CHAPTER 2

Sherry Wines

M. Ángeles Pozo-Bayón and **M. Victoria Moreno-Arribas**[1]

Contents

Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC-UAM), C/Nicolás Cabrera, 9, 28049, Madrid, Spain
[1] Corresponding author: M. Victoria Moreno-Arribas, *E-mail address:* victoria.moreno@csic.es

Advances in Food and Nutrition Research, Volume 63
ISSN 1043-4526, DOI: 10.1016/B978-0-12-384927-4.00002-6

Abstract

Sherry wines are among the most distinctive Spanish wines, mainly produced in the southern Spain (particularly in Jerez and Montilla-Moriles), using traditional practices aimed at ensuring uniform quality and characteristics over time. Several types of Sherry wines are produced depending on the winemaking conditions. Fino-type wines are characterized by a dynamic biological aging, in which a layer of yeast grows in the surface of the wine (*flor* velum). On the contrary, Oloroso-type sherry wines are subjected to an oxidative aging, while Amontillado-type Sherries are produced by combining both production systems. Therefore, these wines undergo different biological and chemical processes that affect distinctively their chemical composition and their aroma and sensory characteristics. Through this review, the main aspects involved in the winemaking technology of sherry wines, and the latest scientific findings related to the microbiota of the *flor* film and other aspects associated to the changes in their chemical and sensory composition during aging will be revised. Some new trends in sherry wine technology focused on the acceleration of the biological aging or the use of organic grapes will be also considered.

I. INTRODUCTION

Biologically aged wines are one of the most distinctive Spanish wines, mainly produced in the south (particularly Jerez and Montilla-Moriles), using traditional practices aimed at ensuring uniform quality and characteristics over time. France (Jura), Italy (Sardinia and Sicily), Hungary (Tokay), USA (California), and various South African and Australian regions are other countries of the world's foremost producers of sherry; its quality is highly regarded.

Sherry wines are obtained from young wines, carefully selected soon after completing fermentation. These are typically fortified by adding vinous alcohol until they reach an alcohol content of 15–15.5°. They are subsequently transferred to oak barrels before being aged. In most sherries, wine aging occurs in the so-called *solera* and *criaderas* system under the *flor* film of yeast. Once alcoholic fermentation is finished, races of *Saccharomyces cerevisiae* that can grow on the surface of the wine switch from fermentative to oxidative (respiratory) metabolism. They spontaneously form a biofilm called *flor* on the wine surface.

The velum (*flor*) that forms isolates and protects the wine from excess oxidation. It is the origin of complex biochemical reactions, resulting from oxidative metabolism of the *flor* yeast and the reducing environment created in the wine. The combination of both these actions donates the main biochemical originality to this unique aging process of great enologic significance.

The most significant metabolic changes occurring during biological aging is acetaldehyde production. It is considered the best marker of biological aging. It has an important organoleptic contribution, together with a marked reduction in glycerol and acetic acid content and a moderate ethanol metabolism. The yeasts use ethanol as carbon source in the absence of glucose. There is also simultaneous consumption of amino acids. The consumption of proline is noteworthy. It is a major amino acid in musts and wines that is otherwise used only to a limited extent under enologic conditions.

Recently, different research teams have conducted important studies to evaluate biologically aged wines, their microbiology, and the chemical and biochemical transformations taking place during winemaking. These works are reviewed in this chapter. The chapter also provides an updated overview of the possibilities offered by new technologies to improve the quality and production of biologically aged wines.

II. WINEMAKING PROCESS

The basic process for making biologically aged wines consists of two consecutive steps. The first consists of grape must fermentation, which produces a ''young'' wine using fermentative yeasts. The next step is a postfermentative treatment, in which this young wine is fortified with wine alcohol to ~15.0–15.5% (v/v) ethanol. This operation is termed *encabezado*. However, in Montilla-Moriles, the favorable climatic conditions and the characteristics of Pedro Ximénez grapes, which constitute the dominant variety in this region, allow musts with natural alcohol content in excess of 15% (v/v). Thus, fortification is not required. The alcohol used for ''encabezado'' is highly rectified (low in congeners such as higher alcohols or other organics), possessing an ethanol strength of around 95.5–96% (v/v).

Flor yeast that can grow in wine with a high ethanol content adapts to these conditions by forming a *flor* film (velum) on its surface. In this position, its metabolism becomes oxidative (Ibeas *et al.*, 1997; Mauricio *et al.*, 1997). Three white grape varieties are mainly used for sherry wine production: Pedro Ximénez, Palomino, and Muscat. In sherry production, a few months after alcoholic fermentation has finished, the young wine is racked to separate the wine from the lees. In Jerez de la Frontera (Southern Spain), the wine is aged before initiation of biological aging. During the intervening period, the wine undergoes malolactic fermentation and a yeast film forms spontaneously. This initiates the acquisition of characteristics typical of biologically aged wines. Wine stored in this way is referred to as *sobretablas* wine (Fig. 2.1). Subsequently, the wine is clarified

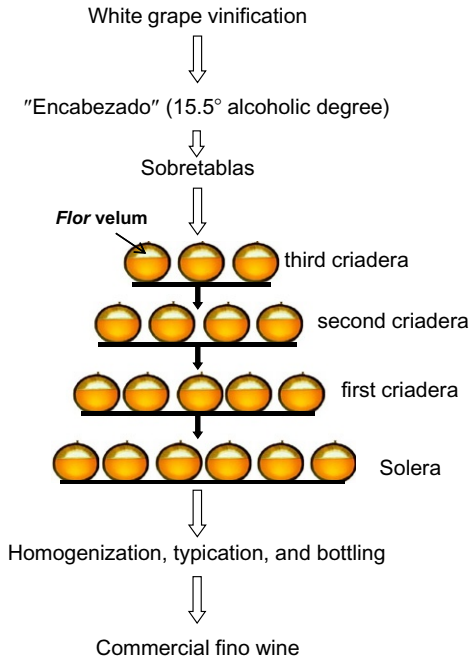

FIGURE 2.1 Scheme of the main steps of the biological aging of sherry wines.

by natural sedimentation, fortified, and placed in oak butts in *sobretablas* location.

Biological aging takes place in American oak casks of variable capacity, depending on their position in a dynamic aging system, consisting of several *criaderas* and a *solera*. This involves stacking the casks in rows, called *criaderas* (scales), such that all casks in any row contain wine of the same type and age. The casks are filled to four-fifths of their capacity to allow a biofilm of flor yeast to develop on the wine's surface. Each series of casks holds maturing wine arranged to facilitate progressive, fractional blending. The row standing on the floor, called the *solera*, contains the oldest wine in the system. It is from this row that the commercial wine is withdrawn for bottling. Extraction never exceeds 40% of the cask's contents per year and may occur three to four times per annum.

The amount of wine extracted from the *solera* is replaced with an identical volume of wine from the upper row. It is designated the first *criadera*. Likewise, the amount extracted from the first *criadera* is replaced with wine from the next row (the second *criadera*), and so forth (Fig. 2.1). Finally, the uppermost *criadera*, which contains the youngest wine, is replaced with *sobretablas* wine. The number of stages typically ranges from 4 to 6. Usually, the number is positively correlated with the quality of the final wine.

The transfer of wine from one stage to the next is termed the *rocío*. It is preceded by a series of operations intended to homogenize the level of biological aging in each stage (Berlanga *et al.*, 2004). The wine extracted from each cask is combined in a tank prior to transfer to the casks in the next (older) row. The operation must be carefully performed to avoid disrupting the *flor* film on the wine's surface. This dynamic process generates uniformity in the character of the wine transferred to the next stage in the *solera* system. This fractional blending of homogeneous mixtures permits wine of similar sensory characteristics to be obtained year after year, irrespective of the particular vintage. In addition, the *rocío* operation, by blending older wines with younger wines, supplements the transfer of nutrients from the old to the young wine. This favors the formation and maintenance of the yeast film. In addition, this process provides aeration, which is highly beneficial for wine and flor yeast (Berlanga *et al.*, 2001, 2004).

