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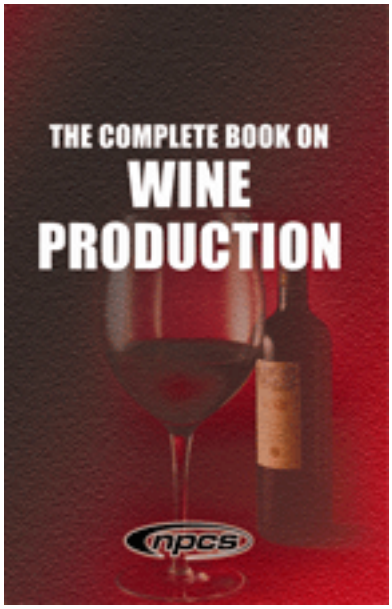
The Complete Book on Wine Production



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Wine is the most loved beverage across the world and a popular accompaniment with food. The popularity of wine in India has started growing rapidly. Wine is the fermented product of the grape. Because crushed grapes contain all that is needed to create wine, ancient wine producers simply allowed nature to take its course. As time went on, people realized that by intervening at certain times, they could make a wine with more predictable characteristics. Grape cultivation is one of the most remunerative farming enterprises in India. Grapes can be eaten raw or they can be used for making wine, jam, juice, jelly, vinegar. Delicate wine grapes are generally produced in frost free and moderate temperature environments. Thousands of grape varieties are grown all over the world; the wine grape varieties represent only a fraction of them. The colour, size, phenolic distribution and acidity of grapes give each wine its own characteristic. Wine quality is affected by the factors such as soil, climate, viticulture and wine making techniques. Wine quality is dictated mainly by the grapevines, not by the winemaker. Wine must be slightly aged to be drinkable. Grape production, linked with wine processing has provided the much-needed impetus for the growth of the wine industry. Indian government plays a crucial role in the current phase of Indian wine industry, supporting the current momentum amongst others through financial assistance and market protection. Gradual reduction of import duty levels will no doubt lead to increasing competition through imports, but will on the longer term result in a competitive industry that is able to export its top quality products to overseas markets.

Some of the fundamentals of the book are wine quality, mold and mold complexes associated with grapes, grape aroma components, soluble solids in winemaking, the molds and yeasts of grapes and wine molds, yeasts of grapes and wine, by-products of fermentation, chemistry of fermentation and composition of wines, outline of red wine making, stuck wines, white table wine, sparkling wine, vermouth and flavoured wines, cider and apple wine, plum wines in Europe, berry wines in pacific coast states, cherry and plum wines in pacific coast states, pomegranate wine from concord grapes, pineapple wine, pear wine, wine from oranges, grapefruit wine, wine from dried fruits, Swiss research on fruit juice fermentation honey wine (mead), etc.

This book provides a complete detail on all aspects of Wine production like describe the varieties of wine available, its manufacturing process, bottling and storage of wine, quality control in wine making and many more. It is hoped that this book will be very resourceful to all its readers, students, scientists, technocrats, existing industries, new entrepreneurs and all those who are related to wine making.

Content:

Sample Chapter:

The Composition of Grapes

The character and quality of a wine is determined by (1) the composition of the raw materials from which it is made, (2) the nature of the fermentation process, and (3) the changes which occur naturally, or are made to occur, during the post-fermentation period. This chapter considers the composition of grapes and the factors influencing it.

How Grapes Ripen

Amerine distinguishes physical and chemical changes which occur during ripening. Obviously, however, there is an intimate interrelationship between the two if changes in volume occur, as they do.

Physical Changes

The grape berry in its early weeks is a tiny, green-colored, very acid pellet. Cell division and a very slow berry enlargement continue for some time. Cell division ceases about the time noticeable changes in berry size, color, and texture occur.

The rate of increase in volume of the berry may show a slight decrease during the period of seed development, Fig. 1. Following this a very rapid increase in berry size occurs so that in a few weeks the tiny berry becomes a plump, sweet, colored fruit. The period of most rapid increase in volume does not exactly coincide with the start of ripening. Fig 1 indicates that the increase in soluble solids precedes this period by about two weeks.

The fruit does not continue to increase in size indefinitely. When the fruit has reached normal maturity there is a rather abrupt cessation of cell enlargement and under very warm conditions there may be a decrease in volume. Peynaud suggest harvesting one week after the fruit reaches its maximum weight. The decrease in weight occurs mainly by withdrawal of water from the fruit to the leaves—especially during periods of soil moisture deficiency. Poux also noted that increase in sugar content paralleled increase in berry weight. The ratio grams sugar per gram/total weight in grams was a constant for a given variety when the fresh weight was at its maximum.

Within the berry there are changes in the relative amounts of skins, seeds, and pulp during maturation. The changes on a per cent basis and on the basis of grams per 100 berries are shown in Fig. 2. On a per cent basis the pulp increases from about 73 to 89 per cent of the weight while the per cent seeds and skins decrease from about 13 to 4 and 8, respectively. On a per berry basis the increase in the weight of pulp is more striking and a slight increase in skin weight occurs. It should be noted that separation of the pulp from the skin and seeds is difficult and subject to manipulative and evaporation errors.

Another physical change which is of practical importance is that in turgidity. Unripe berries are hard and difficult to crush. As the fruit ripens there is an increase in turgidity. During overripening the fruit shrivels and there is a loss in turgidity—making the fruit again difficult to crush.

Another physical change occurs when the grape is attacked by *Botrytis cinerea.* This weakens the attachment of the skin to the flesh and makes the fruit easier to crush.

Besides the increase in glycerol and sugar there is a net decrease in malic and tartaric acids, especially in tartaric. The effect on the acidity and its intensity depends on the climate following botrytis attack. Gluconic acid is a constant product of botrytis attack. Amounts of 0.50 to 2.50 gm. per liter were reported in wines made from botrytised Bordeaux grapes. Normal wines had only about 0.29 to 0.92 gm. per liter. Charpentié suggested a minimum gluconic acid as a measure of the authenticity of botrytised wines. Galacturonic and glucuronic acids have both long been considered to be present in small amounts in musts and in larger amounts in musts or wines of grapes attacked by *Botrytis cinerea.* Tanner reported little glucuronic acid in normal or botrytised musts but considerable galacturonic acid.

The constant presence of glucuronic and galacturonic acids in musts and wines help to explain, the deficit of anions in the acid balance, the high dextro-rotatory condition of certain wines and some of the unknown

compounds that combine with sulfur dioxide. For 100 mg. of free sulfur dioxide, 1 milliequivalent of glucuronic acid will combine with 2 to 3 mg. of sulfur dioxide and 1 milliequivalent of galacturonic acid will combine with 5 to 6 mg. of sulfur dioxide. It is difficult to control field conditions to secure the optimum botrytis growth. Attack by other molds or rains may cause some sugar loss. Even under the best conditions considerable net sugar is lost by respiration—up to 30 per cent. Dextrose is attacked more by the mold than levulose so the dextrose/levulose ratio decreases.

Chemical Changes

The grape consists of a seed (except for a few varieties), surrounding pulp and on enclosing skin. The relative proportion of each of these and their composition changes during maturation. The present concept is that the grape ripens from the exterior to the interior, but there may be varietal differences in this. The pulp near the skin is thus, throughout the season, lower in acid and higher in sugar than that near the seed. Because of the increasing proportion of the fruit in the intermediate pulp zone, its composition dominates that of the total juice more and more as the season advances. This is of some importance in measuring ripening changes. In partially ripe or ripe fruit when the berries are turgid the free-run juice is mainly from the pulp near the skin and will be higher in sugar and lower in acid than the juice from the press. In overripe fruit where both turgid and nonturgid fruit are present the free-run may be lower in sugar and higher in acids than the press juice. This is due to the lesser proportion of juice from near the skin—and from the non-turgid riper fruit—in the press juice.

When whole grapes are pressed, as in the Champagne region of France, the first juice to be higher in sugar and lower in pH *and* in titratable acidity than that of later pressings. This is summarized in Table 1. Intermediate pressings were higher in acidity and lower in pH than the first juice, apparently because more tartrate-buffered material was present. Certainly few non-turgid berries are present so this represents the first case noted above. In California where shriveling is common the second case is more common.

The over-all changes in sugar, total acidity, and pH are shown for two varieties which ripen in mid-season and one which ripens very late in Fig. 3. During the more rapid stages of ripening in the warmer parts of California the Balling may increase 0.1º to 0.4º per day and for each increase of a degree Balling the acidity may drop 0.05 to 0.15 per cent. When ripening starts there is a continuous increase in per cent sugar in the fruit. The two sugars present, levulose and dextrose, do not increase at the same rate, as shown in Table 2. Dextrose is the predominant sugar in unripe fruit while levulose is at least equal and often higher in ripe and overripe fruit. Very little sucrose has been found in *V. vinifera* grapes; more is found in *V. labrusca* and other native species.

The titratable acidity decreases during ripening, whether expressed on a per berry or on a per cent basis. Since the acids are translocated into the fruit from the leaves the changes in acids occurring in the leaves during ripening are noteworthy. Amerine report a continuous increase in malates in the leaves. The tartrate content of the leaves, generally decreases slightly during maturation.

Grape Maturity and Quality

Wine Quality

Quality is a subjective judgment that depends on the degree to which the wine is satisfying and balanced and reflects the character of the grape. It can be described in nine categories: color (hue, strength, purity, and stability), aroma intensity, vitality (purity), complexity, subtlety, palate strength, length, balance, and longevity. Hue refers to the dominant color wavelength, strength to the depth of color, and purity to the degree of “off” or tawny tones. Intensity refers to the magnitude of aromas and vitality to the quality and purity of those aromas. Complexity denotes the harmony of wine components. Delicate, refined flavors, strength of palate, length of finish and balance, or the entire intergration of the wine, and longevity or conservation, are also important quality factors.

Quality components are largely the results of fruit characteristics governed by the parameters shown in Fig.

1. Overlaid on basic grape quality is the mark of the winemaker, who can adjust grape growing and wine-making to emphasize or mute aromas, flavors, and textures to produce a well-balanced, integrated product. Wine styles differ bacause of the tremendous number of variables in grape growing and in winemaking, as discussed below.

Clones

A clone is a population of plants all of which are descendants by vegetative propagation of a single parent vine. many grapevine cultivars may not be clones, but mass selections. However, such a distinction may be academic. When a mutation occurs in a dividing cell, all cells derived from that individual cell carry that genetic change. All vines produced from the mutant shoot constitute a clone, yet are similar enough to their mother vine to have the same varietal name.Clonal variation can affect yield, setting rate, growth, clusters per vine, berry size, fruit rot susceptibility, and berry flavor components. The latter two are of particular interest.

Variations in vineyard and winemaking techniques likely obscure all but the most pronounced effects of clones on wine style and quality. However, the identification of superior grapevines within cultivars remains an important goal.

Climate

Climatologists recognize three levels of climate: macroclimate or regional climate, meso- or site climate, and micro- or grapevine canopy climate. Wine quality is influenced by the mesoclimate, particularly temperature, during the final state (stage III) of berry ripening. Jackson and Jackson have divided grape-growing regions into two temperate zones: alpha zones with mean temperatures between 9-15ºC (48ºF-59ºF) during stage III ripening; and beta zones with mean temperatures greater than 16ºC (61ºF).

The best variety for any region is one that matches the length of the growing season, so that maturation occurs even during the coolest portion of the season. The optimum choice of cultivars should allow for fruit maturity just before the mean monthly temperature drops to 10ºC/50ºF. In warmer climates, the length of the growing season easily allows for adequate fruit maturity but results in fruit development during the warmest part of the season.

Microclimate is determined by the presence of plant cover. Grapevine leaves are the major cause of microclimate variations; the presence of fruit, shoots, stems, and permanent vine parts are less significant. In the sense that grapevine canopy influences microclimate, it is under the control of the viticulturist. Canopy microclimate components include radiation, temperature, humidity, and evaporation. Berries maturing in densely shaded canopy interiors are generally associated with the following when compared with berries in open or exposed canopies: (1) low total soluble solids, (2) high titratable acidity, (3) high malate concentrations, (4) elevated pH, (5) high potassium, (6) low proline, (7) high arginine, (8) low total phenols, and (9) low anthocyanin concentration in red and high chlorophyll vs. flavonoid pigments in white cultivars. These differences are due to exposure to sunlight and heat (see Tables 1 and 2).

