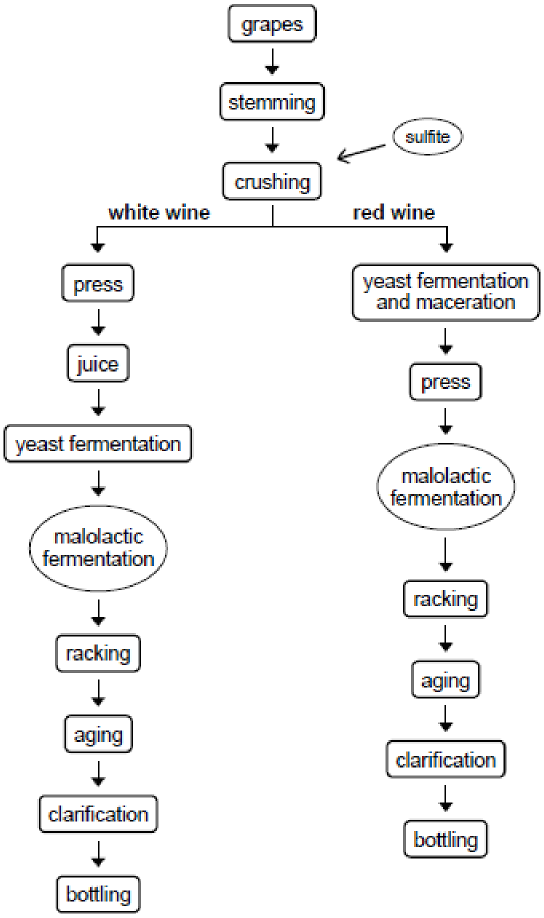

Figure 1. Flow chart of wine manufacture.

The main difference between the manufacture of red and white wine is that the grapes for red wines are fermented in the presence of skins and seeds (maceration), whereas for white wines, these materials are removed after crushing. Although optional, sulfiting agents are added to most U.S. wines. Whether the malolactic reaction is induced or encouraged depends on the grape composition, the style of wine, and the manufacturer’s preferences.

Harvesting and Preparing Grapes for Wine Making

According to both viticulturists and enologists, the first step in wine making is considered to be one of the most important. Grapes must be harvested at just the right level of maturity. This means that the concentrations of sugars and acids (and the sugar/acid ratio), pH, the total soluble solids, and even the phenolic constituents must be at just the right level for the particular cultivar and the type of wine being made. In addition, berry size and weight also influence the time at which grapes are harvested. In general, grapes should be sampled sometime before their expected harvest time and their composition assessed (at minimum °Brix and pH should be measured) to make sure that over-ripening does not occur.

Unfortunately, there is no exact or objective set of rules to ensure or predict the optimum time for harvesting grapes. Rather, grapes are frequently harvested based on more subjective criteria. As grapes ripen on the vine, the sugar concentration, as well as flavor and color components, increase, and acids usually decrease, so identifying the correct moment for harvesting can be a real challenge. It is possible, moreover, for grapes to over-ripen, such that the harvested grapes contain too much sugar or too little acid or be too heavily contaminated with wild yeast and molds. Once the grapes have been deemed properly mature, it is essential that they be picked and harvested quickly, since the composition can continue to change.

Even in this twenty-first century, when so much of modern agriculture has become automated and mechanized, a sizable portion of grapes for wine making is still harvested manually. Only recently has mechanical harvesting begun to displace manual harvesting. In the United States, the majority of grapes are now harvested by mechanical means; however, manual picking of grapes is still done for premium quality American wines and in much of Europe. Manual harvesting is more gentle on the grapes, and bruising and breaking of the grapes is minimized. For certain wines, such as sweet wines made from noble rot grapes, or wines for which grape harvesting methods are regulated (e.g., French Champagne), manual harvesting is required. Anyone who has seen the odd contours and steep terrains of some of European vineyards will also appreciate the necessity of manual grape picking. On the other hand, mechanical harvesters are faster and cheaper, and, unlike hired laborers, deployable on short notice and available around the clock.

Once the grapes are removed from the vines, they must be transported to the winery. It is important that the grapes not be bruised, crushed, or otherwise damaged either during harvesting or transport, since this encourages growth of microorganisms prior to the actual start of the fermentation. For the same reason, transportation time is also important.

Crushing and Maceration

The purpose of crushing is to extract the juice from the grapes. Before the grapes are crushed, however, leaves, large stems, and stalks are removed. Some wine makers may not remove all of the stems to increase the concentration of tannins and other phenolic compounds that are present in the stems and extracted into the juice. Once the extraneous material is separated and removed, the grapes are crushed by one of several types of devices. Roller crushers consist of a pair of stainless steel cylinder shaped rollers. Another type of crusher, called the Garolla crusher, not only performs the crushing step, but also removes stems. It consists of a rotating shaft contained within a large horizontal stainless steel cylinder or cage. Arms on the shaft are attached to paddles or blades such that when the shaft turns, the grapes are moved and pressed against the side of the cylinder. Perforations on the walls of the cylinder allow for the juice (along with the skin, seeds, and pulpy material) to pass through into collection vats, whereas the stems gather at the end.

