Wine Chemistry and Biochemistry

M. Victoria Moreno-Arribas · M. Carmen Polo
Editors

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Springer

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ISBN: 978-0-387-74116-1 e-ISBN: 978-0-387-74118-5
DOI 10.1007/978-0-387-74118-5

Library of Congress Control Number: 2008938361

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Foreword

Winemaking is a most fascinating and complex transformation process of a raw plant material. It starts with the arrival of the harvest at the cellar and ends with the most active and decisive fermentation steps. After this, for some wines, comes the long aging period of the wine, during which the bouquet and taste of the wine is developed and refined. The transformation of grape must in wine is a priori a spontaneous phenomenon. The microbial complex present on the grape berry is exposed to a new ecosystem when the grapes are crushed and pressed. It then evolves spontaneously following the conditions dictated by both the nature of the microorganisms present and the composition of the community.

Without the skill and attention of the oenologist and winemaker, the system would evolve into a fermented product, the quality of which would have little chance of satisfying the consumer. This expertise is based on scientific knowledge of the phenomena that occur in this complex environment. After its beginnings mainly based on observation and empiricism, oenology now uses scientific data derived from research in chemistry, biochemistry and microbiology. Together with biochemical reactions catalyzed by enzymes of yeasts and bacteria, chemical reactions also occur between molecules already present in the must, those gradually extracted from the grape solids during fermentation, those derived from metabolisms and, possibly, also those released by the wood. For many of them the temperature and dissolved oxygen parameters related to technological operations of the winery can have dramatic effects and the quality of the final wine depends on the type and intensity of reactions taking place.

From the beginning of the twentieth century, chemistry and microbiology have been used in an attempt to interpret the observations used by winemakers. These constitute the foundations on which the basic rules for winemaking and aging were established. Hence, as producers' control of the events of winemaking and aging steadily increased, so did wine quality. First, defects and the most critical alterations have been avoided. After that, knowledge has become more accurate and reliable, and more technological tools have been developed, and now the winemaker can control the evolution of the system as a whole with great efficiency.

Continuously, researchers in oenology, both chemists and biologists, appropriate the most efficient analytical methods and data to conduct their research. New molecules of wine aroma, color and flavor have been identified. Sensory analysis,

increasingly present in the laboratory alongside chemical analysis methods, reveals the importance of molecules present even at very low concentrations and the importance of interactions between them. Genomics is used in research on yeast and bacteria and reveals the extraordinary complexity of the microbial consortium, giving microbiologists keys for the optimal use of the natural biodiversity of species involved in fermentation.

The authors, invited by M.C. Polo and M.V. Moreno-Arribas to write this book, are recognized in their own field for their research and ability to transfer scientific results from the laboratory to the winemaking process and storage cellar, and here provide updates on the most recent advances in the field.

With this manual, oenologists will be able to update their knowledge and benefit from a deeper understanding of the phenomena they observe in practice. Moreover, researchers in oenology are now highly specialized, and must conduct their activities at the basic level, while finding in the cellars and caves the elements of their thinking. While in the laboratories, chemists specializing in macromolecules or volatile compounds and microbiologists specializing in yeasts or bacteria must continue their research into the interactions taking place. Working individually without knowledge of research in this field from other specialists their efforts lose all meaning and progress remains erratic or limited. Scientists will, therefore, benefit from this handbook that enables them to contemplate and understand the results and progress made in other specialities related to this area.

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Part II Wine Chemical Compounds and Biochemical Processes

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Introduction

M. Carmen Polo and M. Victoria Moreno-Arribas

The aim of this book is to describe chemical and biochemical aspects of winemaking which are currently being researched. The areas of most interest at present and the subjects in which this interest is likely to continue or to increase in the following years have been selected.

The first part of the book concerns the most important aspects of winemaking technology and microbiology. The second part, the most extensive, deals with the different groups of compounds, how these are modified during the various steps of the production process, and how they influence the wine quality and its sensorial aspects and physiological activity. The third section describes undesirable alterations of wines, including those that affect quality and food safety. Finally, two aspects have been considered which have not yet been tackled in any other book on oenology – automatic analysers used in oenological laboratories for control and research purposes, and the statistical treatment of data. In this last subject, the author not only describes the tools available for analytical data processing but also indicates the most appropriate treatment to apply, depending on the information required. The chapter is illustrated throughout with examples from the oenological literature.