Sunlight can affect vine and grape physiology through photosynthetic and thermal responses. The amount of diffuse solar radiation reaching the interior canopy leaves and fruit decreases geometrically as the number of leaf layers increases, resulting in a reduction in photosynthetic rates. Varying shoot numbers, reducing vine vigor, or adopting training and trellising systems that divide canopies into separate, thin curtains of foliage improve grapevine microclimate and enhance grape quality. For vineyards already planted to conventional trellis designs, alternative methods of decreasing canopy shade may be desired. Canopy microclimate can be improved with selected fruit zone leaf removal which can result in reduced fruit rot incidence. Increased spray penetration, desiccation, and reduction of evaporation potential will likely contribute to rot reduction. For a review of grapevine canopy microclimate evaluation and management.

Terroir

Although the precise contribution of each factor influencing the terroir (or soil-plant environment) is not

known, experience suggests that a ‘good’ terroir encourages slow, yet complete maturation. Seguin suggested that a desirable terroir has adequate but not excessive nitrogen, the ability to moderate the influence of heavy rain, especially after veraison, as well as to moderate drought stress. The influence of soil on wine quality is widely debated. Rankine concluded that soil composition is less important than climate. Jackson reported that soil is known to have several direct influences on plant growth by affecting moisture retention, nutrient availability, heat and light reflecting capacity, and root growth as a result of penetrability. Soils that help promote excessive vegetative growth may negatively influence quality by altering canopy microclimate.

Vineyard Yield

Yield is an important economic and production consideration. The majority of vineyards producing quality wines tend to be those having low to moderate yields. Because high yield may delay maturity, direct effects are not easy to measure. The leaf-to-fruit ratio is considered to be an important factor influencing fruit sugar and the other components. For example, McCarthy found that potential volatile terpenes (PVT) were higher in low-crop vines. Kingston determined that 15 cm2 leaf area per gram of fruit was optimum for soluble solids production. Yields can be estimated if both the average number of clusters per vine and the average cluster weight is known. Cluster counts may be determined on a vine-by-vine basis before bloom when they are easily visible whereas cluster weights are determined on a shoot-by-shoot basis near veraison when weights begin to stabilize.

Fruit Quality and Soluble Solids

Maturity Sampling

Grape maturity assessment as well as fruit quality is critical to the successful production of palatable wines. Part of the grower’s payment for the fruit often is based upon delivery of grapes at agreed-upon parameters: so care must be taken in the maturity monitoring process. Traditionally, degrees Brix (ºB, weight percent sugar in the juice) and titratable acidity (g/L of acid in the juice) have been selected as the harvest parameters most important in monitoring maturity. Because of the importance of fruit maturity to ultimate wine palatability, field sampling of fruit must be performed in an objective and statistically acceptable manner.

Amerine compared sample collection techniques and reported that berry sampling can provide an accurate and economical sampling technique. Theoretically, to be within plus or minus 1º B with a probability level of 0.05 (95 out of 100 samples), two lots of 100 berries each should be examined. In cases where one wishes to be within plus or minus 0.5º B 95% of the time, five lots of 100 berries should be collected.

Berry sampling is difficult because of the time required and variations in fruit chemistry. The chemical composition of grape berries differs with their position on the rachis, the location of the cluster on the vine, and the location of the vine in the vineyard. However, properly performed berry sampling can provide an accurate picture of the overall vineyard fruit chemistry. Jordan recommend collection of berries from the top, middle, and bottom of the cluster. Terminal berries on the rachis may be less mature than other berries. Berry sampling in various locations on the cluster may be significant in the case of larger clusters; but many persons, sample by selecting berries only from the middle of the rachis, a technique that may be acceptable in the case of varieties with small clusters. Additionally, in a berry sampling procedure the side of the cluster from which the berries are taken must be randomized. One should avoid selection of fruit from ends of rows or from isolated vines or those with obvious physiological or morphological differences. Vine suggested an alternating-row method of sample selection. Significant variations in soil type shading, and so on, may play an important role in vine growth and therefore fruit maturity. Ideally, sampling should be designed to reflect differences in soil type, topography, and vine growth. For consistency, some recommend that selected vines within each block be targeted for sampling.

Samples should be collected at approximately the same time period each day. Jordan have made the

following sampling recommendations: (1) edge rows and the first two vines in a row should not be sampled; (2) samples should be collected from both sides of the vine; (3) for each row, estimate the proportion of shaded bunches and sample according to that proportion. This proportion may vary with the side of the row sampled because of variation in leaf cover. Smart demonstrated that white and red grapes exposed to direct sunlight may be as much as 8ºC and 15ºC, respectively, warmer than the ambient air temperature. Such exposure may have a dramatic effect on fruit composition. Kliewer summarizes these differences in Table 1. As the table shows, a sampling technique that does not consider the effect of exposure to the sun may not be accurate.

There is a natural tendency when one is picking individual berries to select those with the most eye appeal. These are often the more mature berries. Such bias in a vineyard sampling technique may result in Brix readings that are as much as 2º B higher than that measured by the winery after crushing the entire vineyard load.

Most growers begin sampling fruit several weeks prior to harvest. Initially, samples are taken once weekly, with a shift to more frequent intervals as harvest nears. Brix and titratable acidity are the traditional harvest parameters most important in monitoring maturity. Recently, the importance of pH, aroma, organic acid, etc., has been established.

Measurements of ºB, titratable acidity, and pH, by themselves, may not be specific indicators of physiological maturity or potential wine character and palatability. These parameters will vary considerably, depending upon the season, crop load, soil moisture, and so on. Maturity is clearly a multidimensional phenomenon, and should be viewed on a relative rather than an absolute basis.

There is considerable variation in the definition of optimum maturity for a particular wine grape variety. If maturity is defined as that point in time when the berry possesses the maximum varietal character consistent with the type and style of wine desired, then the assessment of varietal character is a logical adjunct and extension of conventional definitions of maturity. Further, the optimal maturity may vary somewhat with the style of wine to be produced. Long lists the parameters she considers important in affecting fruit development and maturity assessment, which are summarized in Table 2. The relative importance of each parameter is predicated upon the type and style of wine to be produced as well as the ability of the vine to continue to mature the crop. Several of these parameters are described in more detail below.

The Molds and Yeasts of Grapes and Wine

Molds

Molds are of importance in wine making, first, because of the damage they may do to the grapes before or after picking and, second, because they often grow in empty wooden cooperage imparting a moldy odor and flavor to the wine. They do not grow in wine because of the inhibitory effect of the alcohol.

Yeasts of the genus *Saccharomyces* are necessary in the fermentation of the must, but they and other yeasts may cause the clouding of bottled table wines. The flor yeasts, which represent a special group, are used in the making of Spanish sherries and certain Jura French wines. Wild yeasts, such as the apiculate yeasts and others, may be harmful to wine quality when they develop during fermentation, although in some cases they may produce flavors which give a distinct or unique character to the wines.

General Classification of Microorganisms

The complete Classification and description of the microorganisms of importance and interest to the wine maker would be beyond the scope of this book. A brief discussion of the more important forms only will be given.

Some of the fungi are saprophytes and can utilize only non-living substances for growth; others are parasitic and can attack living tissues. Examples of the former are *Penicillium* mold and wine yeast.*Saccharomyces cerevisiae* var. *ellipsoideus.* Typical fungal parasites are *Botrytis cinerea* (“noble

mold”) and *oidium* of the vine (powdery mildew).

Fungi may be unicellular or multicellular. Yeasts are higher fungi whose dominant form of growth is unicellular. Certain other fungi are always multicellular in growth. Still others may under certain conditions grow as one-celled organisms and later on under changed conditions become multicellular.

This chapter deals with the occurrence, morphology, and some other general properties of the yeasts and molds of importance in wine production.

Molds

Molds are distinguished by the formation of a mycelium. They differ from each other principally in their methods of producing spores and conidia, but there are also easily recognizable differences in the appearance of the mycelium and in the nature of the chemical changes which they induce in media suited to their growth. Variations in external and microscopical appearance, however, are not always reliable for identification and classification as the appearance is often affected profoundly by conditions of growth.

Primitive fungi form a mycelium without cross walls (non-septate), and the mycelium is termed “non-septate” or “coenocytic.” These molds are also termed *Phycomycetes.* The other molds, or higher fungi, possess a septate mycelium.

Some molds form yeast-like cells under certain conditions and may even induce feeble alcoholic fermentation, as do some of the *Monilia* and *Mucor* molds.

Many genera, species, and varieties or sub-species of molds have been described in the literature. They are usually aerobic, although they have been encountered as a feeble growth in bottled juices that have not been thoroughly pasteurized.

Penicillium

Molds of the penicillium group are most troublesome to the California wine maker. In the initial stages of growth *Penicillium* is white in appearance. Later, spores or conidia are formed in enormous numbers and give a powdery appearance to the growth, which is blue, green, or pink, according to the color and age of the conidia and the age of the culture.

P. expansum, (formerly *P. glaucum* is the best known of the penicillium molds and the one responsible for very great losses to fresh fruit shippers and fruit product manufacturers. The asexual spores of conidia are spherical in shape and are formed in great abundance upon upright hyphae or conidiophores. The conidia are light and are carried by air currents. They are universally distributed on surfaces and in the air.

This mold will grow on practically all food materials exposed to the air, if the conditions of moisture content and freedom from antiseptics permit the growth of any microorganism. It prefers sugar-containing substances such as fruits, fruit juices, jams, etc., but will also develop on such material as moist leather or the moist inner surface of empty wooden barrels or tanks. Any acid material affords a more favorable medium for growth than does an alkaline or neutral medium. After early fall rains it often grows in great abundance on cracked grapes rendering them unfit for wine making. The taste of these wines is very unpleasant, and on the basis of our experience very little is required to taint the wine. “Corked” wines may also have *Penicillium* sp. in the cork.

Growth is more abundant at temperatures ranging from 59° to 75ºf. but will occur at temperatures near freezing and slowly at temperatures of 95° to 98°F. It is known as a “cold weather” mold.

Alcoholometry

The alcohol content of a wine, influences its stability as well as its sensory properties. Wines are taxed, in large part, according to their alcohol levels. Careful monitoring of alcohol is important in stylistic wine production, as well as in carrying out accurate fortifications and in formulation of blends for bottling.

Yeast Metabolism

Fermentation

Microorganisms have varying requirements for oxygen. At the two extremes are the microorganisms that

require oxygen, the aerobes, and those that cannot survive in its presence, the anaerobes. Between these extremes are facultative microorganisms such as the yeasts, which are metabolically equipped to handle conditions where oxygen is either plentiful or limiting. Under oxidative (aerobic) conditions where concentrations of utilizable sugars are less than 3%, yeasts utilize the pathway outlined by the solid line in Fig. 1. Glucose is converted to pyruvate via the Embden-Meyerhott-Parnas (EMP) pathway (glycolysis) and subsequently to carbon dioxide and water via the tricarboxylic acid (Krebs cycle) and cytochrome oxidase pathways. Alternately, where oxygen is limiting, and a fermentable carbohydrate source is present, fermentative metabolism ensues, with pyruvate being decarboxylated to acetaldehyde and subsequently reduced to ethanol. This fermentative pathway is outlined by dashed lines in Fig. 1.

Anaerobiosis is not the only condition that favors fermentatitive metabolism. Even under conditions of relatively high oxygen, the presence of glucose at levels of more than 5%inhibits the activity of respiratory enzyme systems. This example of catabolite repression is generally referred to as the “Crabtree” or “counter Pasteur effect.” Under such conditions, fermentative rather than oxidative metabolism is observed. As glucose levels drop, TCA cycle enzymes are induced, and metabolism may shift from anaerobic (fermentative) to aerobic.

The energy differences between metabolic pathways are considerable. Anerobic metabolism yields only 56 kcal/mol of glucose, whereas aerobic metabolism (respiration) of this sugar produces 688 kcal/mol.

The importance of carbohydrate metabolism to the yeast is twofold. On the one hand, the cell is provided with a source of utilizable energy in the form of substrate-level phosphorylations involving adenosine triphosphate (ATP). Secondly, a variety of intermediate carbon compounds are formed in the process that are channeled into related biosynthetic pathways of the cell.

On an equivalent basis, glucose has a much greater energy potential than the energy generated by conversion of ATP to ADP. However, the importance of ATP lies not so much in its endogeneous energy content as in its direct involvement in energy-transfer reactions. The latter attribute resides in very reactive phosphate anhydride linkages.