The crushed grape material, as noted above, contains juice, seeds, and skins. Pigments, tannins, and other phenolic compounds are located in the skins and seeds, and their extraction into the juice takes time. Endogenous pectinases and other hydrolytic enzymes within the grapes enhance extraction and must also be given time to work. This extraction step, where the crushed grape material is allowed to sit, is referred to as maceration.

Maceration conditions are not the same for all wines. For red wines, where pigment extraction is especially important, long maceration times at high temperatures are usually employed. In general, maceration is done at around 28°C for up to five days. The shorter the maceration times and the lower the temperature, the less material will be extracted. Thus, lighter red wines, such as Beaujolais, are macerated for just a few days at no higher than 25°C. In contrast, deeper red wines, such as Bordeaux, are macerated for up to twenty eight days at 30°C. Since fermentation begins shortly after the grapes are crushed, maceration and fermentation essentially occur at the same time. In fact, the ethanol made by fermenting yeasts enhances extraction. This situation only occurs, however, if the musts are not treated with sulfur dioxide.

As noted above, maceration at low temperatures (<15°C) ordinarily results in only moderate pigment extraction and little fermentation. However, if the must is macerated at a low temperature (between 5°C and 15°C), but for longer time, extraction of anthocyanins and aroma and flavor compounds can be enhanced. This technique, called cold maceration, simulates the natural conditions in cooler wine-producing areas, such as the Burgundy region of France. For white wines, the maceration step is done at lower temperature and for much less time. Typically, only a few hours at 15°C is sufficient. For most white wines, the producers remove the seeds and skins immediately after crushing. As for red wine, the maceration conditions used for white wines influence the amount of pigments and tannins that are extracted. Wines made from Sauvignon blanc grapes where little maceration occurs typically have a low phenolic concentration, whereas Riesling and Chardonnay musts, which are often macerated in the cold, may contain appreciable amounts.

Sulfur Dioxide Treatment

As soon as the integrity of the grapes has been compromised by the crushing step, the sugars in the juice are liberated and made available for whatever microorganisms happen to be present. Ordinarily, the must is populated by epiphytic yeasts (that is, yeasts that reside on the surface of the grapes) and by yeasts that have “contaminated” the crushers, presses, and other wine-making equipment. Although the surface of a single grape may contain only about 10^2 to

10^4 yeast cells, after the grapes have been exposed to the contaminated equipment, the number of cells increases about 100-fold, to about 10^4 to 10^6 cells per ml. Whether this resident microflora actually commences a fermentation, however, depends on the intent of the wine maker.

Two options exist. First, a spontaneous or natural fermentation may be allowed to proceed ("natural" simply means that pure starter cultures are not added). In this case, except for temperature control, essentially no other restrictions are placed on the fermentation, and yeast (and bacterial) growth occurs with just a relatively short lag phase. The other option is to start the fermentation, under controlled circumstances, with a defined yeast starter culture selected by the wine maker. The latter option usually requires that the indigenous microflora be inactivated, so that it does not compete with and possibly interfere with the added culture.

When starter cultures are used, the naturally occurring or so-called wild yeasts are inactivated in one of two ways. First, the must can be heat treated, usually via a high-temperature, short-time (or flash) pasteurization process. Although very effective against most organisms found in musts, even moderate heating is often detrimental to the juice and to the wine. Thus, this process is rarely used. The preferred method is to chemically pasteurize the must by adding sulfites. It should be noted that even naturally fermented wines, especially white wines, are often sulfite-treated to control undesirable organisms. The most common sulfating agents are SO_2 gas and potassium bisulfate salts. Sulfites are cheap, effective, and multi-functional. In addition to their effectiveness against wild yeast, these agents also inhibit growth of acetic acid-producing microorganisms, malolactic bacteria, and various fungi. Sulfites can thus be considered as serving a preservative function in wine. Importantly, they also control several deleterious chemical reactions, particularly oxidation and browning reactions.

The amount of sulfite added and when it is added varies depending on the condition of the grapes, the microbial load, must pH and acidity, and the type of wine (red or white). Musts from mature grapes that often contain high levels of wild yeast require more SO_2, but in general, about 80 mg/L is sufficient. Also, the lower the pH, the less sulfite is necessary for antimicrobial activity. Due to human health concerns, however, there are also regulations that dictate how much sulfite can be present in wine. Usually, SO_2 or sulfite salts are added to the must just after crushing.