The main categories of sherry wines are *fino*, *amontillado,* and *oloroso*. Their difference derives from the specifics of the way they are aged. The best known category of biologically aged wine is *fino*, obtained by using the *criaderas* and *solera* system as described above. In contrast, *amontillado* sherry is produced by a two-stage aging process. In the first stage, it undergoes dynamic biological aging, exactly as described for *fino* production. Ethanol is then added to reach 18–20%, and the wine completes its maturation via oxidative aging. In *oloroso* sherries, aging begins under a *flor* velum, then the wines are subjected to fractional blending, which involves only oxidative aging. The addition of alcohol to bring the level in the young wine to between 18% and 20%, at the beginning of fractional blending and solera aging prevents the formation of a yeast velum. The dynamic oxidation associated with fractional blending gives these wines their unique organoleptic characteristics.

Other types of high-quality wines are produced in the Jerez region. These are sweet wines made from the varieties, Pedro Ximenez and Muscat. The wines are produced after the grapes have been sun-dried for about 5–10 days. The resulting raisining produces a very dark must. Further, the musts are partially fermented. Fermentation is arrested by adding rectified ethanol. This produces very sweet, dark wines.

As with sherry wines, the *jaune* (yellow) wines of the Jura, France, are another example of biologically aged wines. Their manufacturing process is similar, although the biological aging process is static. The base wines are produced from Savagnin grapes using techniques traditional for white wine-making techniques. After the wine has completed malolactic fermentation, it is transferred to large containers, which are filled, leaving a gap of 5–6 L, and tightly closed for storage where they are stored for a legislated period of 6 years and 3 months. During this period, *flor* yeast (*S. cerevisiae*) develop on the surface of the wine, altering its sensory

properties. In addition, the acetaldehyde can reach 600–700 mg/L (Pham *et al.*, 1995). As its name implies, the wine acquires a typical golden yellow color. Sherry-like wines obtained in other wine growing areas, such as California or South Africa are produced by a shorter dynamic process in order to reduce costs.

III. MICROBIOTA OF THE *FLOR* FILM

During biological aging, considerable microbial diversity occurs in the velum that develops on the wine. Although the flora consists mainly of yeasts, other fungi and bacteria may occur. However, the restrictive conditions of biological aging (low pH, presence of sulfite, high ethanol and acetaldehyde concentrations, scarcity of sugars, and low oxygen concentration) are compatible with only a few *S. cerevisiae.* Therefore, more than 95% of the film's microbiota usually consists of film-forming *S. cerevisiae* races (Martínez *et al.*, 1997; Mesa *et al.*, 2000). Other yeasts that have been found include species of the genera *Debaryomyces*, *Zygosaccharomyces*, *Pichia*, *Hansenula,* and *Candida* (Suarez-Lepez and Iñigo-Leal, 2004). Guijo *et al.* (1986) also isolated *Torulaspora delbrueckii* and *Zygosaccharomyces bailii*, but they were deemed contaminants in the *flor* films in Montilla-Moriles wines. Some authors have additionally isolated species of *Dekkera* and *Brettanomyces*. They are believed to cause an abnormal increased acidity in casks containing biologically aging wines (Ibeas *et al.*, 1996).

Physiological and molecular characterization has shown that most yeasts present in the velum of sherry wines belong to different races of *S. cerevisiae*, mainly *beticus*, *cheresiensis*, *montuliensis,* and *rouxii* (Martínez *et al.*, 1995, 1997). These ''*flor*'' yeast differ from typical fermentative yeasts (which are unable to grow aerobically in wine), possessing distinct metabolic and genetic characteristics (Budroni *et al.*, 2005; Esteve-Zarzoso *et al.*, 2001, 2004). These strains present a heterogeneous genetic profile, characterized by considerable variability in the DNA content, mitochondrial DNA (mtDNA) restriction analysis, and chromosomal profiles. These facilitate their identification. In addition, the genetic profiles of strains isolated in different cellars vary and/or differ throughout the aging process. mtDNA restriction analysis seems to be a simple but elegant method for studying the dynamics of yeast strain development during specific steps or during the whole process of sherry winemaking (Esteve-Zarzoso *et al.*, 2001; Querol *et al.*, 1992).

Several studies have been aimed at elucidating the relationship between the activity of particular *flor* yeast enzymes during velum production, both in lab-scale and under winery conditions. For example, studies on the activity of alcohol and aldehyde dehydrogenase have

been conducted with the main objective of selecting *flor* yeast strains able to accelerate the biological aging process (Blandino *et al.*, 1997; Mauricio *et al.*, 1997). These enzymes catalyze the oxidation of ethanol to acetaldehyde and acetaldehyde into acetic acid, respectively. Moreover, alcohol acetyltransferase and esterase activities, involved in the production of isoamyl alcohols and ethyl acetate, have been examined in different *flor* yeast strains during biological aging (Plata *et al.*, 1998).

The consumption and release of amino acids, urea, and ammonium ions by flor yeast, as well as the influence of amino acids on the aging process have also received increasing attention (Botella *et al.*, 1990; Mauricio and Ortega, 1997; Mauricio *et al.*, 2001a,b). *Flor* yeast may be able to use amino acids not only as nitrogen sources but also as redox agents to balance the oxidation–reduction potential under conditions of restricted oxygen availability (Mauricio *et al.*, 2001a,b). Taking into account that nitrogen compounds are known to be essential for the vinification process, it is not surprising that more research will be aimed at establishing the details of their metabolic roles in biological aging.

In comparison with *flor* yeast, little research has been focused on the presence and role of bacteria during the biological aging of wines (Suárez and Agudelo, 1993; Suárez *et al.*, 1994). Lactic acid bacteria can play a significant role in wine production through malolactic fermentation. It is an important secondary process that occurs in many wines after yeast-induced alcoholic fermentation has come to completion (Lonvaud-Funel, 1999; Moreno-Arribas and Polo, 2005). Moreno-Arribas and Polo (2008) studied the occurrence of lactic acid bacteria populations during different stages of biological aging. During the production and aging of fino sherry, the population of lactic acid bacteria remained low. However, malolactic fermentation may occur during storage, prior to the commencement of biological aging or during its initiation (Moreno-Arribas and Polo, 2008). Strains of *Oenococcus oeni*, the main lactic acid bacteria responsible for malolactic fermentation in wines, were not found. *Lactobacillus plantarum*, followed by *L. casei*, *L. brevis, and L. zeae*, were the most commonly isolated bacterial species in biologically aged wines (Moreno-Arribas and Polo, 2008; Suárez *et al.*, 1994).

IV. CHANGES IN THE CHEMICAL COMPOSITION OF SHERRY WINES DURING THE BIOLOGICAL AND OXIDATIVE AGING

The production of sherry wines is mainly characterized by a long aging period (from 5 to 12 years, depending on the style) in oak casks, the use of a limited number of white grape varieties (cv. Palomino for dry sherry, and Pedro Ximenez and Muscat for sweet sherries) fermented under

similar conditions, and the application of different aging procedures. It is during aging that the wines undergo their most important changes in chemical composition. Some are due to the aerobic metabolism of *flor* yeast growing on the wine's surface, as with fino-style sherries, when the ethanol content is lower than 15% (v/v). In addition to their metabolic activity, *flor* yeast may undergo autolysis (Charpentier *et al.*, 2004). From an enologic point of view, this process is important due to the enzymatic hydrolysis of biopolymers in the cells. This releases cytoplasmic (peptides, amino acids, fatty acids, and nucleotides) and cell wall compounds (glucans, mannoproteins) into the wine. These modify the wine's chemical composition and, therefore, its sensory characteristics (Charpentier and Feuillat, 1993; Martinez-Rodriguez and Polo, 2000; Pozo-Bayón *et al.*, 2009). However, raising the ethanol content to 18% (v/v) before fractional blending, as in the case of oloroso sherries, prevents the growth of *flor* yeast. Thus, the wine undergoes only oxidative aging. This activates important changes in the wine's chemical composition, such as the oxidation of polyphenols. In amontillado wines, one of the most appreciated of sherry styles, both types of aging are involved in their production. Thus, the chemical changes are much more complex, giving rise to very complex aromatic and other sensory attributes.

Some of the most important chemical changes that occur during the biological and/or oxidative aging of sherry wines are reviewed below.