The free energy of hydrolysis for ATP is reported as 7 kcal/mol, whereas for ADP and AMP the values are 6.4 and 3.0 kcal/mol, respectively. Therefore, ATP serves as an “energy carrier” whereby the energy produced from biological oxidation is harnessed (conserved) in ATP synthesis rather than liberated immediately in the form of heat. As such, this stored energy is available to drive cellular reactions requiring the input of energy. For brevity, only the salient points of the EMP pathway will be discussed further.

1. In addition to glucose, the monosaccharide fructose is fully fermentable. In this case, direct phosphorylation yields fructose-6-phosphate and subsequently fructose-1,6-diphosphate.
2. Formation of the trioses, dihydroxyacetone-phosphate and glyceraldehyde-3-phosphate, occupies a special position of importance to the wine-maker—namely, in the formation of glycerol, which is discussed in greater detail later in this chapter.
3. The initial phosphorylation of glucose to form glucose-6-phosphate and fructose to yield fructose-1,6-diphosphate utilizes two molecules of ATP. Direct energy in the form of ATP is recovered at two locations in the EMP pathway. First, the oxidation of 1,3-diphosphoglyceric acid to 3-phosphoglyceric acid is accompanied by phosphorylation of ADP. A second molecule of ATP is recovered in the formation of pyruvate from phosphoenolpyruvate.

One molecule of glucose produces two molecules of the triose phosphate, so a total of four ATP’s are generated per molecule of initial sugar. Subtracting the two ATP’s utilized in initial phosphorylation reactions, the pathway yields a not gain of two ATP’s. To emphasize the dramatic differences in energy yield between aerobic and anaerobic glycolysis, the major reaction sequences are summarized in Table 1.

The nucleotide NAD+ is reduced to NADH + H+ in formation of 1,3-diphosphoglyceric acid. Because intracellular concentrations of NAD+ are low, the cell must regenerate this compound if metabolism is to

continue. Under aerobic conditions, reduced NAD is reoxidized in the cytochrome oxidase system of the cell, yielding an additional six ATP's.

In the fermentative met-abolic mode, however, NADH + H+ is reoxidized in the reduction of acetaldehyde to ethanol. Fifty-six (56) kcal of energy (7 kcal/ATP) are potentially available in glycolysis, when coupled to oxidative pathways. However, during fermentation only 14 kcal (two ATP's) are produced. This amounts to a net efficiency with respect to ATP production of only 25% in anaerobic glycolysis.

Fermentations may be described as a series of redox reactions in which organic compounds (in this case sugars) are oxidized initially, and, at a later point, their products serve as terminal electron acceptors. Thus a fermentable compound must, ideally, be at some intermediary stage of oxidation; and carbohydrates fit this requirement. Furthermore, the end products and by-products of fermentation (ethanol and organic acids) are, themselves, at an intermediate stage of oxidation. As such, they may serve as reservoirs that may be available to the organisms growing under oxidative conditions (i.e., acetic acid bacteria).

In fermentation, acetaldehyde formed by decarboxylation of pyruvate may also combine with sulfite to form an addition product (Neuberg's second form of fermentation), as shown in Equation 2-1:

GLUCOSE+SO3-2®GLYCEROL+ACETALDEHYDE•+CO2 (1)

Thus the addition of large quantities of bisulfite acts as a block, preventing acetaldehyde from operating as an hydrogen acceptor for NAD. Under these conditions, the triose dihydroxyacetone phosphate replaces acetaldehyde as a hydrogen acceptor, resulting in formation of glycerophosphate in amounts equivalent to the quantity of acetaldehyde bound. Hydrolysis of accumulated glycerophosphate by phosphate enzymes then leads to the formation of glycerol. It has been reported that NAD regeneration occurs in this manner in the early stages of fermentation before the concentration of acetaldehyde reaches the levels needed for alcohol dehydrogenase activity. However, utilization of this pathway does not provide the cell with energy.

Additional alcohols of importance in winemaking include glycerol, methanol, and several 3- to 5-carbon alcohols collectively known as fusel oils. These may, on occasion, be important in sensory and regulatory considerations.

Chemistry of Fermentation and Composition of Wines

Fermentation

Fermentation originally indicated the conversion of grape juice into wine. It is now applied to a variety of processes of anaerobic dissimilation of organic compounds by microorganisms, by living cells, or by extracts prepared from them. It is also used for certain aerobic microbial processes. Alcoholic fermentation is but one of the many chemical processes which may be classified as fermentation. Other industrial fermentations of importance produce antibiotics, acetic acid (vinegar), citric acid, butyl alcohol, etc.

In any dissimilation process organic compounds of higher energy are converted to products of lesser energy with the subsequent release of energy in the form of heat. Dissimilation processes are therefore oxidation-reduction reactions including: (1) addition of oxygen, (2) removal of hydrogen, or (3) loss of an electron. In the usual process hydrogen from the donor is transferred by a series of enzymes and respiratory pigments to a reducible substance, the hydrogen acceptor. Atmospheric oxygen, reducible compounds in the substrate or intermediate compounds may act as acceptors. Aerobic processes involve atmospheric oxygen while the other two types are anaerobic in nature. An important difference between aerobic and anaerobic processes is that the former release much larger amounts of energy.

History

The famous French chemist, Lavoisier, in 1789 made quantitative studies on alcoholic fermentation—one of the first such studies of a natural phenomena. In 1810 Gay-Lussac correctly reported the over-all reaction in the famous equation which bears his name:

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

It became clear in the late twentieth century that the Gay-Lussac equation represented only the over-all

process of alcoholic fermentation.

The relation of yeasts to the process of alcoholic fermentation had been noted by many early investigators. In fact the species name *Saccharomyces* comes from the Greek *sakcharos,* sugar, and *mykos,*fungus. However, Liebig, the great German organic chemist, considered the yeasts to be without significance in fermentation and the weight of his authority halted progress in understanding the process until Pasteur's definitive studies.

Chemistry

Pasteur's work, even though he did not clearly understand the nature of the process, established the essential validity of the Gay-Lussac equation but also showed that a variety of by-products were present which were not accounted for by the equation. Among the common by-products are glycerol, acetic and lactic acids, and acetaldehyde. Since Pasteur's time many biochemists have devoted their time to tracing the complex process from sugar to alcohol, carbon dioxide and by-products. Fig. 1 indicates the general scheme and accounts for glycerol as one of the by-products.

Even though the variety of by-products and the importance of the enzyme system were established before 1900 it was not until 1913 that Neuberg developed the first tenable scheme of alcoholic fermentation. Progress was rapid thereafter leading to the present generally-accepted series of reactions, Fig. 1.

During the initial induction stage the hexose phosphate is converted to a-glycerophosphate and 3-phosphoglycerate, since at first the entire sequence of reactions is delayed because no acetaldehyde is present. Glycerol is produced directly from the a-glycerophosphate. The 3-phosphoglycerate is transformed to pyruvate which is decarboxylated to acetal-dehyde.

As acetaldehyde accumulates it becomes the hydrogen acceptor (in place of dehydroxyacetone phosphate) and reacts with reduced coenzyme I (NADH) to produce ethyl alcohol. During the stationary phase this process predominates and little glycerol is formed. If acetaldehyde is not available (when removed by sulfite, for example) the induction phase continues and glycerol is produced. Most of the reactions are reversible.

In the presence of a high concentration of sulfur dioxide in acid solution acetaldehyde, carbon dioxide, and glycerol are the primary products and alcohol a by-product. If the sulfite solution is alkaline acetaldehyde, glycerol, alcohol, and carbon dioxide are all produced. Other types of fermentation have been reported. The glycolytic sequence clearly shows the complexity of the system. It also shows how glycerol, lactic acid, and acetaldehyde may accumulate as by-products. The tricarboxylic acid cycle (Krebs) which starts with pyruvate can explain by-products such as succinic acid. For the glycolytic cycle note that no less than 22 enzymes are required plus both magnesium and potassium ions and six or more coenzymes.

Red Table Wine

Outline of Red Wine Making

The red, colored pigments of most grapes are localized in the skins. Therefore, in the making of red table wines the juice is fermented on the skins in order to extract this color during fermentation. In the making of white wine, on the other hand, the juice is fermented free of the skins, in order to extract as little color and tannin as possible. As the cellar operations differ somewhat in other respects the two wine types will be considered in separate chapters.

Varieties

There does not seem to be any doubt that Cabernet Sauvignon produces the highest quality red table wines yet made in this state. The grapes normally arrive at the winery in excellent condition and ferment well. If fermented on the skins more than 4 or 5 days, the tannin content may be high and the wines will require longer aging. Both in California and Bordeaux there has been a tendency to press early. This results in wines of less tannin and color but earlier maturity. The best Cabernets may not mature until they have had 10 or more years of bottle aging.

Pinot noir presents special problems in California because it ripens very early in the season. Also, it appears to favor a warm fermentation and in some cases, a malo-lactic fermentation. There are, further, at least two clones—one much less colored than the other. We do not believe the highest possible quality has yet been achieved from Pinot noir in this state. Both cask and bottle aging are recommended.

Zinfandel ripens unevenly and great care in harvesting must be exercised. The best wings appear to come from vineyards on the slopes of hills in regions II and III. In regions IV and V bunch rot is a problem. Contrary to pre-prohibition opinion the best Zinfandels profit by cask and bottle aging. We have tasted excellent Zinfandels of 10 to 15 years of age.

Ruby Cabernet, because of its high total acidity, should be used for red table wines only when grown in regions IV and V. There does not seem to be any more rapid aging for this variety than for Cabernet Sauvignon when produced by the regular procedures from grapes grown in regions II or III. Wines produced from grapes grown in regions IV and V appear to mature more rapidly.

Petite Sirah is highly subject to bunch rot and sunburn. Grapes from regions II and III are most likely to produce the best wines. The same is true of Refosco and Carignane. Grenache produces the best red wines from grapes grown in region I. Elsewhere they should be used for producing rosé wines.

Calzin has been extensively tested for red table wines. It is deficient in color and, suprisingly, exceedingly high in tannin. It is not recommended for planting or wine making in this state. Barbera, because of its high total acidity, is recommended for planting in region IV for use in blending.

Testing The Grapes

As grapes approach maturity, they should be tested frequently in order that they may be picked at the proper stage of ripeness.

As the grapes are received, each load should be tested for Balling degree. If the grapes are found to be excessively high in sugar they should be used for making port wine or for distilling material. The addition of acid is often indicated for table wines. It is best to add the acid early in the history of the wine; for example, at the time of transfer from the fermenting vat to the storage tank. Tartaric acid is preferred in cases of low acidity (below 0.6 percent).

For this reason, Balling (or Brix) tests on the grapes should always be accompanied by titration of the samples for total acidity. There is evidence to indicate that acidification before fermentation results in better development of bouquet and flavor than if it is delayed until fermentation is complete. In fact, addition to the crushed grapes is probably the best procedure, except for the unavoidable loss of some of the added acid in the pomace. It is also desirable to follow the pH during ripening as musts of high pH are unsuitable for making high quality table wines. A pH meter is used for this determination.

Picking

In California harvesting is usually done, often by contract, by crews of itinerant pickers who pick several vineyards in succession. Short, curved knives or short-bladed shears are used in cutting the bunches from the vines but picking shears, such as used for table-grape harvesting, are preferable, as they slash the fruit less and also permit easier cutting out of rotten berries. Lug boxes holding from 45 to 55 lbs. of grapes have been used for picking and for transporting the grapes to the winery in most table-wine vineyards. However, small gondolas which can be moved through the vineyard are becoming increasingly popular. The gondola is taken directly to the winery.In some of the large dessert-wine grape vineyards, the grapes, after picking into buckets or lugs, are dumped into large steel-bodied, hopper-like trucks (called gondolas) and transported long distances in bulk to the winery.

This is objectionable from the standpoint of sanitation and microbiology, for inevitably many of the grapes are crushed in bulk transfer, with consequent fermentation and contamination with fruit flies*(Drosophila melanogaster).* Fermentation, bacterial growth, and volatile acid formation have then been noted in gondola trucks where crushing was delayed. Less objectionable is the practice of picking into moderate sized bulk

containers which are transported directly to the crusher. Economy dictates that as little handling of fruit be employed as possible, consequently direct harvesting into containers which can be mechanically dumped into the crusher probably will be used in the future.