Other Pre-treatments

It is permissible to add other materials, besides sulfites, to the must to enhance extraction, modify the composition, or promote fermentation. For example, pectic enzymes can added during crushing to facilitate extraction of juice from skins and later during pressing to improve clarification. This practice is quite common for white wines manufactured in the United States. As noted above, it is not uncommon for some grapes, and juices from those grapes, to have either too low or too high of a pH. Thus, either acids, such as tartaric acid, or neutralizing salts, such as calcium carbonate, can be added to adjust must pH. Finally, nutrients that enhance yeast growth and fermentation can be added. These yeast growth factors usually are added to white wine juices, since shorter extraction times result in lower nutrient concentrations. Nutrients added to juices include mainly ammonium salts and various vitamins.

Microbial Ecology and Spontaneous Wine Fermentations

In the absence of SO_2 addition, the indigenous microflora is relied upon to initiate and then carry out a spontaneous or natural fermentation. This is one of the most well studied of all fermentations, and much is now known about the ecology of wine and the yeasts that participate in the wine fermentation. In reality, however, the yeast fermentation is but one of two distinct fermentations that occur in wine making. Yeasts, of course, ferment sugars to ethanol, CO_2, and small amounts of other end products. A second fermentation, called the malolactic fermentation, is carried out by specific lactic acid bacteria that are either naturally present or added for this purpose. The malolactic fermentation, to be discussed later, is now regarded as nearly as important to wine quality as the ethanolic fermentation.

As noted above, the surface of grapes usually contain less than 10^4 yeast cells per grape (or per ml of juice).This number may increase during ripening on the vine, especially if the temperature is warm. Although ten or more yeast genera may be represented, the primary organism most frequently isolated from grape surfaces and the fresh must is *Kloeckera apiculata*. In contrast, *S. cerevisiae*, the yeast most responsible for the wine fermentation, is rarely observed on grapes. Rather, *S. cerevisiae* and other related strains are introduced into the must during grape handling and crushing steps directly from the equipment. The must is inoculated, in other words, by the yeasts originating from the grape surface as well as by those residing on the winery equipment.

Despite the large amount of available carbohydrates and other nutrients, the must is actually quite a selective environment. The pH is typically below that which many organisms can tolerate, and the organic acids present in the must have considerable antimicrobial activity. The high sugar concentration and resulting high osmotic pressure can also inhibit many of the indigenous organisms. Eventually, the CO_2 formed during the early stages of fermentation makes the environment anaerobic, restricting growth of aerobic organisms. Likewise, enough ethanol is produced to provide selection against ethanol-sensitive organisms. Finally, sulfites, if added, have antimicrobial activity against a wide range of yeasts and bacteria. Thus, as the environment changes, yeast species that are numerically dominant may be displaced by other species or strains better suited to the environment at any particular time.

Members of the genus *Saccharomyces* represent less than 10% of the initial yeast population. The early yeast population is dominated by *K. apiculata*. This organism produces up to 6% ethanol from glucose and fructose; however, it has a low ethanol tolerance, being inhibited by 3% to 4% ethanol. Thus, even though *K. apiculata* is among the first to grow in the must, its numbers are not usually maintained beyond the first several days. However, it is thought that this organism does produce small amounts of acids, esters, glycerol, and other potential flavor components, some of which may or may not be desirable. As the ethanol concentration increases and the Eh (or redox potential) is reduced, due to CO_2 formation,the environment begins to select for *S. cerevisiae* and other various ethanol tolerant *Saccharomyces* spp.

According to current yeast taxonomy most wine yeasts are now classified simply as *Saccharomyces cerevisiae*. Thus, although different *Saccharomyces* strains may be present in musts during the course of a fermentation or in musts of varying composition (e.g., high sugar, low pH, etc.), these strains are all *S. cerevisiae*. It is interesting to note that *S. cerevisiae* is especially well-adapted to the wine environment and appears to have been the predominant wine strain for thousands of years.

Separation and Pressing

After the maceration step, or in the case of most white wines, almost immediately after crushing, the juice is separated from the seeds, skins, and pulp (collectively referred to as the pomace). For red wines, some fermentation will have already occurred prior to the separation step, whereas for white wine, fermentation follows the separation and clarification steps. The juice that separates from the pomace simply by gravitational forces is called the “free run.” Screens are typically used to catch any large particles. The free run juice is pumped into vats or barrels. Since the free run juice contains less than 75% of the total juice volume and the rest is present within the pomace, the latter is usually pressed to recover the remaining juice. Several types of presses and configurations are used. Hydraulic or pneumatic wine presses squeeze the juice from the pomace. Screw- or auger type devices force the juice against perforated cylinder walls and have the additional advantage of being continuous. The so-called first press juice can be collected and either added back to the free run juice or kept as a separate portion.The free run fraction is considered to have an appreciably higher quality and is used for premium wines. Juices containing mixtures of free run and pressed fractions are used for lower quality wines. Finally, for white wine, the juice is clarified to remove any remaining solids. Clarification is done via either settling and decantation, filtration, or centrifugation.