A. Major alcohols

Ethanol is produced during yeast fermentation of grape sugars, and it is, after water, the major component of wines. Ethanol content is highly variable across wines, depending on the sugar content of the must and on the winemaking technology involved in their production. In the case of sherry wines, and other fortified wines, its content ranges between 15% (in fino wines) and 18–21% (in the case of oloroso wines). Ethanol can positively impact on the sensory characteristics of these wines. Not only does it directly contribute to a wine's aroma, occurring at above its perception threshold (Bayonove *et al.*, 2000), but also can modify solution polarity. This alters the gas–liquid partition coefficient between aroma compounds and the wine matrix, and thereby their relative volatility (Pozo-Bayón and Reineccius, 2009).

During the biological aging of sherry, the concentration of ethanol decreases because of its consumption by *flor* yeast. Its respiration via the tricarboxylic acid pathway (Suarez-Lepez and Iñigo-Leal, 2004) provides the main source of carbon and energy. Acetaldehyde is the main organic by-product of ethanol metabolism, but other volatile compounds, notably acetic acid, butanediol, diacetyl, and acetoin, can also be formed. In addition,

ethanol is lost by evaporation through the oak cask, resulting in a progressive decrease in alcoholic content during aging (Charpentier *et al.*, 2000).

Glycerol is also one of the most abundant components of wines. It can contribute directly to flavor perception, through its sweet taste (Noble and Bursick 1984), as well as viscosity. Thus, it can influence the aroma of the wine when tasted. Glycerol is mainly produced during glycerol–pyruvic fermentation at the beginning of the alcoholic fermentation. Flor yeast can use it as a carbon source; therefore, its concentration decreases during wine aging. This is potentially a useful indicator of the biological aging of wine (Peinado and Mauricio, 2009).

B. Nitrogen compounds

The nitrogen fraction of must and wine consists mainly of amino acids and ammonium compounds. Nitrogen-containing compounds are important not only for yeast growth and metabolism, but deficiency can also lead to sluggish or stuck fermentations (Mauricio *et al.*, 1995, 2001a,b). *S. cerevisiae* can grow on a wide variety of nitrogen-containing substrates. The rate of consumption and their metabolism is largely dependent on the yeast strain, its physiological state, and the physicochemical properties of the wine. *S. cerevisiae* can use amino acids, either directly in the biosynthesis of proteins or as a nitrogen source. Amino acids can be degraded by yeasts and the nitrogen released (generally as ammonia) and used for the synthesis of other nitrogenous constituents. The carbon of the amino acids might also be used by the yeast for synthetic purposes, and in this case, the compound acts as a carbon source that can be excreted into the medium (Large, 1986). The biological aging of sherry wines reduces the content of amino acids and other nitrogenous wine components (ammonium and urea). The main source of nitrogen for the yeast during the biological aging is L-proline, although yeasts differ in the amount of assimilable nitrogen, they can use and have preferences for amino acids consumption (Mauricio and Ortega, 1997; Valero *et al.*, 2003). Short aeration, used in accelerated aging, did not increase the overall consumption of assimilable nitrogen but accelerated the consumption of particular nitrogen compounds, such as L-proline, L-tryptophan, L-glutamic acid, ammonium ion, L-lysine, and L-arginine (Mauricio and Ortega, 1997).

Besides the use of amino acids as a nitrogen source, *flor* yeast may use these compounds to balance the oxidation–reduction potential under conditions of restricted oxygen availability. This can be achieved by releasing amino acids into the medium to restore the intracellular redox balance by oxidation of excess NADH (Mauricio *et al.*, 2001a,b; Moreno-Arribas and Polo, 2005; Valero *et al.*, 2003).

C. Organic acids

After fortification (*encabezado*), the base wine is stored for a variable period (*sobretablas*) prior to biological aging (*criadera* system). It is during this period that it undergoes malolactic fermentation. Therefore, most of the malic acid is converted into lactic acid before biological aging commences. The wine's lactic and pyruvic acids content can decrease during aging due to metabolism by *flor* yeast (Charpentier *et al.*, 2000). The tartaric acid content also declines due to its precipitation as potassium bitartrate. The gluconic acid content can be used as a measure of the amount of rot in the harvested grapes—concentrations below 1 g/L being considered suitable for sherry production (Peinado *et al.*, 2003, 2006a). *Flor* yeast can metabolize this acid without provoking changes in the sensory quality of the wines. Acetic acid is produced by yeast during fermentation, although its accumulation in sherry is usually low, occurring at below 0.7 g/L (Peinado and Mauricio, 2009). This acid is metabolized by *flor* yeast during biological aging by incorporating it (via acetyl-CoA) into the Krebs cycle or in the synthesis of fatty acids (Peinado and Mauricio, 2009).

D. Polyphenols

As noted previously, the main types of sherry (fino, oloroso, and amontillado) are produced employing different conditions. These differences result in significantly different polyphenolic composition. In the case of fino-type sherries, the layer of yeast that grows in the surface of the wine (*flor* velum) preserves its pale color. The velum limits the exposure of the wine from oxygen (Baron *et al.*, 1997). Hence, fino wines mature in a markedly reductive environment.

On the contrary, oloroso-type sherry wines are subjected to an oxidative aging. Their higher ethanol content (between 18% and 20%) does not allow yeast growth. The absence of a *flor* covering subjects the wine to extended oxidation, giving these wines particular organoleptic characteristics. Oloroso wines are characterized by a dark color, resulting largely from the oxidation of phenolic compounds (Ortega *et al.*, 2003). Flavan-3-ol monomers and oligomers may form brown pigments via several chemical pathways (Es-Safi *et al.*, 2000, 2003; Fulcrand *et al.*, 1997; Simpson, 1982, among others). Basically, the oxidation of phenolic compounds produces quinones. Their polymerization leads to compounds generating a reddish-brown color. The rate of this process under cellar conditions depends on various factors, including the acidity of the wine, its SO_2 content, the presence of metals (Fe), the amount of available O_2, and the temperature (Ortega *et al.*, 2003). As aging progresses, polyphenol oxidation increases the wine's dark coloration. Phenolic polymerization also results in a decrease in their low-molecular-weight monomers. However,

the concentration of other phenolic compounds might increase due to their extraction from the wood barrels. The specific nature of these phenolics depends, for example, on the type of wood used, the storage temperature, etc. (Cadahia *et al.*, 2001; Chatonet and Dubourdieu, 1998; Fernandez de Simon *et al.*, 1996). In addition, the concentration of some phenols of low molecular weight may increase as a result of the hydrolysis of oligomers, in particular, flavan-3-ol derivatives (Dallas *et al.*, 1995; Haslam, 1980). The concentration of phenolic compounds also can be influenced by evaporative losses of water and ethanol through the cask (Singleton, 1995).

Amontillado-type sherry is produced in a two-stage process. In the first stage, it undergoes dynamic biological aging, exactly the same for fino sherry production. Then, ethanol is added to bring its alcoholic degree up to 18–19%, and it completes its aging oxidatively, similar to oloroso sherries. Therefore, amontillados exhibit a phenolic composition between that of fino and oloroso sherries.

Figure 2.2 illustrates some of these differences in phenolic profile resulting from the different production technologies involved in sherry production. In fino sherries, phenolic aldehydes, typically associated with wines aged in wood are very low or almost absent. In addition, they do not show marked changes during aging. The only phenolic compounds that increase during biological aging are the benzoic acids (Garcia-Moreno and Garcia-Barroso, 2002). This is attributed to lignin breakdown and by the deamination of nitrogen compounds produced during *flor* yeast autolysis (Estrella *et al.*, 1987).

In oloroso sherries, benzoic and cinnamic acids remain at constant values through oxidative aging. However, other phenolic acids, such as gallic, syringic, and caffeic acids, experience greater changes during aging. In addition, the content of esterified derivatives is lower than in fino sherries. Oloroso wines are also characterized by their high content in 5-(hydroxymethyl)-2-furaldehyde (HMF), notably toward the end of the aging process.