Lug boxes or picking buckets or tubs should be clean; not moldy or vinegar sour. Washing and steaming such containers during the vintage, especially after a rain, is necessary.

Only sound (not moldy, or mildewed, or vinegar-soured) grapes should be taken by the pickers. Some varieties develop a considerable quantity of second crop bunches that ripen 2 or 3 weeks later than the main crop. If the main crop is overripe, it is often desirable to pick the second crop along with the first in order that the second crop will furnish much needed acidity. On the other hand, if the grapes are not overripe, it is better that the second crop be left on the vine to ripen. The Zinfandel usually sets a good second crop which if picked with the first crop in a cool region will make the must unduly acid.

White Table Wine

White wines are not simply colorless wines. They differ fundamentally from red wines in production, composition, and sensory quality. Since they are not produced by fermentation on the skins the tannin and extract contents are lower. Whereas red table wines are usually dry or nearly so white table wines may be very sweet, as with French Sauternes or the *Auslese* wines of Germany.

Process

White wines are usually more delicate in flavor than red and, owing to the lack of tannin and coloring matter, defects in taste and appearance are more apparent in them. Red grapes are usually fermented in open vats, whereas white must is preferably fermented for dry wine in covered tanks or casks. White wine fermentations are usually allowed to run to completion in tank or cask, and then the casks or tanks kept full until the first racking in December. Clarification and bottling may take place in 6 to 24 months—the lighter (lower-alcohol) types being sold first.

However, in the making of bulk standard wines in California some of the larger wineries ferment the white must in open vats until the Balling drops to 0° before transferring it to storage tanks for the after-fermentation. The fermentation of white musts in open vats results in loss of bouquet and flavor if there is a quality potential in the grapes employed.

Varieties

The recommended varieties for planting in California have already been listed. Some further comments here regarding their enological characteristics seem desirable.

White Riesling is thevariety for Riesling wine if one can afford it. It is a shy producer, sunburns easily, and requires a low fermentation temperature. In California it is most often erroneously named Johannisberg (or Johannisberger) Riesling but White Riesling is preferable. Sylvaner (Franken Riesling) and the so-called Grey Riesling have little Riesling character, either in this country or abroad. The Walschriesling (Italian Riesling) is not grown commercially in this country and does not produce a Riesling wine in the countries where it is grown (Italy and Yugoslavia) but its wine is pleasant. The Emerald Riesling has a distinctive aroma, more reminiscent of its muscat than its Riesling parent. Its tendency to darken, as noted by Berg is a serious defect but its high acidity is a more than compensating factor, especially in regions III and IV in California where the warm climatic conditions lead to low acidity in other varieties. The Sylvaner, Grey Riesling, and Emerald Riesling each vinified separately and properly cared for have a place in our wine industry if planted in the correct region. Because of their tendency to darken musts of Grey and Emerald Rieslings should probably be well settled before fermentation.

Chardonnay produces excellent wines but is a poor producer. It should be fully matured before harvesting if the characteristic ripe grape aroma is to be developed. Ballings of 23° are desired. Another low producer is the Gewurztraminer. However, its distinctive aroma makes it useful. Very careful harvesting is necessary to secure sufficient maturity for flavor but one must avoid low acidity and excessive sugar by too late

harvesting. Flora, a new release of the California Agricultural Experiment Station is now being extensively tested as a supplement to or replacement of Gewürztraminer.

Sémillon is one of the best all-purpose varieties now available, if it is not overcropped and is not picked too early. Picked in mid-season at Ballings of 22.5 to 23.5, it is the basis of a good standard white table wine. At slightly higher Ballings, even under California conditions, it can produce sweet table wines. In years of early rainfall immediate harvesting is advisable to prevent excessive botrytis rot.

Sauvignon blanc is an excellent variety but must be mature if its wine is to have a characteristic aroma. This means harvesting at a Balling of at least 22.5°. Overcropping is possible and delays maturity. Wines from overcropped vines also have less flavor. Some of the best white table wines of California have been made from this variety.

For standard white wines French Colombard, Folle Blanche Chenin blanc, and Veltliner are all useful. Some rot may develop in Chenin blanc and Folle Blanche in rainy years. Delay in maturity owing to overcropping is a fault of Veltliner. Still under trial is Helena, a new and promising hybrid of the California Agricultural Experiment Station. Aligoté is probably about as useful as these varieties.

Not recommended for general planting for white table wines are Trebbiano (Ugni blanc or St. Emilion), Palomino (darkens), Sauvignon vert (low acidity), Green Hungarian (thin, neutral wines), and Burger (thin but possibly useful as a sparkling wine stock); or table grape varieties such as Thompson Seedless, etc.

Picking and Transporting

The proper time of harvest varies from variety to variety, region, season, amount of crop, and the prospective use of the fruit. The only way to fix the time accurately is to determine the maturity of the grapes in the vine-yard as outlined. For early-maturing, fruity, white table wines harvesting can begin at 20° to 21° Balling. For richer more flavorful slower-maturing wines harvesting may be delayed to Ballings of 22° to 23°. The acidity and pH must also be considered. Wines of better flavor and keeping quality and easier clarification are produced from musts with an acidity of over 0.70 per cent (as tartaric) and pH of 3.3 or lower.

In California the white grapes are preferably picked into clean lug boxes or aluminum tubs; carried to the end of the row to be picked up by truck or conveyed into small or large metal containers for transport to the winery. If the vineyard is on a steep slope the boxes are moved by tractor-drawn sled which delivers them to the truck. Increasing amounts of grapes are transferred in the vineyard to small or larger gondola trucks. Where the transfer is carefully made, the gondolas clean, excessive crushing of the grapes avoided, and the movement to the winery rapid, the system works well. However, it is difficult to transfer the delicate white grapes long distances in gondola trucks without considerable crushing. Harvesting directly into large metal containers which can be unloaded by power lifts is also common. Similar systems using wooden tanks are used in Europe, particularly in Germany.

Great care should be exercised in picking in order to avoid moldy bunches, particularly late in the season. In some European vineyards, Champagne, for example, each bunch is inspected and if necessary, unfit individual berries are removed by small shears. Such extreme care is not necessary or economically possible in California; however, after early rains the bunches which become moldy should not be picked for wine making.

The boxes or baskets should be scrupulously clean and free of all mold.

Sherry

Sherry is the most important California wine type. Sweet white wines of low acidity containing unfermented sugar readily develop on exposure to air a peculiar characteristic flavor known as rancio *(goût de rance* in France). The excessive caramelized odor of some baked sherries is not, a true rancio flavor.

There are three types of wine sold under the name of sherry. The first is that of the flor sherries of Jerez de la Frontera in Spain which owe their characteristic flavor and bouquet to the growth and action of flor yeasts

that develop on the surface of the wine. Similar types are produced in Australia, California, Canada, the Jura region of France, the Soviet Union, and South Africa. The second type is California sherry that owes its-flavor and bouquet to baking. This type resembles the wine of the island of Madeira more than any other. The third type is that which is aged in small cooperage for several years without flor yeast or baking. The aged non-flor sherries of Australia and California are of this type, as are some of the wines of Banyuls in the south of France and the Prioratos of northern Spain.

Sherry, particularly if dry, is used traditionally as an appetizer wine or cocktail hour beverage.

California Sherry

The origin of the California baked sherry process is not definitely known. One might assume that it began as an attempt by a California wine maker to produce wines similar in flavor and bouquet to certain kinds of Spanish sherry. If so, the attempt failed. It is known that some California sherry in the last century was baked in glass hot houses in barrels or puncheons, heat being furnished by the sun. Later, artificially-produced heat was employed.

Grapes

In Spain the Palomino is the principal variety grown for sherry production. It is low in acidity but the sugar content is very acceptable for sherry, when well ripened. It is rather neutral in flavor and aroma and the wines darken in color after production,. In California other white varieties available in abundance are also used. These include the Thompson Seedless variety (Sultanina) grown extensively in the San Joaquin Valley for raisin production and for fresh shipment for table use; Malaga, an important white table and shipping variety; Emperor, a shipping grape of light red skin color and white juice; and the Tokay (Flame Tokay), a red grape of white juice grown extensively in the Lodi area. In New York and other eastern states labrusca varieties characterized by their pronounced varietal flavor are used. By the Tressler method the foxy flavor is partially eliminated.

In the making of baked sherry, the grape variety is probably of less importance than in the making of any other California wine, because the flavor and bouquet of the final product depend chiefly on the baking process and other cellar operations. However, varieties of marked flavor, such as the Muscat of Alexandria, should not be used, because sherry should not possess a Muscat varietal flavor.

Picking and Delivery

The grapes for sherry making should be well ripened, as the final acidity should not be as high as for table wines and the corresponding pH value may be somewhat higher. Berg made sherries of various pH values by acidifying new white wine made from Tokay grapes, the adjusted pH values being 4.0, 3.8, 3.6, 3.4, and 3.2.

Experienced tasters gave the sherry of pH 3.2 the highest score and those of 3.4 and 3.6 a score that was a close second to that of pH 3.2. The sherry of pH 4.0 was given the lowest rating. The chief defect of the Palomino variety is its relatively high pH.

Some sherry is made from the grapes sorted out at packing houses as unsuitable for shipping fresh for table use. These grapes are usually sound, but early in the shipping season may be lower in Balling degree and higher in acidity than is desired. However, sherries made from such grapes may be useful for blending.

In the principal sherry producing districts of the State the grapes are picked into large pans or lug boxes and transferred to gondola trucks for delivery to the winery, or are first placed in small gondolas which in turn are emptied into large gondola trucks.

Crushing

Sherry making in California is usually a large-scale operation. The grapes are crushed and stemmed in the same manner as for other wines. Garolla-type crushers and stemmers described in an earlier chapter have superseded roller crushers and orthodox stemmers.

To the crushed grapes sulfur dioxide in the form of the gas from a cylinder of the liquid is added, or

potassium metabisulfite is used.

Fermentation

The crushed grapes are allowed to drain and the free-run is pumped to fermenters. They are large, usually of 60,000 gallon capacity or even larger. In smaller plants concrete vats or redwood tanks of 10,000 to 20,000 gallons are used. The drained crushed grapes are generally employed to produce fortifying brandy. A starter of fermenting must from another fermentation or of must fermenting with pure yeast is usually added to the juice.

During fermentation, it is customary to artificially cool the must to maintain the temperature below 85°F. Ten of 19 plants surveyed stated that 80°F. is a more desirable maximum.

Usually the must is fermented dry or to a low sugar content. A small amount of sugar is considered desirable during baking, but is often added later in the form of angelica or fortified sherry material of high sugar content. In a survey of Martini 11 of the 16 cellars fortified at —1º or lower Balling, one at 0º Balling, and two “when dry.”

Port and Other Dessert Wines

In the preceding chapter the production of sherry was discussed. The other principal fortified dessert wines are port, angelica, Malaga, Madeira (of the island of Madeira), Marsala, California tokay and muscatel, and similiar types produced in many countries.

Port

The production of sweet red wines in Portugal has been one of that country’s most important industries for more than two centuries. Red sweet wines, often called port, or port-type, are also produced in Australia, California, Chile, South Africa, and the Soviet Union. California is the principal producing region in the United States.

At present wine makers must use such red wine varieties as are available. Probably the Alicante Bouschet is the poorest of these, owing to its low sugar musts, the tendency of its wines to lose color, and to its slightly unpleasant aroma. Carignane, Zinfandel, Mataro, and Petite Sirah are satisfactory when picked before raisining occurs; however, Mataro is deficient in color. The Mission and Grenache, grown in the same area, are lacking in acid and color but are of pleasing flavor. Blends with Salvador and Alicante Bouschet often have to be employed in order to bring up the color. It is to be hoped that more suitable grape varieties will be planted in the Fresno area, such as Tinta Madeira, Souzäo, Royalty, and Rubired. The latter are two new hybrids released by the University of California. They were created by Prof. H.P. Olmo specifically for red sweet wine production. In the hot interior valleys of California, where most of the sweet dessert wines are made, many varieties of red wine grapes fail to develop sufficient color for production of port of satisfactory tint, unless special methods of vinification are used. There is great need for the planting of varieties of maximum red color in these area to bring up to a desirable depth the color of ports made from the varieties grown at present. The Salvador is widely used for this purpose but its flavor is poor. The Souzão, Rubired and Royalty should help supply this deficiency without the undesirable flavor. There may be a problem of color stability with the Rubired.