Fermentation

The wine fermentation begins as soon as the grapes are crushed. However, when a starter culture is used and SO_2 is added to control the indigenous organisms, limited ethanol fermentation will occur prior to addition of the culture. In the case of white wine production, the culture is added to the must after pressing and clarification, whereas for red wine, culture addition is done prior to seed and skin removal. Thus, for red wines, fermentation occurs during maceration, just as it would for a natural fermentation. The amount, concentration, and form of the culture depends on the type of wine being produced, the composition of the grapes, and other considerations specific to the wine manufacturer. Of course, the culture’s main responsibility is to produce ethanol from sugars, but the criteria for culture selection actually includes many other properties. For most wines, the culture inoculum, whether in a rehydrated dried or active liquid form, should provide about 10^6 cells per ml of must.

Traditionally, fermentations were performed in open barrels or vats with a capacity of 500 L or less. Such barrels still are used today; however, enclosed stainless steel tanks are now more common. The latter have several advantages. They are easy to clean and disinfect and often can be sterilized. Airborne microorganisms are less likely to contaminate the wine. Various control features, including temperature control and mixing and pumping activities, are easily incorporated into the design, and are usually computerized. Finally, modern tanks can be quite large, with capacities of more than 250,000 L.

The temperature of incubation depends on the type of wine being produced. In general, white wines are fermented at lower temperatures than red wines. For example, many wineries control the temperature between 7°C and 20°C for white wine and between 20°C and 30°C for red wines. Some wineries prefer low incubation temperatures for all wines, because less ethanol is lost to evaporation and fewer of the volatile flavors are lost. In addition, low temperature incubations result in overall higher ethanol concentrations and less sugar remaining at the end of the fermentation (assuming time is not a factor). Although *S. cerevisiae* has a lower growth rate as the temperature decreases, the diversity of metabolic end products may actually increase, enhancing flavor development. In addition, lower temperatures may favor growth of *K. apiculata* and other wild yeasts (at least when they are not inactivated by SO_2) that produce various volatile compounds, making the wine aroma and flavor appear more complex.

It is critical to recognize that the wine fermentation is exothermic and a considerable amount of heat may be generated. Some of this heat is gradually lost or dissipated into the environment without ill effect. However, in large volume fermentations, in particular, much of this heat is retained, raising the temperature of the wine. For example, if the initial temperature starts at 20°C, the temperature can increase 10°C or more. If the temperature were to rise above 30°C, the yeast may become inhibited or stop growing altogether. The wine will contain less ethanol and more residual sugar. Such fermentations are said to be “stuck.” Although other factors may cause a wine fermentation to become stuck, high temperature is the most common reason. It is, therefore, essential that the appropriate temperature is maintained. For fermentations conducted in modern, stainless steel, jacketed vats, coolant solutions can easily be circulated, externally. Alternatively, internal cooling coils can also achieve the same effect. The cooling requirement can also be met, especially for white wines, by simply locating fermentation barrels in cold rooms or cellars. However, the need for adequate cooling has led even some traditional wine manufacturers to abandon oak barrels and casks in favor of stainless steel vats.

The actual fermentation period is not long. After culture addition, the yeasts enter a short lag phase (from a few hours up to a day or two) that is then followed by a period of active growth (log phase) that lasts for three to five days. If the fermentation is conducted at lower temperatures (10°C to 15°C), the lag and log phases can be extended for several days. Conversely, if the yeast culture is highly active at the outset, by virtue of having been previously propagated under ideal growth conditions, the cells will almost immediately enter log phase. Although one might expect that a natural fermentation would take longer, in fact, growth of the indigenous yeast begins so soon after crushing, that the lag phase is barely noticeable.

During the log phase of growth, when an active fermentation is occurring, a layer of CO_2 forms across the surface. In red wine production, some of the pomace will float to the top and be trapped within this CO2 layer, forming a dense blanket or cap. Since the pigments and tannins are present in this thick cap layer, a mixing step is required to return these substances back into the fermenting must. The temperature in the cap can also become elevated, supporting growth of undesirable thermophilic bacteria; thus, mixing serves to maintain a more uniform temperature. Various techniques exist for this mixing step (called pigeage). In the "pumping over" technique, a portion of the must is periodically removed from the vat and pumped onto the cap. Alternatively, the cap can be "punched down," either manually or via mechanical means. In modern wineries, automated pumping and punching systems are used to mix the pomace cap into the fermenting must.

The fermentation of white wine occurs after the must is pressed and clarified. After about the seventh or eighth day of fermentation, cell numbers may begin to decline, representing the end of the primary ethanolic fermentation. Some yeast strains will flocculate or clump together, causing them to settle to the bottom of the tank. This is a desirable property that enhances their removal later during the racking and clarification processes. The fermentation is considered complete when all or most of the sugars are depleted, as determined by a decrease in the Brix value. For red wines this may take as long as five to six weeks. If less than about 0.5% sugar is present, and there is no apparent perception of sweetness, the wine is considered to be dry. Of course, not all wines are intended to be dry. If some sugars are present in the wine after fermentation (or if sugars are added), a sweet wine results.