Amontillado sherries are characterized by a phenolic composition between fino and oloroso wines. Wines from the youngest stage in the criadera system (stage 7) show a similar profile to the samples of Fino wine taken from its final stage (Ortega *et al.*, 2003). However, in subsequent stages, their character begins to resemble more that of oloroso than fino wines. In amontillados, aldehydes such as vanillin and *p*-hydroxybenzaldehyde show considerable increases during aging, whereas syringaldehyde remains constant. In addition, HMF shows a considerable increase during aging.

Differences in the phenolic composition of these types of sherries enable their differentiation, even during their earliest stages of production. In fact, discriminate variables, obtained by Linear Discriminant

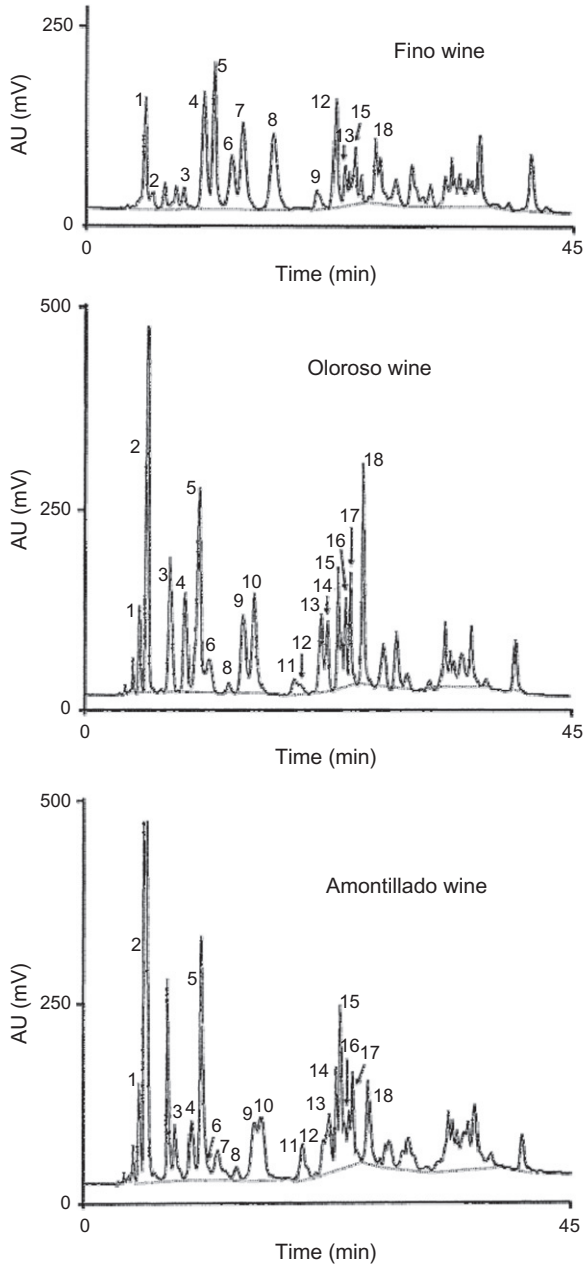

FIGURE 2.2 Chromatograms of sherry wines. Peaks: 1 = gallic acid; 2 = hydroxymethylfurfural; 3 = protocatechuic acid; 4 = caftaric acid; 5 = tyrosol; 6 = *cis-p*-coutaric acid; 7 = hydrocaffeic acid; 8 = *p*-hydroxybenzoic acid; 9 = *trans-p*-coutaric acid; 10 = *p*-hydroxybenzaldehyde; 11 = vanillic acid; 12 = chlorogenic acid; 13 = caffeic acid; 14 = vanillin; 15 = syringic acid; 16 = *cis-p*-coumaric acid; 17 = syringaldehyde; 18 = *trans-p*-coumaric acid; (- - -) basis line taken. (Reprinted with permission from Garcia-Moreno and Garcia-Barroso (2002). Copyright 2002. American Chemical Society.)

Analysis (LDA), have shown that the most effective indicators of differences between these wines are syringaldehyde, *trans*-*p*-coumaric, caffeic, *trans*-*p*-coutaric, syringic and vanillic acids, and *p*-hydroxybenzaldehyde. Three of them (HMF, *p*-hydroxybenzaldehyde, and syringaldehyde) were not detected in finos, and one (hydrocaffeic acid) was not detected in olorosos. The other compounds presented different behaviors, depending on the type of aging system (Ortega *et al.*, 2003).

V. AROMA AND SENSORY CHARACTERISTICS OF SHERRY WINES: EVOLUTION DURING AGING

Volatile compounds are responsible for the aroma of wines. They are, therefore, directly linked to wine quality and consumer preferences. The different sherry production technologies permit the evolution of wines with distinct volatile compositions and sensory characteristics. Fino wines, having undergone biological aging, acquire much of their typical and distinguishable flavor from the present volatile compounds derived from *flor* yeast metabolism. They are absent in wines such as oloroso wines, which undergoes an oxidative aging process. They possess a different slate of volatile compounds and aroma. Amontillado wines, in which both biological and oxidative aging occur are the oldest and most valued of these three wines styles. They also possess a more complex flavor than the other two (Zea *et al.*, 2001).

Acetaldehyde constitutes one of the most important volatile compounds produced during biological aging. Besides contributing to ethereal and overripe, apple notes, it is responsible for the pungent aroma of fino sherries (Zea *et al.*, 2007). Its acetaldehyde content also allows fino sherries to be differentiated from the other sherry styles (Moreno *et al.*, 2005). Its concentration can reach values between 350 and 450 mg/L, and occasionally 1000 mg/L (Martínez *et al.*, 1998). Acetaldehyde is produced by *flor* yeast, mainly as a result of the oxidation of ethanol by alcohol dehydrogenase II (ADH II). The enzyme is repressed by glucose. The acetaldehyde content increases during aging, although the most important changes occur during the earliest stages. This correlates with the period when *flor* yeast show their most intense metabolism (Peinado and Mauricio, 2009). Acetaldehyde also is involved in different biochemical reactions during biological aging, such as its combination with ethanol to produce 1,1-diethoxyethane. It can accumulate to concentration above 100 mg/L. This acetal has been shown to contribute to the wine's aroma, donating fresh, fruity, and green aromatic notes (Etievant, 1991). Acetaldehyde is also involved in the formation of other aroma compounds, such as acetoin, 2,3-butanediol (Peinado and Mauricio, 2009), and sotolon (Guichard *et al.*, 1997; Pham *et al.*, 1995). In addition, it is an important

molecule involved in different reactions in wines. For example, it has the ability to combine with sulfite ions, increasing the proportion of bound sulfite; it combines with some polyphenols (procyanidins) to form different pigments; and can oxidize to acetic acid. Nonetheless, this latter reaction only occurs to a limited extent and has little influence on wine composition and quality (Peinado and Mauricio, 2009).

Flor yeast also increase the content in other aroma compounds, such as higher alcohols, ethyl esters, lactones, and terpenes (Zea *et al.*, 1995). For instance, higher alcohols are very important contributors to the aroma of fino wines, although the concentration of most of them (e.g., isobutanol, 2-phenylethanol, and isoamylic alcohols) is quite stable throughout aging. One exception is propanol, which can dramatically increase during aging (Moreno *et al.*, 2005). The biosynthesis of higher alcohols is mainly produced in the fourth, third, and second *criadera* stages, from their corresponding amino acids. This coincides with maximal yeast activity (Peinado and Mauricio, 2009). In addition, it has been suggested that their production may increase due to yeast autolysis (Peinado and Mauricio, 2009).

Regarding esters, their concentration depends on balance between synthesis and hydrolysis reactions during aging, as well as the enzymatic activity of yeast. It, in turn, depends on features such as the type and strain of yeast and its physiological state (Mauricio *et al.*, 1993, Plata *et al.*, 1998). Many esters contribute to fruity aromas. In general, the concentration of higher alcohol acetates decreases through hydrolysis during the first few months of aging, whereas ethyl esters of organic acids (lactic, succinic) increase (Martinez de la Ossa *et al.*, 1987; Useglio-Tomasset, 1983). These changes are similar to trends found in other types of wines aged in contact with yeast, for example, sparkling wines (Hidalgo *et al.*, 2004; Pozo-Bayon *et al.*, 2003, 2010; Riu-Aumatell *et al.*, 2006).