Normal Vinification of Port

The principal problem in making port is extraction of sufficient color during a restricted period of fermentation.

The grapes should be well ripened, 23º to 25º Bailing and should be picked to eliminate moldy fruit and handled with care to avoid bruising. They should be crushed as soon as possible after picking. Shipping loose in bulk in gondolas with long delays in crushing is not conducive to quality. Use of rain-damaged grapes that have molded on the vines is also undesirable, as such grapes give wines of poor flavor and unstable color.

Crushing and stemming are conducted as for grapes for dry red wine. The crushed grapes are pumped into

large fermentation vats holding 10,000 gallons or more. Some sulfur dioxide should be added during filling of the vat to give about 100 p.p.m. in the must. This will insure a cleaner fermentation, help to stabilize the color, and assist in extraction of the color.

Early in the season a 1 or 2 per cent starter of pure yeast is added to the crushed grapes. Later in the season, a similar quantity of fermenting must from a vat in active fermentation will answer the purpose but it is better to continue to use a pure yeast culture. Flanzy reported better quality dessert wines when the must was fermented at 59° to 68ºF compared to 86ºF. He believed sulfur dioxide reduced the quality of the dessert wines. Both these results need confirmation.

Frequent pumping over of must in large vats is essential to extraction of the color. In Portugal color extraction is accomplished by intermittent treading of the fermenting grapes; an effective but not very aesthetic procedure.

If the grapes have good color, and the wine maker has been lucky, his must will have attained fair color when it has fermented to the point at which it is ready for fortification to give a standard port of 20 × 7 composition (20 per cent alcohol and 7° Balling) after fortification. Thus, if the original grapes tested 24° Balling and were fermented and fortified at 13° to 20 per cent alcohol, the resulting fortified wine should be about 6°. As a matter of fact, in this case the fermenting must would be drawn from the vat at about 15° Balling, since there may be nearly 2° drop during drawing off, pumping to the fortifying tank, measuring, and fortifying. In warm regions the 15° Balling will be reached in 24 to 48 hours after crushing.

The free run will naturally not be very deep in color. When possible the drained skins and seeds (pomace) should be pressed, preferably in a basket or Willmes press. However, a continuous press is generally used because of its convenience and economy of operation. The press wine is often fortified and is of deeper color. One can readily see the difficulty in extracting sufficient color in such a short period of fermentation, especially with grapes grown near Fresno, or in other hot localities. Hence, “normal vinification” of port is usually not practicable. Other methods are discussed in the next section.

Most San Joaquin wineries draw the free-run colored fermenting must off at the proper stage and pump it to the fortifying tanks. The residual pomace is then watered and fermented for distilling. It is next passed to a Metzner still, to a hammer mill for grinding in order to use a pomace still, to a continuous press, or to a “scalping” apparatus (a spray of water used to remove the alcohol). Countercurrent extraction has also been used to recover the residual sugar. In some cases the pomace is transferred to other tanks for completion of fermentation.

The relation between initial Balling (or Brix) degree of the grapes, Balling of the must, and final Balling after fortification to 20.5 per cent alcohol. Wine makers commonly pay too little attention to fortification at the proper sugar content. The result is that some lots are fortified too soon and more too late. Closer attention to this table would reduce this problem.

Sparkling Wine

Sparkling wines, those which contain a visible excess of carbon dioxide are difficult to define precisely. The present maximum for still wines is 0.277 gm. per 100 ml. of carbon dioxide (at 60ºF.). This is equivalent to 7 lbs. per sq. in. pressure. According to Protin most countries now distinguish between slightly gassy wines *(pétillants* or *perlants)* from wines with a full pressure. He reports the unofficial position of various countries as follows:

Country	*Type*	*Pressure*	*Tem-perature*
		Pounds/Sq. inch	*°F.*
Austria	*Pétillant*	1.1 Maximum	68
	Sparkling	10.0 Minimum	68
Chile	Sparkling	10.0 Minimum	59

France	*Pétillant*	3.3 Maximum	59
	Sparkling	10.0 Minimum	59
Germany	*Pétillant*	1.1 Maximum	68
	Sparkling	6. 6 Minimum	68
Spain	*Pétillant*	2.2 Maximum	?
	Sparkling	7 .7 Minimum	?
Switzerland	*Pétillant*	1.1 Maximum	59
	Sparkling	8.8 Minimum	59

The problem is complicated in this country because of the higher taxes on sparkling compared to still wines. In practice the excess carbon dioxide may originate from fermentation of residual or added sugar, from a malo-lactic fermentation, or from added carbon dioxide.

Definition

No classification based on method of production is adequate to define the types of sparkling wine. The consumer, however, is not interested in the method of production as much as in the recognizability of the various types of sparkling wines.

Therefore, we arbitrarily define as sparkling wines those which have more than 1.5 atmosphere pressure at 50°F. The amount of dissolved carbon dioxide at this pressure and temperature is approximately 3.9 gm. per liter. If the carbon dioxide is kept at this figure the pressure at 60ºF. will be about 1.8 atm., at 70°F. 2.1 atm., and at 80°F. 2.4 atm., see Fig. 1. This is about half the minimum suggested by the Office International de la Vigne et du Vin for sparkling wines, 4 atm. at 68ºF.

The enologist, tax expert, and connoisseur will need a more detailed classification of the many different types on the market. In the classification which follows there are some overlappings in carbon dioxide content. The *source* of the carbon dioxide is thus the basis of this subdivision. In some cases there may be no sensory test which will distinguish between the types!

Type I. Excess carbon dioxide produced by fermentation of residual sugar from the primary fermentation. This includes many Alsatian, German, Loire, and Italian wines as well as the muscato amabile in California.

Type II. Excess carbon dioxide from a malo-lactic fermentation. The Vinho Verde wines of northern Portugal are the best representative of this type, but there are many examples in Italy and elsewhere in Europe.

Type III. Excess carbon dioxide from fermentation of sugar added after the process of fermentation. Most of the sparkling wines of the world are produced by this procedure.

Type IV. Excess carbon dioxide added. This includes the so-called carbonated wines.

Carpenè distinguished four types of fermented sparkling wines: (1) slow bottle fermentation, long aging on yeast, disgorging, (2) same but transferred and filtered, (3) rapid bottle fermentation, no aging on lees, transferred and filtered, and (4) tank fermented. He stresses the importance of aging on the yeast. He agrees with Schanderl that while the fermentation is the same in tanks or bottles the products of methods (1) and (3) or (4) are different.

Type I Sparkling Wines

Almost any wine can be made sparkling by stopping the fermentation before all of the must sugar has fermented and then, later, bottling the wine. If even a few viable yeasts are in the wine at the time of bottling, and if the sulfur dioxide content is not excessive, the sugar will later most likely ferment and the wine will become gassy. If the fermentation is slow at a low even temperature the amount of yeast cells produced may be surprisingly low. In some cases, when the yeast deposit is excessive, the wines are treated as Type III sparkling wines and clarified in the usual way. One reason why more wines of this type are not produced in California is that it is most difficult to stop the fermentation with the desired residual

sugar content.
However, with the increasing technological control of fermentation (temperature, pressure, DEPC, depletion of amino acids, etc.) and with the generally high sugar content of our musts it should not be difficult to produce wines by such procedures. The addition of high, quality grape concentrate before fermentation also offers interesting possibilities.
Sparkling wines as we know them probably originated in this manner. It is no accident that the first centers of sparkling wine production were in northern France. It is in such cold regions that the fermentation is slow and incomplete. When the temperature increased the following spring the fermentations restarted and gassy wines resulted.

Vermouth and Flavoured Wines

Vermouth consists of a fortified wine flavoured with a characteristic mixture of herbs and spices, some of which impart an aromatic flavor and odor and others a bitter flavor. Two classes are recognized in the trade, the sweet or Italian-type vermouth and the dry or French type. Dubonnet, Byrrh, Bonal, and Cap Corse are flavored wines that are usually classed with vermouth and will also be discussed in this chapter. In addition, there are on the market several wines that are lightly flavored with certain herbs, spices, fruit juices, essences, aromatics, and other natural flavorings. They have attained considerable popularity, such as Thunder-bird, Silver Satin, etc. Many formulas for the preparation of each type exist. According to Joslyn the Italian or sweet vermouth contains from 15 to 17 per cent of alcohol by volume and 12 to 19 per cent of reducing sugar: and the French or dry vermouth usually contains about 18 per cent of alcohol and about 4 per cent of reducing sugar. The quantity of herbs and spices used in making the dry vermouth is less per unit of vermouth than for the sweet; customarily about 0.5 to 0.75 oz. per gallon of the dry and 0.75 to 1 oz. per gallon of the sweet. While vermouth is served principally “straight” in European countries it is used in America principally in mixed drinks such as Martini and Manhattan cocktails. Some is served mixed with sherry as a “sherry cocktail.”

Origin

The name is probably derived from “Wermut,” the German word for wormwood, a frequent ingredient of vermouth. The “w” in German is pronounced like “v” and “u” as “oo”; hence the natural tendency to change the German spelling in English. The German word is probably based on the alleged beneficial properties of wines containing wormwood. The addition of wormwood to wine appears to date from early Roman and probably early Greek times, although the production of vermouth itself in Italy did not begin until the eighteenth century. The quality and type of vermouth depend upon the quality and nature of the base wine and on the kind, quality, and amounts of the various herbs used. According to Valaer the formulas for the European-made vermouths are closely guarded secrets, whereas there is less secrecy among the American producers. However, Valaer occupied a privileged position in the Internal Revenue Service. Few producers are willing to divulge their vermouth formulas.
Before passage of the 18th Amendment, only a limited amount of vermouth was produced in the United States; most of that then on the market came from Italy and France. After repeal of the amendment the demand greatly increased and production in America rose accordingly. According to Valaer California produced about 2,000,000 gallons of vermouth. New York State is also an important producer of vermouth.
Flavored wines, euphemistically and legally entitled “Special Natural Wines,” have come on the market in the past ten years in considerable quantities. These are not vermouths and they do not resemble the usual aperitif wines very closely, although classed as such. They represent a new type of wine. They usually contain the same amount of alcohol as dessert wines, 18 to 20 per cent by volume, and are sweeter than dry vermouth but not so sweet as Italian-style sweet vermouth. The essence used for flavoring these wines must be approved by the Alcohol and Tobacco Tax Division of the Internal Revenue Service. The flavoring is often mild. Only small amounts are made at present in other states. These products sell at only slightly

higher prices than dessert wines. They are served usually “straight,” often with ice (“on the rocks”), rather than as an ingredient of mixed drinks; in that respect differing from vermouth. A few, for special markets, are made as unfortified wines with the same flavor and are sold under the same name as the fortified. Recently some of these products have resembled the standard cocktails both in name and flavor.

Herbs and Spices

The herbs and spices used in vermouth are furnished in dry form and represent different parts of various plants such as the seeds, wood, leaves, bark, or roots. Until World War II practically all of the herbs used for vermouth production were imported, but during the War successful attempts were made to obtain some of the herbs from plants growing wild and to grow some of the others in this country. Considerable quantities of these are now grown in the United States, although most of the herbs and spices now used are imported. Some species are obtained from the tropics and others from the Near East, but most from European countries such as Italy, France, and Belgium.

Information on the classification of the more important herbs and spices used in vermouth production is given by Pilone as bitter, aromatic, or bitter-aromatic. Bitter plants include aloe, angelica, blessed thistle, cinchona, European centaury, germander, lungwort, lungmoss, quassia, and rhubarb. Aromatic plants are anise, bitter almond, cardamon, cinnamon, clove, coriander, dittany of Crete, galingale, marjoram, nutmeg, Roman, camomile, rosemary, summer savory, thyme, tonka bean, and vanilla bean.

The bitter-aromatic plants include allspice, elder, elecampane, gentian, juniper, bitter orange, sweet orange, saffron, sage, sweet flag, speedwell, wormwood (common), wormwood (gentile), wormwood (pontico), and yarrow.