For red wines, much (if not all) of the fermentation occurs in the presence of the pomace. When the extraction of pigments, tannins, flavor compounds, and other materials is considered sufficient, or when the desired ethanol concentration is reached, the free run juice is separated from the pomace and moved into another tank. The fermentation is then completed (if not already). In the meantime, the pomace is pressed and is either fermented separately from the free run or is mixed with the free run for the final fermentation. Since the pressed wine is rich in pigments and tannins, adding a portion back to the free run wine makes the final product richer in color and flavor.

Adjustments, Blending, and Clarification

After the fermentation is complete, the wine will contain little or no sugar and about 12% to 14% ethanol. Still, because of differences in grape composition, microflora, and wine manufacturing practices, variations in wine composition and sensory quality are to be expected. Therefore, adjusting the wine after fermentation (and sometimes before) is a normal step. The pH and acidity, in particular, can vary markedly, as can the color and flavor. Therefore, some wineries adjust the acidity of wine by acidification or deacidification steps (e.g., by adding acids or neutralizing agents).

When acidity of wine or must is due to malic acid, deacidification is managed via the malolactic fermentation. Adjustment to wine color and flavor can also be done, if legally permitted, by filtration and enzyme treatments, respectively. Filtration techniques, for example, are most often used to "decolorize" wine by removing undesirable pigments.

Except for very small wineries, which may have only a few vats of wine, most modern wineries have many individual vats of wine. Each one is unique, in that a particular vat may contain wine made from grapes harvested at a time or place different from the grapes in a neighboring vat. Wines within a single winery may be made from different grape varieties. Therefore, another common procedure, especially for premium wines, is to blend different wines to optimize or enhance the organoleptic properties. Blending also produces wines with consistent flavor, aroma, and color from year to year. Perhaps more so than any other winemaking step, however, blending is a tricky business, and is a highly subjective process. Success relies on the imagination, creativity, and skill of the wine blending specialist.

At the end of the fermentation, the wine contains non-soluble proteins and protein-tannin complexes, as well as living and dead microorganisms. These materials give the wine a cloudy, hazy, undesirable appearance. The clarification step removes these substances from the wine without removing desirable flavor and aroma components. It is particularly important that the cells are removed. If left in the wine, these cells can lyse, releasing enzymes that may catalyze formation of off-flavors and odors (although an exception to this rule exists, as described below). Inducing precipitation of tartrate salts and tannin-protein complexes is also commonly done to facilitate their removal before they precipitate later during aging. It is important to recognize that, in some cases, cell lysis may be a good thing. Intracellular constituents released during cell lysis include amino acids and nucleotides, providing nutrients that are later used by bacteria in secondary fermentations or that contribute to the sensory properties.

According to traditional practices, wine is clarified by simply allowing the sediment, containing the yeasts and bacterial cells, as well as precipitated material, to settle naturally in barrels or vats. The wine could then be removed from the sediment (or "lees") by decantation. This process, called "racking", is usually done for the first time after three to six weeks following the end of the fermentation. Racking can be repeated several times over a period of weeks or months until the wine is nearly crystal clear. During the racking step, the wine is also aged (see below). Racking can now be done in enclosed tanks using automated transferring systems.

Filtration is another method used to clarify wine. This can be especially effective if fining agents, such as bentonite, albumin, or gelatin, are used as filtration aids. If micropore filtration membranes are used, it is even possible to sterilize wines. Clarification may occur after racking or after aging.

In contrast to removing the sediment shortly after the fermentation has ended, some wines are intentionally left in contact with the lees for an extended time before the first racking occurs. This traditional maturing practice, known as "sur lies," enhances the flavor, character, mouth feel, and complexity of the wine. It is more common for white wines than red.

Aging

Aging actually begins just after fermentation. Thus, aging occurs when the wine is racked, as well as beyond. Aging conditions vary considerably. Some wines are aged for several years, whereas others are "aged" for only a few weeks. Some wines are aged in expensive oak barrels, others in stainless steel, and yet others depend on bottle-aging, or a combination of all of the above. Whether a wine is aged for a long time in oak barrels or is quickly bottled and sent to market depends, in part, on marketing considerations, but also on the original composition of the grapes and how they are made into wine. Thus, long, careful aging should be reserved for only premium wines made from high quality grapes. By analogy, Cheddar cheese manufactured for the process cheese market cannot be expected to develop into a flavorful, two-year Cheddar, no matter how carefully it may have been aged. Of course, some excellent quality wines, like excellent cheeses, are meant to be consumed in a "fresh" or un-aged state, so whether or not a wine is aged does not distinguish wine quality, per se. For example, Beaujolais nouveau, a popular wine from the Burgundy area in France, is meant to be drunk after only a few weeks after the grapes are harvested. These wines are fruity and "gulpable"; no amount of aging will lead to their improvement.