'Wine chemistry and biochemistry' is scientifically written including current trends but also in a style that is easy and clear to understand. It is hoped that it will serve as a most useful text and reference source for wine researchers and oenologists alike, as well as for winemakers and other professionals of the sector, and students of oenology, food technology and similar disciplines.

The editors would like to express their thanks to Springer and all the authors who contributed their expertise and know-how to the success of this book.

Part I
Chemical and Biochemical Aspects of Winemaking

Chapter 1
Biochemistry of Alcoholic Fermentation

Fernando Zamora

Contents

1.1 Introduction

Alcoholic fermentation is the anaerobic transformation of sugars, mainly glucose and fructose, into ethanol and carbon dioxide. This process, which is carried out by yeast and also by some bacteria such as *Zymomonas mobilis*, can be summarised by this overall reaction.

$$\underset{\text{Hexoses}}{C_6H_{12}O_6} \rightarrow 2\ \underset{\text{Ethanol}}{CH_3CH_2OH} + \underset{\text{Carbon dioxide}}{2CO_2}$$

However, alcoholic fermentation is fortunately a much more complex process. At the same time as this overall reaction proceeds, a lot of other biochemical, chemical and physicochemical processes take place, making it possible to turn the grape

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M.V. Moreno-Arribas, M.C. Polo (eds.), *Wine Chemistry and Biochemistry*,
DOI 10.1007/978-0-387-74118-5_1, © Springer Science+Business Media, LLC 2009

juice into wine. Besides ethanol, several other compounds are produced throughout alcoholic fermentation such as higher alcohols, esters, glycerol, succinic acid, diacetyl, acetoin and 2,3-butanediol. Simultaneously, some compounds of grape juice are also transformed by yeast metabolism. Without the production of these other substances, wine would have little organoleptic interest.

At the start of the winemaking process, several species of yeast may be present in the grape juice. This biodiversity depends on several factors such as grape variety, the ripening stage at harvest, the antifungal treatments, the climatic conditions of the year, the development of grey rot or other fungal plagues and the viticultural practices (Sapis-Domerq 1980; Pretorius et al. 1999). However, other factors are also important. All contact of grapes and must during harvest, transport and, in particular winery operations significantly influence the final distribution of yeasts at the beginning of alcoholic fermentation (Constantí et al. 1997; Mortimer and Polsinelli 1999).

Different yeast species participate in spontaneous alcoholic fermentation even when sulphur dioxide is present (Constantí et al. 1998; Beltran et al. 2002). Usually *Kloeckera*, *Hanseniaspora* and *Candida* predominate in the early stages of alcoholic fermentation. Later, *Pichia* and *Metschnikowia* prevail in the middle stages. Finally, during the latter stages of fermentation, *Saccharomyces cerevisiae* is the predominant yeast because of its greater resistance to high ethanol concentration (Fleet 1993; Fleet and Heard 1993). Some other yeast, such as *Torulaspora*, *Kluyveromyces*, *Schizosacchaomyces*, *Zygosaccharomyces* and *Brettanomyces* may also be present during alcoholic fermentation and even in the wine itself, which may cause some organoleptic defects (Peynaud and Domercq 1959; Ribéreau-Gayon et al. 2000a).

Evidently, the succession of these different yeast species throughout alcoholic fermentation influences the final composition of wine in a way that, depending on which yeasts have grown, may be positive in some cases or negative in others (Chatonnet et al. 1995; Ribéreau-Gayon et al. 2000a). To prevent undesirable yeasts developing, wineries add sulphur dioxide to the grape juice and inoculate selected strains of dry yeasts (*Saccharomyces cerevisiae*). Sulphur dioxide has a drastic selective effect on yeast development. As *Saccharomyces cerevisiae* is more resistant to sulphur dioxide than most other yeasts, using this additive favours its development (Lafon-Lafourcade and Peynaud 1974; Romano and Suzzi 1993).