The major flavoring constituents of the herbs and spices used in vermouth manufacture have been given by Brevans as follows:

1. Hydrocarbons (such as styrol, cymene, pinene, and other terpenes)
2. Aldehydes (such as citral, citronellal, furfural, benzoic aldehyde, vanillin, cinnamaldehyde)
3. Ketones (such as methyl heptenone, carvone, luparone, thujone)
4. Lactones (such as alantolactone)
5. Oxides (such as cineole or eucalyptol)
6. Phenols and phenol derivatives (such as luparol, thymol, cadinone, caryophyllene)
7. Alcohols, particularly terpenic alcohols (such as calamenol, citronellol, borneol, anethol, eugenol, terpineol, safrol)
8. Alkaloids (such as quinine, cusparine, absotin)
9. Glucosides (such as absinthin, gratiolin, quassin, aloin)
10. Saccharides (such as gentinose)
11. Tannins
12. Coloring matters
13. Gums and pectins
14. Resins (such as humulon)
15. Esters (such as amyl valerianate)
16. Simple acids (such as citric)
17. Complex acids (such as angelic, alantolic)

The herbs and spices are usually purchased in dried form. Therefore quality will be affected by the care given them in harvesting and storage. They should be purchased only from a reliable supplier who furnishes products of the highest quality. Specimens of the same variety of plant grown under different climatic or cultural conditions may differ markedly in character and quality. The longer the dried products are stored before use, the poorer will be their flavor and aroma, as these depend to a great extent on volatile compounds that slowly evaporate during storage.

Furthermore staling of the flavor through oxidation and other chemical reactions occur during storage. For these reasons, the dried herbs and spices should be as fresh as possible. During prolonged storage, insects may infest the dried products and render them completely unfit for use in vermouth. If the moisture content of the storage room or of the dried products during storage is too high, molding is apt to occur with more or less damage to quality. Fumigation at suitable intervals with methyl bromide or odier effective fumigant is advisable to control insects if the products are to be stored for an appreciable period.

If they are in tightly sealed containers such as friction top cans, jars, or moisture proof plastic bags, observation must be made occasionally to make certain that moisture has not distilled from the product and condensed on the walls of the package or on the product causing a local rise in moisture content with resultant spoilage by mold. Vermouth producers should carefully inspect all herbs offered for sale before purchase.

It is preferable to purchase the dried plant materials in the whole form, as they can be examined more satisfactorily than if powdered or in granular form. When the whole plant is available it is easier to determine whether the material is from an old or new crop. Also, the storage life of the powdered and granular products is shorter than that of the whole materials because volatilization of flavor and aroma is more rapid from ground material.

There seems to be an increasing use of fluid and solid extracts, concretes, absolutes, oils, gums, balms, resins, oleoresins, waxes, and distillates in the production of vermouth. These may be used in amounts not to exceed the amount reasonably required to accomplish their intended physical, nutritional or other technical effect. Microscopic detection of impurities in or falsification of *Artemesia absinthium* with other *Artemesia* or with *Achillea* is described by Griebel.

Fruit Wines

Considerable wine is now made in the Pacific Coast states and in British Columbia from apples, berries, and plums. Also some sweetened Concord grape wine similar in composition to berry wines is made on the Pacific Coast and in several Eastern states.

In England and in several European continental countries apple wine (hard cider) is produced in important quantities. In fact, the cider of Normandy is nearly as famous as French Burgundy or Roquefort cheese. Berry wines are made in several European countries, particularly in Switzerland, Germany, and the Scandinavian countries.

Cider and Apple Wine

In Great Britain the term “cider” means apple wine, hard cider, or fermented apple juice and nothing else. Unfortunately in the United States it may designate either unfermented apple juice, or the fermented, hence is ambiguous. In France the fermented juice of the apple is *cidre* and in Germany it is *Apfelwein.*

Statistics

According to Anon, France produced over 250 million gallons of cider, of which a considerable proportion was distilled for apple brandy such as Calvados. Charley reported that England at the time produced about 20 to 25 million gallons and Germany about 6 million gallons per year. According to Kroemer there was produced in Switzerland at that time about 12,000,000 gallons of apple cider per year. Cider and other fruit wines are also made in most other European countries and in Canada. The U.S. Treasury Department has reported 21.5 millions of bushels of apples and over 1,750,000 gallons of cider were used for making commercial apple wine in the United States or a total of about 3,475,000 gallons in a single year. This total does not include the hard cider made in the home.

Apples for Cider in Europe

In Switzerland and Germany many of the apple and pear trees are not grown in orchards as in America and Canada, but are found as border trees or are scattered through the pastures, along the roadside or in back yards. In England the trees are usually grown in orchards with grass forming a sod between and under the

trees. The trees are usually headed quite high. In England, Switzerland, and France a large proportion of the apple crop is of varieties grown expressly for the production of cider rather than for table use. Such varieties are usually high in sugar content, of medium to low fixed acidity and higher in tannin content than table apples.

Composition of Cider Apples

Many analyses of apples used for cider production in various countries have been published and while all of these cannot be reviewed here the data given in Table 1 will illustrate fairly well the range in composition that has been observed. Certain varieties are grown in France, Switzerland, and England for cider making, whereas in the United States, Germany, and Canada table varieties are usually employed.

It will be seen that some of the French cider varieties are higher in sugar content than the apples used in the other countries listed in the table and both the French and British cider apples are higher in tannin content than the apples analyzed from the United States, Germany, and Canada.

French Methods

According to Kroemer cider is made in France about as follows: the apples are stored in bins for a few days to develop aroma. They are then washed, sorted to remove rotten fruit, crushed and pressed in a rack and cloth press. In some plants, according to Charley, the crushed apples are not pressed at once but are allowed to stand for 3 to 24 hours to develop color and flavor before pressing. The crushed fruit is allowed to drain during this period of maceration. It is then pressed. The maceration greatly improves the “pressability” of the crushed apples. To the juice is added sulfur dioxide or metabisulfite to give 50 to 100 mg. of sulfur dioxide per liter (50 to 100 p.p.m.). It is then allowed to cool to 32° to 46°F. and settle until fairly clear through the action of natural pectic enzymes. This practice is termed “keeving.” The juice is then racked and allowed to ferment at 40º to 50ºF. Fermentation is slow at this temperature, but it is believed that a low temperature during fermentation is essential for the production of cider of best quality. Temperature during the apple season is low and the cider producers have no means of controlling it.

The pomace is often mixed with water, allowed to stand several hours, and is then pressed. Sugar and sulfur dioxide are usually added and the “juice” fermented to give a product of rather low quality called*cidre marchand.* The resulting pomace may be watered and pressed a second time to give *petite cidre.*

After the primary fermentation is completed the cider is drawn off from the yeast lees and below the cap, *“chapeau brun.”* It then undergoes a slow secondary fermentation for several months in casks at about 40°F. It is then racked, bottled, and develops some carbon dioxide pressure in the bottle. Procedure varies considerably, however. For example, Charley reports that in some cases some of the juice is fermented completely. It is then filtered and blended with sweeter cider or with unfermented juice preserved at about 32°F.

Pure yeast starters may be used in the production of French ciders in some plants, but, according to Charley, natural fermentation is the more common procedure. Sparkling cider is made by the bottle fermentation procedure or by the Charmat bulk fermentation process.

The Bottling and Storage of Wines

The bottling of wines is arguably the most important of all winemaking operations since it determines the condition in which the wine is delivered to the market. It is the culmination of the sequence that began long before, starting with grape development, harvesting, fermentation, and aging. Mistakes are costly to rectify and quality control is of primary importance.

The glass bottles used for wine are generally the 750-mL size, of clear or colored glass and in a number of traditional shapes. Other volumes, smaller and larger, are also used depending on the interest in further aging, the setting in which it is likely to be consumed, and the value of the wine. The inertness and protection offered to wines by glass bottles has been verified by many years of usage. The most vulnerable aspect of bottled wine is the nature of the closure or seal that is employed. For many years corks have

been unquestioned as the closure of choice due to their compressible, relatively inert nature. However, in recent decades, the elimination of many defects due to improved winemaking technology has made the incidence of defects attributable to corks to be a major problem in some wines.

The preparation of wines for bottling, the steps involved in bottling and the aspects of their behavior under bottle storage conditions are the subjects addressed in this chapter. The addresses of equipment companies mentioned in this chapter can be found in Appendix I.

Preparation for Bottling

The preparation of wines for bottling involves any final adjustments of chemical composition, final filtration, and modification of the dissolved oxygen and carbon dioxide levels in the wines. The preparation of blends, fining, stabilization, and adjustments of acidity should not be considered as finishing operations and will generally have been attended to some period before the time of bottling.

Final Filtration

The type and style of wine will somewhat influence whether filter pads or a membrane are to be employed as the final filtration. The use of nominally sterile pads is widely practiced, particularly with dry wines, while wines containing residual sugar, or those in which the malolactic fermentation has been prevented, will generally be membrane fitered. The assumption that dry wines that have completed malolactic fermentation will not support additional microbial growth is not always true in practice. While the incidence of later microbial spoilage is lower in such cases, it is not eliminated entirely. The continuing trend for the use of lower levels of chemical additives and the desire to use minimal concentrations of sulfur dioxide only enhance the recommendation of membrane filtrations as the means to prevent unwanted microbial action in bottled wines.

Concerns about color removal from red wines by membrane filters have no sensory basis since the material collected on such filters has already precipitated from solution and insoluble particles have no taste or flavor associated with them. Such material will usually deposit in the bottle within the first months after being bottled and the collection of it on the filter is simply deferring the onset of such a deposit. The removal of yeast and bacterial cells, which also have no taste contribution in themselves, is for reasons of quality control and the assurance that the wine that is consumed closely resembles that which was put into the bottle. The point is, that only soluble components that can be sensed by taste receptors on the tongue or volatile ones reaching the nasal cavity can have a sensory impact and there is no evidence that soluble components and small volatile molecules are significantly removed by such filtrations.

The need to remove all microbes by filtration is a far more acceptable approach to controlling unwanted microbial activity than the chemical additive approach. The variation in quality due to microbial effects can often be seen in wines that have not been filtered, within the first two years after bottling. Tasting of a series of wines from different vintages made in this style by the same producer will generally show such changes and they are undesirable in vintage-dated varietal wines.

The notion of stripping of wine components by filtration has little scientific basis. While some individuals claim to have shown this to be real, there are no panel tests or published results to support it. It has become fashionable, in some circles, to claim that unfiltered or unfined wines are superior, but this has no basis in fact. There are wines that will not need to be fined and perhaps not need to be filtered, but they are not necessarily any better than those that have been. These arguments are generally driven by public relations efforts that try to distinguish wineries from one another or by wine writers who try to be controversial rather than educational in their comments. Bottle sickness, a temporary lowering of flavor in freshly bottled wines, appears to result from the disturbance of an established vapor-liquid equilibrium and not from filtration.

Microbiological Spoilage of Wine and Its Control

This chapter includes the descriptions and origins of various kinds of microbiological spoilage

organisms—and the prevention of their presence and the control of their growth it present. It is important for the winemaker to know which spoilage has occurred in any given instance and to understand potential spoilage problems, but obviously it is better to forestall spoilage than to diagnose it. The taxonomic identifications of the yeasts are given in Chapter 4, and the lactic acid bacteria in Chapter 6. For the aerobic bacteria, the taxonomies are given at the end of this chapter.

Definitions of Microbiological Spoilage

Microbiological spoilage organisms can be said to be any of those which are unwanted at a particular place and time. Obviously, this includes those organisms which produce off-flavors, odors, colors, or precipitates, or have the potential to do so, under the conditions of the present and future storage of the wine. However, this definition also includes bonafide desirable wine yeast and bacteria when they are unwanted in a particular wine, for example, Montrachet yeast in semidry bottled wine or *Leuconostoc oenos* ML34 in bottled wine susceptible to malolactic fermentation. To complicate further the definition of microbiologic spoilage, one has to come to a decision on with which flavors, odors, colors, and turbidities are to be considered “off.” Sediments may be acceptable in aged red wines; oxidized, aldehydic tones and brown hues are required in sherries; and slightly reduced, sulfurous notes might be found in aged sparkling wines. Another complication is that the distinct scents of wine from certain geographic regions, while being expected in those wines, are unacceptable—spoiled—in others, but have nothing to do with microbial spoilage: for example, foxiness or muscadine flavors in wines made from American grape varieties. Another complication is that sometimes the acceptance of distinct odors and flavors caused by certain microbes is controversial, as those seem to be which are be caused by *Brettanomyces*yeast. And finally, one more complication to add to the list is that winemakers may become so accustomed to their own wines that unusual flavors and odors—unacceptable to other tasters—are unnoticed. Winemakers are admonished habitually to taste the wines of other producers and to have their own wines habitually tasted by sensitive colleagues.