Of lactones, sotolon is an important by-product of the biological aging of fino wines, as well as aging under oxidative conditions. The compound is produced from an aldolization between α-ketobutyric acid (from the deamination of L-threonine) and acetaldehyde, through a mechanism proposed by Pham *et al.* (1995). Because of its low perception threshold (10 μg/L), this compound has been described as an important odor impact compound. It adds nut, curry, and candy cotton notes to biologically and oxidatively aged sherries (Cutzach *et al.*, 2000; Escudero and Etievant, 1999; Kosteridis and Baumes, 2000). Its concentration in sherry wines depends on the duration of aging, but normally occurs at concentrations above 200 μg/L (Guichard *et al.*, 1997; Moreno *et al.*, 2005).

Other lactones detected include α-butyrolactone and pantolactone (2,4-hydroxy-3,3-dimethylbutyrolactone), both of which are typical of sherries. Besides the duration of aging, their concentration is closely linked to yeast strain (Zea *et al.*, 1995). Another lactone, solerone (4-

acetyl-γ-butyrolactone), was thought to be an important by-product of biological aging; however, its sensory impact on sherry aroma has been shown to be very low (Martin and Etievant, 1991).

Lactones derived from oak constitute an important flavorant in most wines aged in barrel. However, because sherries are aged in cask that are rarely emptied or cleaned, they derive few lactones from the wood. Their detection is possible only in stages containing the oldest wine (Chatonnet *et al.*, 1990).

Nonetheless, other compounds released from these casks used can be important contributors to the aroma of biological aged sherries and are absolutely essential to the aroma of oxidatively aged sherries. For example, Z-whisky lactone, also known as wood lactone, contributes to vanilla notes of olorosos. In addition, other compounds such as the phenols, eugenol, and 4-ethylguaiacol contribute to clove-like spicy fragrance. Both are derived from precursors extracted by ethanol from the casks. Their concentrations increase relative to contact time (Moyano *et al.*, 2002). The origin of the oak, the ethanol content of the wine, and the cellar temperature are the main factors influencing the efficiency of their extraction in all type of wines (Moyano *et al.*, 2009)

One way to quantify the odor impact of a compound is to determine the aroma value or odor activity value (OAV). This is calculated by dividing the concentration of the compound by its perception threshold. Therefore, the odor impact of a compound increases in proportion to its OAV when this value is >1. Thus, compounds exhibiting higher OAV values are more likely to contribute to the aroma of wine and have an important influence on its sensory characteristics.

Based on these criteria, Zea *et al.* (2001) were able to discriminate among the aroma fractions of the three types of sherries. They showed that the volatile compounds contributing the most to the flavor of fino wines were acetaldehyde, β-citronellol, and β-ionone. During oxidative aging (oloroso and partially amontillado sherries), esterification reactions are specially strong. Their high ethanol content, favor esterification and the accumulation of ethyl acetate and ethyl lactate. Using calculated OAV values and odor descriptors, the above-mentioned authors showed differences in the sensory profile between the three types of sherries. Fino wines were markedly floral and fruity (because of the presence of compounds such as farnesol, β-citronellol, and β-ionone). They also had cheesy, rancid (butanoic acid), and pungent (acetaldehyde) aromatic notes. Oloroso wines exhibited smoky and ethereal notes, associated with the presence of ethyl guaiacol and ethyl acetate, respectively. Amontillado wines were characterized by the presence of flavor notes from both aging processes, and correspondingly have a more complex fragrance.

More recently, Moyano *et al.* (2010) have evaluated the evolution of the odor-active compounds in amontillado sherries during the aging process.

They used gas chromatography–olfactometry (GC–O) to measure olfactory intensity. In addition, they calculated the odor spectrum value (OSV), which corresponds to OAV values normalized in respect to a reference value, corresponding to the strongest odorant compound. OSVs are, therefore, concentration-independent and more representative of the relative significance of an aroma compound (Moyano *et al.*, 2010). In this work, they identified 25 odor-active compounds, mainly associated with fruity and fatty notes. In addition, they found that changes in aroma profile largely occurred during the first years of the oxidative aging. Ethyl octanoate was the most powerful odorant, followed by ethyl butanoate, eugenol, ethyl isobutanoate, and sotolon (Table 2.1). All of them maintained a similar relative aroma contribution to the aroma profile of amontillado wines during oxidative aging. In addition, they found that most odorants analyzed increased their concentration over time, leading to an augmentation of flavor.

VI. NEW TRENDS IN SHERRY WINEMAKING TECHNOLOGY

A. Accelerated biological aging

The most distinctive feature of sherry production is the prolonged biological aging process conducted in vast maturation cellars. Aging is carried out in partially filled (~80%), American oak casks, staked in rows that correspond to individual stages (*criadera*) in fractional blending. The process involves the development and maintenance of a *flor* yeast biofilm on the wine's surface for at least 4 years, essential to obtaining high-quality fino sherries.

The prolonged storage, and complexities associated with the development and the maintenance of the yeast biofilm, substantially adds to the sherry production costs. Therefore, different strategies have been proposed to reduce aging time (Muñoz *et al.*, 2007). One suggestion has been to increase the surface/volume ratio of wines by using stainless steel trays. This, however, has disadvantages related to the handling and processing of individual trays and the greater amount of biomass produced, resulting in a depreciation in wine quality. Other strategies noted in Muñoz *et al.* (2007) have focused on increasing aeration, for example, providing steel tanks with stirrers (Ough and Amerine, 1972), or other related procedures (Ough, 1992; Rankine, 1997). However, these systems disrupt formation of a surface *flor* velum. This could affect the metabolic activity of the *flor* yeast, and accelerate oxidation phenomena, resulting in a lower quality product. Other systems involve submitting the wine to periodic, short, microaerations, carried out after film formation. This avoids disrupting the structural integrity of the *flor* velum (Cortes *et al.*, 1999). Muñoz *et al.* (2007) have

TABLE 2.1 Average odor spectrum values of the active odorant compound in Amontillado wines.[a] Reprinted with permission from Moyano *et al.* (2010). Copyright 2010. American Chemical Society.

	Odor spectrum value[b]						
Compounds	AS6[c]	AS7	AS8	AS12	AS18	AS24	RCI
Ethyl octanoate	100	100	100	100	100	100	1
Ethyl isobutanoate	41.2	39.2	38.9	41.3	59.3	44.7	1.15
Eugenol	41.0	46.3	76.3	54.3	59.2	53.8	0.705
Ethyl butanoate	34.4	43.1	55.9	67.5	82.0	66.7	1.19
Sotolon	30.0	29.8	18.7	61.4	56.8	54.5	2.91
Ethyl hexanoate	29.2	29.4	22.7	30.0	34.7	32.2	1.41
Acetaldehyde	20.2	22.0	26.9	22.4	26.9	24.8	0.923
Isoamyl acetate	12.1	5.5	7.1	9.9	13.9	13.7	1.93
Z-oak lactone	11.7	10.1	11.9	15.7	20.0	19.2	1.61
1,1-Diethoxyethane	11.3	14.1	19.4	23.0	28.5	25.5	1.31
Isoamyl alcohols	10.2	11.0	11.6	14.7	16.8	15.2	1.31
Phenethyl alcohol	9.9	11.1	11.7	15.3	18.4	17.6	1.50
4-Ethylguaiacol	9.6	12.3	13.4	16.9	25.0	22.5	1.68
Ethyl acetate	9.4	8.1	7.2	25.9	36.6	33.0	4.58
Methionol	8.7	9.7	4.9	4.3	0.0	0.0	–
3-Methylbutanoic acid	7.4	6.8	6.7	5.5	6.8	4.3	0.642
Methyl butanoate	5.7	6.2	9.0	7.9	8.2	8.8	0.978
Isobutanol	5.4	5.8	6.3	7.1	8.8	8.1	1.29
2,3-Butanedione	5.3	5.7	7.2	18.9	29.4	25.7	3.57
Ethyl lactate	5.2	4.3	3.5	13.5	16.9	16.6	4.74
Acetoin	4.3	4.1	6.6	5.2	7.2	7.0	1.06
Butanoic acid	3.5	3.1	2.9	3.2	3.9	3.0	1.03
Ethyl 3-hydroxyhexanoate	3.2	3.8	3.5	12.3	12.3	10.6	3.03
Phenethyl acetate	2.8	3.2	4.8	8.8	12.1	12.0	2.50
Octanal	1.7	2.1	3.2	3.5	5.0	3.8	1.19

[a] The relative contribution index (RCI) was calculated by dividing the OSV of each compound at the end of oxidative aging into its OSV at the end of biological aging.
[b] Normalized odor activity value with an approximate Steven's law exponent of $n = 0.5$.
[c] AS6, AS7, AS8: age of the wine under biological aging; AS12, AS18, AS24: age of the wines under oxidative aging.

shown the process to generate chemical changes similar to those of the traditional process. The main differences were in compounds extracted from the cask wood. Thus, it may be possible to shorten the biological aging step by periodic microaeration in stainless-steel containers, followed by aging in oak casks under cellar conditions. This may result in wines with high quality in less time, thus reducing both the wine's production cost and retail price for the consumer.