To say that the actual events that occur during aging are complicated would be quite the understatement. Hundreds of enzymatic, microbiological, and chemical reactions occur, and as many as 400 to 600 volatiles, including esters, aldehydes, higher alcohols, ketones, fatty acids, lactones, thiols, and other compounds are formed (Table 1).The wine interacts with the wood and wood constituents in the barrel, oxygen in the air, and even the cork. It is important to recognize that not all of these reactions are beneficial in terms of wine quality, and some wines may actually deteriorate during aging. In fact, long aging is not good for most wines.

Table 1. Effects of aging on wine.

Reaction or step	Effects
Tannin precipitation	Color darkens; astringency increases initially, then decreases.
Wood cooperage	Phenolic and other flavors extracted.
Ester hydrolysis	Fruitiness decreases.
Oxidation	Browning and flavor reactions induced.
Evaporation	Concentration of nonvolatile solutes; color and flavor intensifies, but aroma volatiles decrease.

Ordinarily, in large wineries, fermentations occur in large tanks (exceeding 250,000 liters), and then the wine is moved into wooden 200 liter barrels for aging. However wine can also be aged, at least initially, directly in tanks, and then later moved into oak barrels for final aging.

In many European and other traditional wineries, in contrast, the entire aging period is conducted in oak barrels. The oak barrel or "cooperage" is so important to wine quality that entire industries devoted to oak tree production and cooperage construction have developed. This is because the oak barrels are not inert containers used simply to store wine, but rather they are a source of important flavor and aroma compounds. In fact, one of the major steps in barrel construction involves heating or “toasting” the barrels to promote pyrolysis. This generates a number of flavor and aroma volatiles. In the presence of wine, these compounds, along with tannins, phenolics, lignins, and lactones, are extracted from the wood and solubilized in the wine. Some of these compounds impart unique flavor notes, including vanilla and coconut. Aging wine in oak cooperage is not, however, without a downside. Oak barrels are expensive, and, even if carefully maintained, do not last forever. Loss of wine volume (and hence profit) due to evaporation can also occur. Thus, alternative materials, in particular, stainless steel, have displaced oak cooperage in many wineries. While it certainly does not contribute flavor and aroma compounds, stainless steel is less expensive, easier to clean and maintain, and can be fabricated to accommodate size and shape preferences. It is still possible for wine aged in stainless steel cooperage to obtain desirable oak-derived flavors by adding oak shavings or chips to the aging wine.

Malolactic Fermentation

A certain amount of acidity is expected and desirable in wine. Red wines typically have a pH of 3.3 to 3.6; white wines are usually slightly more acidic. Some grapes, and the musts made from those grapes, however, may contain high levels of organic acids, such that the pH is too low (i.e.,<3.5).Wines made from those grapes will suffer from excess acidity, a serious and readily noticeable flavor defect.

Of the organic acids ordinarily present in grapes, malic acid is particularly important because of its ability to influence pH. This is because malic acid is a four carbon dicarboxylic acid, meaning it contains two carboxylic acid groups and can release or donate two protons. Thus, musts containing high concentrations (0.8% to 1.0%) of malic acid are acidic and have a low pH. High malic acid concentrations are especially common in grapes grown in cooler, more northern climates, such as those in Oregon, Washington, northern California, and New York. Although many of the vineyards in Europe are located in warmer regions and produce grapes with less malic acid (a situation that may lead to the opposite problem—too little acidity), grapes from Germany, Switzerland, and even some regions in France can still contain significantly high malic acid levels. Also, some grape cultivars ordinarily contain more malic acid than others.

One way to reduce the malic acid levels and to “deacidify” the wine is to promote the biological decomposition of malic acid. This deacidification process occurs via the malolactic fermentation pathway that is performed by specific species and strains of lactic acid bacteria. These bacteria may be naturally present in wine and may, therefore, initiate the fermentation on their own. It has now become common to add selected malolactic strains, in the form of a pure culture, directly to the must.

The malolactic fermentation has been the subject of extensive research in the last two decades. The pathway was initially thought to involve two separate reactions (Figure 2A), in which malate is first decarboxylated by an NAD-dependent decarboxylase yielding pyruvate and reduced NADH. In the second reaction, pyruvate serves as the electron acceptor yielding lactic acid. Is it now known that the reaction is catalyzed instead by a single malolactic enzyme that decarboxylates malic acid directly to lactic acid (Figure 2B).Although this enzyme requires NAD (and manganese), no intermediate is formed. The net effect of the malolactic reaction is that malic acid, a dicarboxylic acid, is converted to lactic acid,a monocarboxylic acid, thereby reducing the acidity of the wine.