Origins of Wine Spoilage Microorganisms

The source of microorganisms, good and bad, in the winery comes mainly from infection, in the winery cooperage and winery equipment, especially the equipment used at the grape reception area and used for the transport of must or juice into the winery.

This idea flies in the face of the assumption that most of the natural microorganisms found in wine must arise from the vineyard. However, on sound, healthy, and intact grapes, the berry surface is not much different than that which would be found on any inert surface outdoors.

It is true that the wild yeasts, such as *Kloeckera* and *Hansenula,* are exceptional; they are found on healthy berries—near the pedicel. Their presence may be related to the expectation that the grape skin surface at this region seems to allow some contact with the nutrients within. These nutrients, having a high content of sugars and a low pH, are selectively attractive to these yeasts. Why the presence of a correspondingly high concentration of wine yeast, that is, strains of *Saccharomyces cerevisiae* is also not found is not clear.

Of course not all of the grapes are healthy; breaks in the skin arise from normal conditions such as strong winds brushing berries against each other or against the woody parts of the vine. These breaks in the skin then allow unrestricted growth of all sorts of microorganisms. Other sources of breakage of the skins are bird pecks, hail, or even heavy rainstorms. So it could be expected that even under the best conditions of berry ripening, a substantial portion of the berries would give some exposure of the contents and allow enough growth of all sorts of organisms giving an incipient infection in the juice or must when it arrives inside the winery.

The description given several years ago of the origins of *Brettanomyces* spoilage in some wines in South Africa can serve as the scenario for the origins of many kinds of infections, including those of both good and bad wine microbes. In the *Brettanomyces* work it was discovered that during the crushing and

destemming operations there was some buildup of the infecting organisms in the pools of juice associated with this equipment. Acceptance of only the most healthy fruit and interruption of the crushing operations and washing of the equipment from time to time would tend to minimize this buildup. However, as mentioned above, even the most healthy fruit is not completely devoid of all unwanted organisms. Furthermore, the washing operations, if not done thoroughly enough, might only aggravate the situation. That is, the dilution of the grape juice brings about a lowered concentration of sugar and an increased pH, and an encouragement of growth of various yeast and bacteria.

This is especially true when pools of diluted grape juice are left standing for an extended time, such as overnight. To continue with the scenario, the diluted pools of juice can serve as ideal starter culture media for all sorts of microorganisms Contamination from them eventually reaches into the winery, into fermenting juice and eventually into stored wine. When this sort of starter culture comes in contact with undiluted juice and wine, then only those that thrive on anaerobic conditions at low pH and probably cooler temperature, that is to say, the wine-related microorganisms, will survive. And if nutrients are available, they will grow. The conclusion of the scenario is that if even a single, viable organism under these conditions finds its way into a demijohn, barrel, or tank of wine, with enough time, this organism can multiply to spoilage concentrations.

This same scenario can apply to all sorts of wine spoilage organisms, and even to desirable wine organisms, including wine strains of *Saccharomyces cerevisiae* and coveted malolactic bacteria.

In the *Brettanomyces* studies, the proper prevention was found to be in very thorough cleaning of the crushing equipment and of the piping or hoses from the reception area into the winery, including judicious use of sulfur dioxide to aid in sanitizing. This means that every several hours, there should be a complete halt to the operations and thorough enough washings of the equipment to leave no diluted pools of juice. The washing operations needed to be especially thorough at the end of the day, and at the beginning of the next. Where continuous crushing is done, very thorough washing operations should be made several times during each 24-hour period. Such washing is not a sterilization procedure, but helps prevent buildup of contaminants.

We have found that the piping or hoses transporting the juice and must into the winery can be very susceptible to accumulation of contaminating microbes. In one winery with an especially difficult situation with*Brettanomyces* infection, the piping was underground and made a right angle from the reception to the winery. Over the years, the bend in the piping had allowed a collection of a large mass of material, which sheltered all sorts of contaminants, some seemingly carrying over from season to season. The contamination problem was solved only by unearthing and replacing the piping, and redirecting it to give only gentle curvatures.

Carbohydrates : Reducing Sugars

Carbohydrates are polyhydroxy aldehydes, ketones, and their derivatives, composed of carbon, hydrogen, and oxygen in the ratio Cn(H2O)n. On a molecular basis, carbohydrates exist as monosaccharides, such as glucose and fructose, disaccharides, such as sucrose, and long-chained forms, the polysaccharides. Polysaccbarides may be hydrolyzed to di-and trisaccharides and, ultimately, to monosaccharides. Examples of polysaccharides that are of potential importance to the winemaker include pectin, and starch as well as the alginates used in fining. Other compounds that qualify as carbohydrates include deoxy- and amino sugars, sugar alcohols, and acids.

Reducing Sugars (Hexoses)

To the enologist, the most important carbohydrates are the six-carbon sugars, glucose and fructose, utilized by yeast in alcoholic fermentation. These two sugars also are referred to as reducing sugars. Reducing sugars may be operationally described as those sugars containing functional groups capable of being oxidized and, in turn, bringing about reduction of other components under specific analysis conditions

(copper, as Cu II, used in their analysis). Thus, certain pentoses also are classified as reducing sugars, even though they are unfermentable by wine yeasts.

Glucose and fructose may be differentiated on the basis of the location of their respective functional carbonyl group. As seen in Fig. 1, the carbonyl group of glucose is located on the first carbon and thus is defined as an aldo-group. In fructose, the carbonyl function is located on the second carbon; thus fructose is an example of a keto-sugar. Intramolecular bond angles create molecular structures for these sugars so that they normally do not exist as straight-chained molecules but rather in cyclic configurations called hemiacetals (glucose) or hemiketals (fructose).

Cyclization does not involve the gain for loss of atoms by the sugar molecule. Thus the straight-chained and cyclic forms are isomers, with the cyclic form representing the more important (prevalent) configuration. Glucose, for example, exists both in solution and in crystalline form almost entirely as the cyclic hemiacetal. The fact that sugars display most of the reactions considered typical of aldehydes is the result of an equilibrium established between the open-chained and cyclic configurations present in solution.

Cyclization introduces another structural consideration into the chemistry of sugars. In solution, sugars can occur in rings composed of four carbons and one oxygen or five carbons and one oxygen. The former is termed a furanose ring and the latter a pyranose ring (see Fig. 1).

In grapes, glucose and fructose occur in approximately equal concentrations, each contributing approximately 10 g/100 g to juice. The disaccharide sucrose is the third most abundant sugar, accounting for 0.2 to 1.0 g/100 g. Although glucose and fructose normally are present in a ratio of 1 : 1 in the mature fruit, the proportions may vary significantly. Climatic conditions during the growing season may affect the glucose-fructose ratio; Kliewer found that it decreased in warmer seasons and increased during colder periods. Amerine reported ratios ranging from 0.71 to 1.45 in California's 1955 vintage, whereas Kliewer cited ratios of 0.74 to 1.05 in *Vitis vinifera* wine varieties. During maturation, the ratio of glucose to fructose usually decreases.

In their review of wine microbiology, Kunkee cite differential utilization of glucose and fructose by yeast. At must reducing sugar levels of 17 to 20%, glucose was reported to be fermented faster, whereas at higher reducing sugar levels (> 25%) the rate of fructose utilization was greater. Between 20 and 25% reducing sugar levels, both sugars fermented equally well. Peynaud notes that the ratio of glucose to fructose declines during fermentation from near 0.95 at the start to 0.25 near the end of fermentation. Thus, it,can be seen that fructose usually is present in greater amounts than glucose. As fructose is nearly twice as sweet as glucose, the cited ratios explain the observation that wines sweetened with grape concentrate or mute appear less sweet than wines with the same *analytical concentration* of reducing sugar produced by arresting the fermentation.

Reducing sugar analyses play multiple roles in wine processing considerations. The winemaker needs to know the quantity of fermentable sugar remaining in the wine to determine if the fermentation is complete. This may be important so that provision can be made for dealing with microbial stability as well as potential blend preparations. Additionally, monitoring the fermentable sugar content in pomace, distilling material, and so on, is of concern in overall plant efficiency. Traditionally, one attempts to obtain a measure of the residual fermentable sugar by analysing for all remaining reducing sugars. Thus, although one might expect "dry" table wines to have close to zero residual sugar upon completion offermentation, typical analytical reducing sugar results are higher because of the contributions of nonfermentable pentoses.

As a result, dry wines traditionally have been defined as having reducing sugar levels of 2.0 g/L (0.2%) or less. In contrast, McCloskey defines the sugar content of a "dry" wine as ranging from 0.15 to 1.5 g/L (when determined by enzymatic assay specific for glucose and fructose). Because the primary reducing sugar content in a dry wine is attributed to pentoses which are not fermentable by yeast, a dry wine (< 0.02% reducing sugar) generally is considered stable with respect to yeast refermentation.

Sucrose

The disaccharide sucrose serves as an important energy storage compound in most plants and vegetables. Although sucrose itself is unfermentable, the products of its hydrolysis, glucose and fructose, are utilized readily. In the case of grapes, upon translocation to the berry, hydrolysis by invertase enzymes yields glucose and fructose. Thus, sucrose levels in grape berries, at maturity, range from 0.2 to 1 %. Because yeasts produce their own invertase enzyme, chaptelization of sugar-deficient musts with sucrose does not cause problems relative to fermentability.

Yeast and Biochemistry of Ethanol Fermentation

The transformation of grape juice into wine is essentially a microbial process. As such, it is important for the enologist to have an understanding of yeast and fermentation biochemistry as the fundamental basis of the winemaking profession. The alcoholic fermentation, the conversion of the principal grape sugars glucose and fructose to ethanol and carbon dioxide, is conducted by yeasts of the genus *Saccharomyces,*generally by *S*. *cerevisiae* and *S*. *bayanus.* The current use of the old term *bayanus* for the yeast closely related to *S*. *cerevisiae* is controversial but we expect *bayanus* to become once more an accepted appellation.

Definition, Origins, and Identification of Wine-Related Yeasts

Definition of Wine-Related Yeasts

By wine-related we mean those yeasts which have been found on grapes or in vineyards; in wines, table or dessert, sound or spoiled; or associated with wineries or winery equipment. Comprehensive listings of these organisms have been published. The taxonomies of the wine-related yeast genera grapes in this section are based on these listings, and include some 18 genera—the more obscure and rare being omitted.

Wild yeasts are those *non-Saccharomyces* fermentative yeasts found on grapes, which may take part, if not hindered, in wine fermentations, at least at the outset, and include *Kloeckera, Hanseniaspora, Debaryomyces, Hansenula,* and *Metschnikowia.* Wine yeasts then are the many strains of *Saccharomyces,* which not only can carry out a complete fermentation of grape juice, or other high sugar-containing medium, but also provide the fermented product with pleasant, winelike flavors and odors. Species of *Schizosaccharomyces* can also completely ferment grape juice, and in special cases they have been suggested as wine yeast substitutes of *Saccharomyces:* however, more often than not, the fermentations produced by *Schizosaccha-omyces* are unappealing. Using the above subjective definition for wine yeast, several Other genera could also be included: *Brettanomyces, Dekkera,* and *Zygosaccharomyces.*

We make these distinctions between wild yeasts and wine yeasts for convenience; it would be just as sensible to call “wild yeasts” those yeasts which have never been isolated and grown in vitro and placed in laboratory storage conditions, and wine yeasts could mean all wine-related yeasts. Furthermore, we are not using the term *wild yeasts,* in the genetically correct way to indicate the parent strain from which various mutant strains have been derived.

Origins of Wine-Related Yeast

The presence of many yeast genera on grapes in the vineyard at ripeness has long been established. Indeed electron scan microphotographs of the surface of grape skins showing distinct and intact bipolar budding yeast have been made. The presence of multilateral budding wine yeasts also on grapes has been supposed for as long. There are many reports of the presence of strains of *S. cerevisiae* on grape skins, but generally these have either been vague as to actual numbers or have been the result of enrichment culturing. For enrichment culturing, whole berries, or skin washings, are used to inoculate a selective nutrient broth. The presence of wine yeast in the incubated medium indicates that at least one viable yeast cell was initially present. These reasonable assumptions have lead to extensive written and oral speculations, anecdotally based, on the importance of both wild yeasts and wine yeasts found on the

grapes with respect to subsequent natural fermentations.