B. Accelerated drying conditions for sweet sherry wine production

In addition to focus on reducing the time involved in sherry production, there has been interest in improving the technology associated with producing sweet sherries. These are made primarily from raisined Pedro Ximenez in Montilla-Moriles (southern Spain). Recently, their consumption has increased considerably (Ruíz *et al.*, 2010). Production involves a sun-drying of the grapes for 5–10 days. The grapes (with a potential alcohol degree of at least 13.5% v/v) are spread onto mats. These are periodically turned by hand during the drying process to achieve a uniform concentration of their constituents. The end-product are raisins that give a very dark musts, due to strong browning during raisining. Because evaporative water loss is close to 50% in weight, their sugar content reaches above 400 g/L.

Proper raisining requires high diurnal temperatures and very low humidity levels. In recent years, climatic changes in many wine producing regions, such as Montilla-Moriles, in which many of these wines are produced, have resulted in slower and less efficient raisining. This has increased the risk of ochratoxin A formation in the grapes, and contamination of the must and wine (Amézqueta *et al.*, 2009).

An alternative process that has been proposed recently involves the use of hot air driers. They blow hot air over a wide surface, facilitating rapid water loss from the harvested grapes. To avoid potential problems associated with sun-drying, such as the growth of fungal toxin producers, dust, or insect contamination. Ruíz *et al.* (2010) have investigated its effect, compared to traditional sun-drying, on the aroma composition of musts obtained from Pedro Ximénez grapes. Their results showed that musts from chamber-dried grapes exhibited the same aroma attributes as those from sun-dried grapes. They differed only in generally possessing higher OAVs, resulting in musts of higher aroma intensity.

C. Production of wines from organic grapes

Growing consumer interest in environmental protection has promoted increased emphasis on more ecological sustainable agricultural methods. In addition, concern about health and its relationship to food supply has promoted the demand for organic products. So-called organic or ecological wines are produced from grapes cultivated with limitations on the use of chemical fertilizers, insecticides, and other synthetic pest-control substances. In addition, sustainable agricultural practices such as cover crops and natural products such as manure or compost are used (Moyano *et al.*, 2009).

Moyano *et al.* (2009) have performed the first study comparing the effects of ecological versus conventional procedures on the aroma of sherry wines. They showed that ecologically cultivated grape produced wines showing a sensory profile similar to that of the traditional fino wines, except for lower odor intensity (equivalent to traditional wine aged for <3 years). This finding may result from differences in the nitrogen fraction of the grapes, arising from differences in viticultural practice. This could lead to reduced synthesis of alcohols and esters by the *flor* yeast. Alternately, synthesis of aroma compounds might be favored by the higher concentration of SO_2 used in traditional sherry production.

VII. CONCLUSION AND FUTURE TRENDS

As reported in this chapter, an extensive amount of new research has focused on microbiological and chemical aspect changes associated with biological and oxidative aging in sherry production. However, traditional procedures involve prolonged storage in vast cellars, and labor-intensive operations to ensure the homogeneity essential to the wine's ultimate quality. This substantially raises production costs. In this regard, most current studies are being devoted to attempts to substantially shorten the aging period, while keeping the high quality and special flavor characteristics traditional to these wines.

In addition, genetic improvement in *flor* yeast, such as studies on the genes responsible for flocculation, such as *FLO11*, may lead to advances in cell-immobilization technology. So far, a strain of *S. cerevisiae* var. *capensis* has been successfully coimmobilized with *Penicillium chrysogenum*, in order to obtain biocapsules for potential use in a number of fermentation processes (Peinado *et al.*, 2006b). In addition, expression of genes tolerant to conditions found maturing *flor* films, such as *SOD1* or *MUC1*, may facilitate the establishment of a more stable velum and shortening aging times.

ACKNOWLEDGMENTS

The authors are grateful to the Spanish Ministry for Science and Innovation (AGL2009-13361-C02-01 and CSD2007-00063 Consolider Ingenio 2010 FUN-C-FOOD Projects), and the Comunidad de Madrid (ALIBIRD P2009/AGR-1469 Project).

REFERENCES

Amézqueta, S., González-Peñas, E., Murillo-Arbizu, M., and López de Cerain, A. (2009). Ochratoxin a decontamination: A review. *Food Control* **20**, 326–333.

Baron, R., Mayen, M., Merida, J., and Medina, M. (1997). Changes in phenolic compounds and browning during biological aging of sherry wine. *J. Agric. Food Chem.* **45**, 1682–1685.

Bayonove, C., Baumes, R., Crouzet, J., and Gü.nata, Z. (2000).(Claude Flanzy, Coordinator) *In* ''In Enología: Fundamentos Científicos y Tecnológicos''. pp. 137–176. AMV and Mundi-Prensa Editions, Madrid.

Berlanga, T. M., Atanasio, C., Mauricio, J. C., and Ortega, J. M. (2001). Influence of aeration on the physiological activity of flor yeasts. *J. Agric. Food Chem.* **49**, 3378–3384.

Berlanga, M. T., Peinado, R., Millán, C., Mauricio, J. C., and Ortega, J. M. (2004). Influence of blending on the content of different compounds in the biological aging of sherry dry wines. *J. Agric. Food Chem.* **52**, 2577–2581.

Blandino, A., Caro, I., and Cantero, D. (1997). Comparative study of alcohol dehydrogenase activity in flor yeast extracts. *Biotechnol. Lett.* **19**, 651–654.

Botella, M. A., Pérez-Rodríguez, L., Domecq, B., and Valpuesta, V. (1990). Amino acid content of Fino and Oloroso sherry wines. *Am. J. Enol. Vitic.* **41**, 12–15.

Budroni, M., Zara, S., Zara, G., Pirino, G., and Mannazzu, I. (2005). Peculiarities of flor strains adapted to Sardinian sherry-like wine aging conditions. *FEMS Yeast Res.* **5**, 951–958.

Cadahia, E., Muñoz, I., Fernández de Simón, M. B., and Garcia-Vallejo, M. C. (2001). Changes in low molecular weight phenolic compounds in Spanish, French, and American oak woods during natural seasoning and toasting. *J. Agric. Food Chem.* **49**, 1790–1798.

Charpentier, C. and Feuillat, M. (1993). Yeast autolysis. *In* ''Wine Microbiology and Biotechnology'', (G. Fleet, Ed.), pp. 225–242. CRC Press, New York.

Charpentier, C., Etiévant, P., and Guichard, E. (2000). Vinificación de los vinos de velo: vino Jaune, Jerez y Otros. *In* ''Enología, Fundamentos Científicos y Tecnológicos'', (C. Flanzy, Ed.), pp. 531–539.

Charpentier, C., Dos Santos, A. M., and Feuillat, M. (2004). Release of macromolecules by Saccharomyces cerevisiae during aging of flor Sherry wine ''vin jaune''. *Int. J. Food Microbiol.* **96**, 253–262.

Chatonet, P. and Dubourdieu, D. (1998). Comparative study of the characteristics of American white oak (*Quercus alba*) and European oak (*Querqus petraea* and *Q. robur*) for production of barrels used in barrel aging of wines. *Am. J. Enol. Vitic.* **30**, 61.