Several lactic acid bacteria have the enzymatic capacity to perform this fermentation. The most well-studied are species of *Oenococcus*, particularly *Oenococcus oeni* (formerly *Leuconostoc oenos*). Several species of *Lactobacillus* also have malolactic activity.Although some of these bacteria are found naturally in musts, commercial cultures are now available and are commonly used.

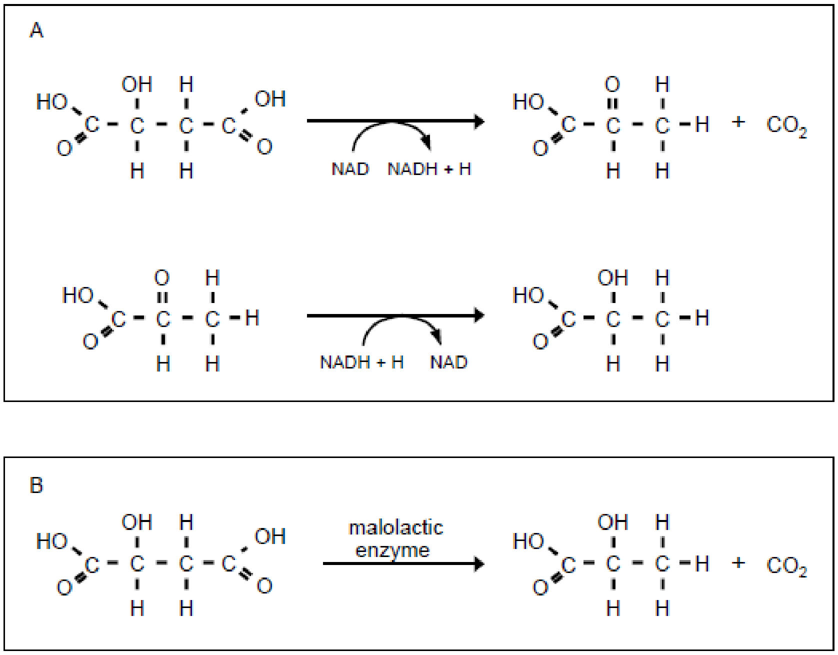

Figure 2. The Malolactic Reaction. The conversion of malic acid to lactic acid was originally thought to be catalyzed by two separate reactions, with pyruvic acid formed as an intermediate, and with NAD required as a co-factor (A). It is now well established that the malolactic reaction occurs as a single step, with lactic acid formed directly from malic acid via a decarboxylation reaction catalyzed by malolactic enzyme (B). No intermediate is formed.

The malolactic fermentation was one that interested microbiologists, not only because of its industrial importance, but also because there seemed to be no obvious reason for bacteria to perform this conversion. In other words, how do malolactic bacteria benefit, or more to the point, how do they gain energy, from the conversion of malic acid to lactic acid? The pathway, after all, contains no substrate level phosphorylation step that would lead to ATP formation, nor is there a change in the redox potential. As it turns out, there is a means of generating ATP via the malolactic pathway, but it is indirect.

As shown in Figure 3, malic acid is transported into the cell via one of two ways. In *Oenococcus*, the malate permease is a uniporter, whereas in lactococci and lactobacilli, uptake of malate is mediated by an antiporter that exchanges an incoming malic acid for an outgoing lactic acid. No energy is spent for malate transport in either system. The exchange reaction, however, is not electroneutral. This is because one extracellular molecule of malic acid, carrying a net electric charge of -2, is exchanged for one of lactic acid that carries a net charge of only -1. This charge difference arises as a result of the decarboxylation reaction, which consumes an intracellular proton. The bottom line is that the cell is able to extrude a proton (or its equivalent) without having to spend energy to do so. That is, the cell conserves energy, in the form of ATP, that it would ordinarily spend to pump protons from the cytoplasm to the extracellular medium.The proton gradient that forms, or the proton motive force, can then either perform other work (e.g., nutrient transport) for the cell or be used to drive ATP synthesis by the proton-translocating F_0 F_1 ATPase.