It has been advocated by others that the distinct characteristics of wines from various long-established, and often famous, wineries come from the yeast in residence in the vineyards associated with wineries. The wild yeasts are thought to provide their own special flavor nuances before being overwhelmed by the wine yeasts, and the wine yeasts add their own distinctive flavor notes. Both of these postures are now generally discredited, with some possible exceptions.

Alternatively there has been a renewal of the suggestion that perhaps there are no wine yeasts on the grapes at all, and that the inoculations in a natural fermentation come from yeast indigenous in the winery. It is true that most of the evidence for wine yeast actually present on the skins of ripe berries comes from enrichment culture studies. Furthermore, we have demonstrated long delays in commencement of fermentations of juice from grapes prepared outside the winery, whereas when the juices from the same grapes were prepared (stemmed and crushed) in the winery, there was little delay in the start of the fermentation. The question arises, how does the yeast become resident in the winery? The answer is essentially the same as for the infections of wineries by spoilage yeast and bacteria, but it is based on the simple idea that whenever there is an environment favorable enough for the survival or growth of a microorganism, given enough time, the population will become established.

We have demonstrated the presence of strains of *S. cerevisiae* on skins of ripe grapes without resorting to enrichment culturing. The main difficulty with this kind of demonstration is that the number of wine yeasts cells is so low that the grape skins must themselves be applied directly to the solid nutrient 12px or a minimal volume of liquid must be used to wash the surface of the grapes, and then plated, that is, spread directly onto solid medium. The problem comes from the great susceptibility of the plating method to contamination by molds, which are fast growing. The growth of a single mold cell on a plate can overrun any other colonies within a day or two. For the demonstration of the presence of wild yeast, such as *Kloeckera* or *Hansenula,* which are here in much higher numbers, samples from the grapes can be greatly diluted so that the chance of getting even a single mold cell on a plate is substantially diminished. Chemicals such as biphenyl and thymol have been touted as useful for prevention on mold growth under these conditions, but we have not found them to be effective. Rather, we have developed a selective medium, containing sorbate and ethanol which, when incubated mildly anaerobically, will (after some two weeks' delay) allow the formation of wine yeast colonies. Further identification needs to be made because this method could also allow, for the growth of *Brettanomyces* (and presumably *Dekkera), Schizosaccharomyces,* and *Zygosaccharomyces.*

Identification of Wine-Related Yeasts

It is important to be able to identify the genus and species of wine-related yeasts in order to compare them and their contribution to a given wine or winery, to assess problems arising during fermentation and microbial spoilage, and to evaluate different yeasts as inocula.

Phenolic Compounds and Wine Color

Variations in wine types and styles are largely due to the concentration and composition of wine phenols. From the vineyard to production and aging, fine wines can be viewed in terms of management of phenolic compounds. Phenols are responsible for red wine color, astringency, and bitterness; they contribute to the offactory profile; serve as important oxygen reservoirs and as substrates for browning reactions.

Representative Grape and Wine Phenols

Grapes and wine contain a large array of phenolic compounds derived from the basic structure of phenol (hydroxybenzene). Representative structures of the major classes are presented in Fig. 1. Two distinct groups occur in grapes and wine: the nonflavonoid and flavonoid phenols. The total phenol content of wine is less than that present in fruit. Traditional fermentation following crushing and destemming leads to a maximal extraction of up to 60%. Microbial activity may lead to increases in the concentrations of certain

phenols. Fermenting and/or storage in oak provides additional sources of phenolics. Singleton reported the average values for phenolic fractions and total phenols (see Table 1). Due to the broad chemical diversity of phenolic compounds, total phenols in must and wines are usually presented in arbitrary units of a phenolic standard such as amount of gallic acid necessary to produce the same analytical response or gallic acid equivalents (GAE). As a result of changes in winemaking style, wines produced in the Unites States tend to be lower in tannin and with more supple tannins.

Nonflavonoid Phenols

In wines not stored in oak, the primary nonflavonoid phenols are derivatives of hydroxycinnamic and hydroxybenzoic acids, the most numerous of which are esterified to sugars, organic acids, or alcohols. The nonflavonoid component arises principally from juice extraction and secondarily from post-fermentation activity, including exposure to oak coopperage.

The levels of benzoic acid and its derivatives in red wines range from 50 to 100 mg/L and in whites from 1 to 5 mg/L. Salicylic acid is present at levels of more than 10 mg/L and other derivatives are present in only trace amounts.

Most nonflavonoids are present at levels below their individual sensory threshold; however, collectively members of the group may contribute to bitterness and harshness.

Nonflavonoid Content of Juice

The phenol content of juice is largely nonflavonoid. The levels of nonflavonoid phenols are relatively constant in red and white wines, because of their extractability from grape pulp. The major source of nonflavonoids from grape solids is the hydrolysis products of anthocyanins, hydroxycinnamic acyl groups. Hydroxycinnamate derivatives comprise the majority of this class of phenolics in both white and red wines. These compounds are present in juice and wine as the free acids and ethyl esters and in the form of tartrate or tartrate-glucose esters. Hydroxycinnamates serve as the primary substrate for polyphenol oxidase activity.

Nonflavonoids Derived from Fermentation and Extraction from Oak

During alcoholic fermentation, slow (incomplete) hydrolysis of nonflavonoid esters occurs, resulting in free acid and ester forms. Caftaric and similarly bound or acylated phenols are hydrolyzed to varying degrees, yielding the corresponding free cinnamic acids. Somers reported that fermentation caused total hydroxycinnamates to decrease by nearly 20% due to adsorption by yeasts. Cinnamic acid may be involved in the formation of microbially produced compounds such as 4-ethylcatechol. This transformation involves decarboxylation of the acid to yield 4-vinyl-catechol and subsequent reduction to 4-ethylcatechol. Similar microbially induced transformation of benzoic, shikimic, or quinic acids to yield catechol and protocatechuic acid has been reported. Ethyl phenols are important sensory compounds of red wines.

Some produced by *Brettanonyces/Dekkera,* are responsible for phenolic or leathery off odors. Analysis of 4-ethyl phenol can be used as a marker for *Brettanonyces/Dekkera*. Tyrosol is produced by yeast from tyrosine during fermentation and is the only phenolic compound produced in significant amounts from nonphenolic precursors.

Nonflavonoid Component Arising from Oak

In wines not exposed to oak cooperage, the nonflavonoid phenol fraction is about the same as in the juice from which the wine was produced. Phenols extracted from oak are present almost entirely as hydrolyzable nonflavonoids. Vanillin, sinapaldehyde, coniferaldehyde, and syringaldehyde are reported to be the major species present in barrel-aged wines. (See section on Oak Barrel Components.)

Flavonoid Phenols

Much of the structure and color in wine is due to flavonoids that are found in skins, seeds, and pulp of the fruit. The base structure (aglycone) of flavonoids consists of two aromatic rings, A and B, joined via a pyran ring. (The base structure and standard numbering system are seen in Fig. 2.) Changes in the oxidation

state result from variations in hydrogen, hydroxyl, and ketone groups associated with carbons 2, 3, and 4 leading to different members of the family.

Flavonoids may exist free or polymerized to other flavonoids, sugars, nonflavonoids, or a combination of these. Those esterified to nonflavonoids or sugars are referred to as acyl and glycoside derivatives, respectively. Polymerization of catechin and leucoanthocyanidin flavonoids produces the procyanidin class of polymers. Their classification is based on the nature of the flavonoid monomers, bonding, esterification to other compounds, or functional properties. The most common functional class of procyanidins is the condensed tannins.

Monomeric flavonoids react to yield dimeric and larger forms, resulting in an array of heterogeneous structures. Polymeric flavonoids make up the major fraction of total phenolics found in all stages of winemaking. Polymerization, either oxidative or nonoxidative, yields tannins and condensed tannins, respectively. Further polymerization may eventually lead to precipitation.

Processing protocol will significantly affect the phenolic composition of wine. Flavonoids are derived primarily from the seeds, skin, and stems of grapes. Anthocyanins and flavonols (Fig. 2) are extracted mainly from the skins, and catechins and leucoanthocyanins from the seeds and stems. Increasing the skin contact time and temperature and the extent of berry breakage increases the flavonoid content. The distribution of phenols in a red wine with 1,400 mg/L (GAE) is seen in Table 2. Flavonoid phenols usually account for 80 to 90% of the phenolic content of conventionally produced red wines and about 25% of the total in whites crushed but without skin contact.

Oxygen, Carbon Dioxide and Ascorbic Acid

Oxygen

Oxygen contact with must and wine is a concern to the winemaker throughout the winemaking process. In some instances (e.g. in juice processing, during barrel aging, etc.), selected and controlled exposure to oxygen may play an important and beneficial role in wine quality. In situations such as bottling, oxygen levels should be as low as possible to prevent premature deterioration.

Since the early 1970s, results of research as well as commercial wine production have suggested that limited oxygen contact of the must prior to fermentation may not be as detrimental as once thought. Studies indicate that oxidative browning occurring in juice may be reversed during fermentation. Oxidative polymerization of phenols in white juice reduces the phenolic content of the subsequent wine and aids in buffering the wine against further oxidative degradation.

Derivatives of hydroxycinnamates are the primary phenols responsible for enzymatic browning in juice. Utilizing these substrates, polyphenoloxidases catalyze oxidation leading to browning. S-Glutathionyl caftaric acid is the major product formed, and is itself resistant to further attack by polyphenoloxidases and browning. The rate and extent of browning depend on the concentration of hydroxycinnamates. However, the reaction is intimately related to all the conditions existing in the must and fermenting wine (pH, oxygen levels, increasing alcohol content, etc.).

There is mounting general concern regarding medical ramifications of sulfur dioxide usage. As a result, emphasis has been directed toward reducing use levels and, eventually, eliminating sulfur dioxide in processing altogether. In addition to medical concerns, proponents of sulfite elimination in wine processing point to several other areas of concern relative to its use. (1) Total phenolics are higher in musts receiving prefermentation additions of sulfur dioxide. (2) sulfur dioxide reacts rapidly with several compounds present in juice and wine, chief among them acetaldehyde. The latter reaction product is rather stable and has no or questionable activity in prevention of oxidation or inhibition of most microorganisms.

Further, formation of the addition compound reduces the levels of free molecular sulfur dioxide present, thereby creating a need for further additions in order to achieve the desired levels of molecular sulfur dioxide needed for microbiological and oxidative stability. Because of the reactivity of SO_2 and

acetaldehyde, the timing of sulfite additions has a major influence on final concentrations of both components in wine. Wines produced from sulfited juice, or which have had sulfite additions made during fermentation, have significantly higher levels of acetaldehyde than do those produced with no added sulfite. (3) Where a malolactic fermentation is considered desirable, high levels (> 50 mg/L) of total sulfur dioxide used in juice processing may inhibit the potential for microbial growth.

To reduce the levels of sulfur dioxide used while maintaining high standards of wine quality, careful control of virtually every facet of wine production is necessary, from the vineyard to the bottled product. Concern regarding the potential for excessive oxidative degradation begins in the vineyard. Relevant factors include grape variety, climatological conditions, harvest maturity, and fruit temperature at harvest, in transport, and during processing.

Grape chemistry and integrity also play roles in oxidation. High-pH musts and wines tend to oxidize at a faster rate than low-pH lots. Winemakers forced to deal with mold-damaged fruit expect to observe more oxidation than is seen in must produced from sound fruit. In these cases, the use of higher levels of sulfur dioxide may be needed to control further deterioration by bacteria and wild yeast. In some instances, microbially produced oxidases may not be inhibited by sulfur dioxide used at reasonable levels. During its growth, *Botrytis cinerea* produces laccase that is relatively insensitive to sulfur dioxide and alcohol. One technique for dealing with oxidase activity employs prefermentation juice fining. Although a variety of enzymes are present in sound fruit, fermentation and processing reduce their activity substantially. Polyphenoloxidase activity in wine is not considered to play a major role in further oxidative degradation, although laccase activity may continue. Browning of wines thus is mainly a function of nonenzymatic oxidation of phenolic compounds.

The color of wine is one of its most important characteristics, so a departure from what is generally accepted as the “normal” color of a wine can be a serious problem. The potential for color changes leading to browning in wines may be closely tied to grape variety, because of differences in concentration and activity of polyphenoloxidase. Grapes grown in warmer regions tend to darken faster than the same variety grown in cooler areas. Also, maturity seems to affect the tendency toward browning. It would appear that controlled oxygen contact with the must plays an important, and probably beneficial, role in wine quality.

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