Chatonnet, P., Boidron, J. N., and Pons, M. (1990). Élevage des vins rouges es fut de chene: évolution de certains composés volatils et de leur impact aromatique. *Sci. Aliment.* **10**, 565–578.

Cortes, M. B., Moreno, J., Zea, L., Moyano, L., and Medina, M. (1999). Response of the aroma fraction in sherry wines subjected to accelerated biological aging. *J. Agric. Food Chem.* **47**, 3297–3302.

Cutzach, I., Chatonet, P., and Dubourdieu, D. (2000). Influence of storage conditions on the formation of some volatile compounds in white fortified wines during the aging process. *J. Agric. Food Chem.* **48**, 2340–2345.

Dallas, C., Ricardo-Da Silva, J. M., and Laureano, O. (1995). Degradation of oligomeric procyanidins and anthocyanins in a Tinta Roriz red wine during maturation. *Vitis* **34**, 51–56.

Escudero, A. and Etievant, P. (1999). Effect of antioxidants on the flavour characteristics and the gas chromatography/olfactometry profiles of champagne extracts. *J. Agric. Food Chem.* **48**, 2340–2345.

Es-Safi, N. E., Guerneve, C., Cheynier, V., and Moutonet, M. (2000). New phenolic compounds formed by evolution of +-(catequine) and glyoxylic acid hydroalcoholic solution and their implication in color changes of grape-derived foods. *J. Agric. Food Chem.* **48**, 4233–4240.

Es-Safi, N. E., Guerneve, C., Cheynier, V., and Moutonet, M. (2003). Effect of copper on oxidation of (+)-catequine in a model solution system. *Int. J. Food Sci. Technol.* **38**, 153–163.

Esteve-Zarzoso, B., Peris-Torán, M. J., García-Maiquez, E., Uruburu, F., and Querol, A. (2001). Yeast population dynamics during the fermentation and biological aging of Sherry wines. *Appl. Environ. Microbiol.* **67**, 2056–2061.

Esteve-Zarzoso, B., Fernandez-Espinar, M. T., and Querol, A. (2004). Authentication and identification of Saccharomyces cerevisiae 'flor' yeast races involved in sherry ageing. *Anton. Leeuw. Int. J. G.* **85**, 151–158.

Estrella, I., Alonso, E., and Revilla, E. (1987). Presence of flavonol aglycones in Sherry wines and changes in their content during aging. *Z. Lebensm. Unters. Forsch.* **184**, 27–29.

Etievant, P. (1991). Wine. *In* ''Volatile Compounds in Food'', (H. Maarse, Ed.), pp. 483–546. Marcel Dekker Inc., New York.

Fernandez de Simon, B., Conde, E., Cadahía, E., and García-Vallejo, M. C. (1996). Low-molecular weight phenolic compounds in woods of Spanish, French and American oak. *J. Sci. Technol. Tonnellerie* **2**, 13–23.

Fulcrand, H., Cheynier, V., Oszmianski, J., and Moutonet, M. (1997). An oxidized tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation. *Phytochemistry* **46**, 223–227.

Garcia-Moreno, M. V. and Garcia-Barroso, C. (2002). Comparison of the evolution of low molecular weight phenolic compounds in typical Sherry wines**:** Fino, Amontillado, and Oloroso. *J. Agric. Food Chem.* **50**, 7556–7563.

Guichard, E., Pham, T. T., and Charpentier, C. (1997). Le sotolon, marqueur de la typicité de l'arome des vines du Jura. *Rev. Oenol.* **82**, 32–34.

Guijo, S., Millán, C., and Ortega, J. M. (1986). Fermentative features of vinification and maduration yeasts isolated in the Montilla-Moriles region of Southern Spain. *Food Microbiol.* **3**, 133–142.

Haslam, E. (1980). In vino veritas: Oligomeric procyanidins and the aging of red wines. *Phytochemistry* **19**, 2577–2582.

Hidalgo, P., Pueyo, E., Pozo-Bayon, M. A., Martinez-Rodriguez, A. J., Martin-Alvarez, P., and Polo, M. C. (2004). Sensory and analytical study of rose sparkling wines manufactured by second fermentation in the bottle. *J. Agric. Food Chem.* **52**(21), 6640–6645.

Ibeas, J. I., Lozano, I., Perdigones, F., and Jiménez, J. (1996). Detection of *Dekkera-Brettanomyces* strains in sherry by a nested PCR method. *Appl. Environ. Microbiol.* **62**, 998–1003.

Ibeas, J. I., Lozano, I., Perdigones, F., and Jiménez, J. (1997). Effects of ethanol and temperature on the biological aging of sherry wines. *Am. J. Enol. Vitic.* **48**, 71–74.

Kosteridis, Y. and Baumes, R. (2000). Identification of impact odorants in bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. *J. Agric Food Chem.* **48**, 400–406.

Large, P. J. (1986). Degradation of organic nitrogen compounds by yeast. *Yeast* **2**, 1–34.

Lonvaud-Funel, A. (1999). Lactic acid bacteria in the quality improvement and depreciation of wine. *Anton. Leeuw. Int. J. G.* **76**, 317–331.

Martin, B. and Etievant, P. (1991). Quantitative determination of solerone and sotolon in flor sherries by two dimensional *GC*. *J. High Resolut. Chromatogr.* **14**, 133–135.

Martínez, P., Codón, A. C., Pérez, L., and Benítez, T. (1995). Physiological and molecular characterization of flor yeasts: polymorphism of flor yeast populations. *Yeast* **11**, 1399–1411.

Martínez, P., Pérez Rodríguez, L., and Benítez, T. (1997). Evolution of flor yeast population during the biological aging of fino Sherry wine. *Am. J. Enol. Vitic.* **48**, 160–168.

Martinez de la Ossa, E., Caro, I., Bonat, M., Pérez, L., and Domecq, B. (1987). Dry extract in Sherry and its evolution in the aging of sherry. *Am. J. Enol. Vitic.* **38**, 321–325.

Martínez, P., Valcárcel, M. J., Pérez, L., and Benítez, T. (1998). Metabolism of *Saccharomyces cerevisiae* flor yeast during fermentation and biological aging of fino sherry: By-products and aroma compounds. *Am. J. Enol. Vitic.* **49**, 240–250.

Martinez-Rodriguez, A. and Polo, M. C. (2000). Enological aspects of yeast autolysis. *In* ''Recent Research Developments in Microbiology'', (S. G. Pandalay, Ed.), Vol. 4, pp. 285–301. Research Signpost, Trivandrum.

Mauricio, J. C. and Ortega, J. M. (1997). Nitrogen compounds in wines during its biological aging by two flor film yeast: An approach to accelerated biological aging of dry-sherry type wines. *Biotechnol. Bioeng.* **53**, 159–167.

Mauricio, J., Moreno, J. J., Valero, E. M., Medina, M., and Ortega, J. M. (1993). Ester formation and specific activities of in *in vitro* alcohol acetyltransferase and esterase by *Saccharomyces cerevisiae* during grape must fermentation. *J. Agric. Food Chem.* **41**, 2086–2091.

Mauricio, J. C., Ortega, J. M., and Salmon, J. M. (1995). Sugar uptake by three strains of *Saccharomyces cerevisiae* during alcoholic fermentation at different initial ammoniacal nitrogen concentrations. *Acta Horticult.* **388**, 197–202.

Mauricio, J. C., Moreno, J. J., and Ortega, J. M. (1997). *In vitro* specific activities of alcohol and aldehyde dehydrogenases from two flor yeasts during controlled wine aging. *J. Agric. Food Chem.* **45**, 1967–1971.

Mauricio, J. C., Valero, E., Millán, C., and Ortega, J. M. (2001a). Changes in nitrogen compounds in must and wine during fermentation and biological aging by flor yeast. *J. Agric. Food Chem.* **49**, 3310–3315.

Mauricio, J. C., Valero, E., Millán, C., and Ortega, J. M. (2001b). Changes in nitrogen compounds in must and wine during fermentation and biological aging by flor yeasts. *J. Agric. Food Chem.* **49**, 3310–3315.

Mesa, J. J., Infante, J. J., Rebordinos, L., Sanchez, J. A., and Cantoral, J. M. (2000). Influence of the yeast genotypes on enological characteristics of Sherry wines. *Am. J. Enol. Vitic.* **51**, 15–21.