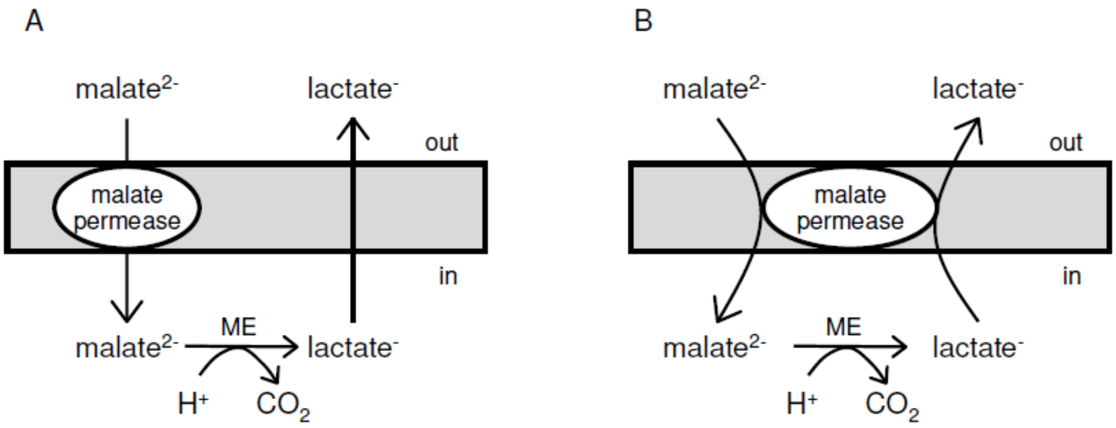

Figure 3. The Malolactic Reaction. The conversion of malic acid to lactic acid was originally thought to be catalyzed by two separate reactions, with pyruvic acid formed as an intermediate, and with NAD required as a co-factor (A). It is now well established that the malolactic reaction occurs as a single step, with lactic acid formed directly from malic acid via a decarboxylation reaction catalyzed by malolactic enzyme (B). No intermediate is formed.

It is important to note that in low-acid grapes, the malolactic fermentation is undesirable, since some acidity is desired in wine. Thus, under some circumstances, the presence of naturally occurring malolactic bacteria is undesirable and the source of potential defects. In contrast, the malolactic fermentation not only is performed for deacidification, but also to promote flavor stability and balance. Moreover, malolactic bacteria often produce diacetyl from citrate, which may, at the appropriate concentration (generally between1 mg/L and 4 mg/L), be desirable in some wines.

Types of Wine

Aside from the rather broad distinction of classifying wines based on their color—red, white, or rose—there are obviously more descriptive means for grouping different types of wine. Similarly, the procedures used for manufacture of wine have, so far, been described in a mostly generic manner. In this section, the manufacture of several well-known categories or styles of wine will be discussed, including sweet wines, fortified wines, sparkling wines, and distilled wines.

Sweet wines

Sweet wines are simply those that contain unfermented sugar (either fructose, glucose, or sucrose).There are several ways to produce a sweet wine. The easiest (and least expensive) way is to simply add sugar (up to 2% to 4%) or sucrose syrup to a dry wine. This results in a wine that is definitely sweet, but not necessarily one that would be acceptable to many consumers. A similar and more common approach is to add unfermented juice, preferably from the same grapes used to make the wine. Alternatively, sweet wines can be made by stopping the fermentation before all of the glucose and fructose have been fermented. In some cases, sugar may be added to the juice prior to fermentation, such that when the fermentation is complete, residual sugar (and sweetness) remain. This is one of the more common practices in the United States, and many of the sweet wines from New York state are produced this way. In either case, arresting the fermentation is the key step. Generally, this is done by rapidly cooling the wine, and then filtering out the yeast.

Fortified wines

Fortified wines are those to which distilled spirits (containing as much as 95% ethanol) are added to raise the total ethanol concentration to 15%. Not only do these wines contain higher concentrations of ethanol, the source of the ethanol (e.g., brandy) is also important since they may contribute unique flavor compounds to the finished product. Aside from this common feature, however, a variety of quite different fortified wines exist. Included are whites and reds, dry and sweet. Fortification usually occurs during or just after the fermentation. In some wines, the added ethanol may inhibit the yeast and prevent the complete fermentation of sugars, resulting in sweet dessert wines. The most well-known of the fortified wines are sherry and port.

Sparkling wines

Sparkling wines are those which contain carbon dioxide, providing bubbles and effervescence. For some sparkling wines, CO2 pressures as high as 600 kPa atmospheres can be reached (by comparison, the pressure inside a can of soda pop is less than 200 kPa). Although sparkling wines are made throughout the world, there are several manufacturing methods that are used to produce the CO2, and these methods define, to a certain extent, the type of sparkling wine being produced. Clearly, the most well known sparkling wine is Champagne, which is traditionally made, not surprisingly, via the Champagne method.

Champagne

Champagne is made from Chardonnay, Pinot Noir, and Pinot Meunier grapes grown in the Champagne district. This is a northern grape growing region and the still wines made from individual cultivars (the Pinots make red wines and the Chardonnay is used for white) are not particularly remarkable (some might call them insipid).However, when the base wines are appropriately blended (a skill first perfected by the monk Dom Pérignon), the wine assumes the best qualities of each individual cultivar.

Brandy

Brandy is produced by distilling wine. The wine can be made from other fruits, but when made from grapes, white wines are used as the base. The most well known brandy is Cognac, made from the Cognac district of France. In the United States, brandy must conform to a standard of identity that describes the starting fruit or juice, the ethanol concentration, the duration of aging, and other compositional and manufacturing details. Most American beverage-type brandies contain less than 50% ethanol (100° proof). In contrast, brandy used for fortification purposes usually contains 70% to 95% ethanol (140° proof to 190° proof). Due to evaporation, long aging can substantially reduce the ethanol concentration.