Moreno, J. A., Zea, L., Moyano, L., and Medina, M. (2005). Aroma compounds as markers of the changes in sherry wines subjected to biological aging. *Food Control* **16**, 333–338.

Moreno-Arribas, M. V. and Polo, M. C. (2005). Winemaking, biochemistry and microbiology: Current knowledge and future trends. *Crit. Rev. Food Sci. Nutr.* **45**, 265–286.

Moreno-Arribas, M. V. and Polo, M. C. (2008). Occurrence of lactic acid bacteria and biogenic amines in biologically aged wines. *Food Microbiol.* **25**, 875–881.

Moyano, L., Zea, L., Moreno, J., and Medina, M. (2002). Analytical study of aromatic series in sherry wines subjected to biological aging. *J. Agric. Food Chem.* **50**, 7356–7361.

Moyano, L., Zea, L., Villafuerte, L., and Medina, M. (2009). Comparison of odour active compounds in sherry wines processed from ecologically and conventionally Brown Pedro Ximenez grapes. *J. Agric. Food Chem.* **57**, 968–973.

Moyano, L., Zea, L., Moreno, J., and Medina, M. (2010). Evaluation of the active odorants in Amontillado Sherry wines during the aging process. *J. Agric. Food Chem.* **58**, 6900–6904.

Muñoz, D., Peinado, R., Medina, M., and Moreno, J. (2007). Biological aging of Sherry wines under periodic and controlled microaerations *with Saccharomyces cerevisiae* var. *capensis*: Effect on odorant series. *Food Chem.* **100**, 1188–1195.

Noble, A. C. and Bursick, A. C. (1984). The contribution to glycerol to perceived viscosity and sweetness in white wine. *Am. J. Enol. Vitic.* **35**, 110–112.

Ortega, A. F., López-Toledano, A., Mayen, M., Merida, J., and Medina, M. (2003). Changes in color and phenolic compounds during oxidative aging of sherry white wines. *J. Food Sci.* **68**, 2461–2468.

Ough, C. S. (1992). Fermentation and wine composition. *In* ''Winemaking Basics'', (R. E. Gough, Ed.), pp. 92–145. Food Products Press. The Haworth Press, Inc. Publisher.

Ough, C. S. and Amerine, M. A. (1972). Further studies with submerged flor sherry. *Am. J. Enol. Vitic.* **23**, 128–131.

Peinado, R. and Mauricio, J. (2009). Biologically aged wines. *In* ''Wine Chemistry and Biochemistry'', (M. C. Polo and M. V. Moreno-Arribas, Eds), pp. 81–101. Springer Life Sciences Publisher, New York.

Peinado, R. A., Moreno, J. J., Ortega, J. M., and Mauricio, J. C. (2003). Effect of gluconic acid consumption during simulation of biological aging of sherry wines by a flor yeast strain on the final volatile compounds. *J. Agric. Food Chem.* **51**, 6198–6203.

Peinado, R. A., Mauricio, J. C., and Moreno, J. J. (2006a). Aromatic series in Sherry wines with gluconic acid subjected to different biological aging conditions by *Saccharomyces cerevisiae* var. *capensis*. *Food Chem.* **94**, 232–239.

Peinado, R. A., Moreno, J. J., Villalba, J. M., González-Reyes, J. A., Ortega, J. M., and Mauricio, J. C. (2006b). Yeast biocapsules: A new immobilization method and their applications. *Enz. Microb. Technol.* **40**, 79–84.

Pham, T. T., Guichard, E., Schilch, P., and Charpentier, C. (1995). Optimal conditions for the formation of sotolon from α-ketobutyric acid in the French ''vin jaune''. *J. Agric. Food Chem.* **43**, 2616–2619.

Plata, M. C., Mauricio, J. C., Millán, C., and Ortega, J. M. (1998). *In Vitro* activity of alcohol acetyltransferase and esterase in two flor yeast strains during biological aging of sherry wines. *J. Ferment. Bioeng.* **85**, 369–374.

Pozo-Bayón, M. A. and Reineccius, G. (2009). Interactions between wine matrix macro-components and aroma compounds. *In* ''Wine Chemistry and Biochemistry'', (M. C. Polo and M. V. Moreno-Arribas, Eds), pp. 417–435. Springer Life Sciences Publisher, New York.

Pozo-Bayon, M. A., Pueyo, E., Martin-Alvarez, P. J., Martinez-Rodriguez, A. J., and Polo, M. C. (2003). Influence of yeast strain, bentonite addition, and aging time on volatile compounds of sparkling wines. *Am. J. Enol. Vitic.* **54**, 273–278.

Pozo-Bayón, M. A., Martínez-Rodríguez, A., Pueyo, E., and Moreno-Arribas, M. V. (2009). Chemical and biochemical features involved in sparkling wine production: From a traditional to an improved winemaking technology. *Trends Food Sci. Technol.* **20**, 289–299.

Pozo-Bayón, M. A., Pueyo, E., Martín-Álvarez, P. J., Moreno-Arribas, M. V., and Andujar-Ortiz, I. (2010). Impact of using Trepat and Monastrell red grape varieties on the volatile and nitrogen composition during the manufacture of rosé Cava sparkling wines during the manufacture. *LWT-Food Sci. Technol.* **43**, 1526–1532.

Querol, A., Barrio, E., Huerta, T., and Ramón, D. (1992). Molecular monitoring of wine fermentation conducted by active dry yeast strains. *Appl. Environ. Microbiol.* **58**, 2948–2953.

Rankine, B. C. (1997). Winemaking methods. Making Good Wine. Pan Macmillan, Sydney.

Riu-Aumatell, M., Bosch-Fuste, J., Lopez-Tamames, E., and Buxaderas, S. (2006). Development of volatile compounds of cava (Spanish sparkling wine) during long aging time in contact with lees. *Food Chem.* **95**(2), 237–242.

Ruíz, M., Zea, L., Moyano, L., and Medina, M. (2010). Aroma active compounds during the drying of grapes cv. Pedro Ximenez destined to the production of sweet sherry wine. *Eur. Food Res. Technol.* **230**, 429.

Simpson, R. F. (1982). Factors affecting oxidative browning of white wine. *Vitis* **21**, 233–239.

Singleton, V. L. (1995). Maturation of wines and spirits: Comparisons, facts, and hypotheses. *Am J. Enol. Vitic.* **46**, 98–115.

Suárez, J. A. and Agudelo, J. (1993). Characterization of yeast and lactic acid bacterial species in ropy wines. *Z. Lebensm. Unters. Forsch.* **196**, 152–154.

Suárez, J. A., Callejo, M. J., and Colomo, B. (1994). Lactic acid production in Sherry-type wines from the Rueda Appellation of Origin Region. *Bull. OIV* **755–756**, 15–24.

Suarez-Lepez, J. A. and Iñigo-Leal, B. (2004). Microbiologia enológica. Fundamentos de vinificación. Ediciones Mundi-Prensa, Madrid, Spain (pp. 673–716).

Useglio-Tomasset, L. (1983). Influence de la température de conservation sur les caractéristiques physico-chimiques et organoleptiques des vins (Vins aromatiques). *Bull. OIV* **623**, 19–34.

Valero, E., Millásn, C., Ortega, J. M., and Mauricio, J. C. (2003). Concentration of amino acids in wine after the end of fermentation by *Saccharomyces cerevisiae* strains. *J. Sci. Food Agric.* **83**, 830–835.

Zea, L., Moreno, J., and Medina, M. (1995). Characterization of aroma fractions in biological aging of ''fino'' white wines produced in the Nmontilal-Moriles appellation d'origine. *Acta Horticult.* **388**, 233–238.

Zea, L., Moyano, L., Moreno, J., Cortes, B., and Medina, M. (2001). Discrimination of the aroma fraction of sherry wines obtained by oxidative and biological aging. *Food Chem.* **75**, 79–84.

Zea, L., Moyano, L., Moreno, J., and Medina, M. (2007). Aroma series as fingerprints for biological aging in fino sherry-type wines. *J. Sci. Food Agric.* **87**, 2319–2326.