On the other hand, the inoculation of selected dry yeasts greatly increases the initial population of *Saccharomyces cerevisiae*. Nowadays, most wineries inoculate selected dry yeast in order to guarantee alcoholic fermentation without any deviation. However, other wineries, especially traditional wine cellars, continue to use spontaneous alcoholic fermentation because they believe it gives their wines greater complexity.

1.2 Yeast Development During Alcoholic Fermentation

At the beginning of the winemaking process, the yeasts start to metabolize the sugars and other nutrients present in the grape juice. The yeasts use all these nutrients to obtain energy and increase their population (Boulton et al. 1996; Ribéreau-Gayon

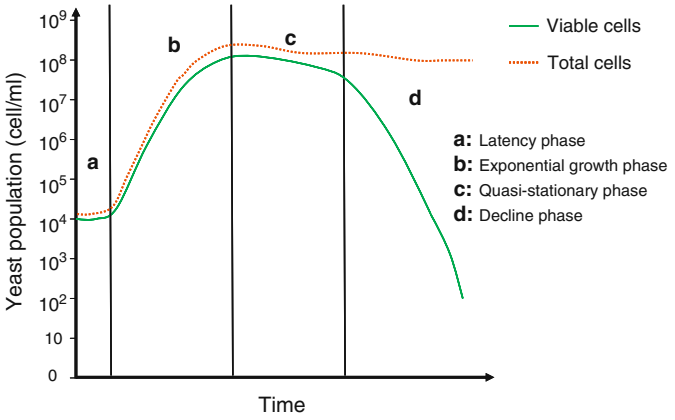

Fig. 1.1 Yeasts growth cycle

et al. 2000b). Figure 1.1 shows the classic yeast growth cycle under standard conditions (Fleet and Heard 1993; Del Nobile et al. 2003).

During the first hours the yeast population does not increase. During this period, also called the latency phase, it is necessary for the cell to adapt to the new environmental conditions. The initial population depends on several factors. If no yeasts are inoculated, the population is around 10^4 cells/ml. However, this population can be higher if the grapes have been attacked by grey rot or other fungal plagues. On the other hand, if selected dry yeasts were inoculated, the initial population would also be higher (around 5×10^6 cells/ml).

Once the yeasts have adapted to the environmental conditions, they begin to grow. This period, named the exponential growth phase, is highly influenced by temperature (Ough 1964), by the concentration of ammonia, amino acids and other nutrients (Lafon-Lafourcade 1983; Sablayrolles et al. 1996) and by the presence of oxygen (Sablayrolles and Barre 1986). During the exponential growth phase, the yeasts increase their population up to 10^7-10^8 cells/ml. This phase can last from 3 to 6 days. After that, yeast stops growing because some nutrients became deficient. During this new phase, called the quasi-stationary phase, the population of yeast remains nearly stable and can last from 2 to 10 days. Later, the decline phase begins and the population of yeast gradually decreases until it has almost completely disappeared. During this period yeasts die because of the lack of nutrients and also because ethanol and other substances produced during alcoholic fermentation are toxic to them (Lafon-Lafourcade et al. 1984).

The success of an alcoholic fermentation depends on maintaining the population of viable yeast at sufficient levels until all the fermentable sugars have been fully consumed (Bisson 1999; Zamora 2004). Otherwise, the winemaker is faced with the serious problem of stuck and sluggish fermentations. The causes and the ways to avoid stuck and sluggish fermentations are discussed later (Bisson and Butzke 2000).

1.3 Glycolysis

The word glycolysis comes from the Greek terms γλυκύς (glucus = sweet) and λύσις (lysis = rupture) and the process consists of the intracellular transformation of glucose (and fructose) into pyruvate. This biochemical pathway is the initial process of carbohydrate catabolism in most organisms and it takes place completely within the cytoplasm. This pathway was fully described in 1940 due, in great part, to the contributions of Gustav Embdem and Otto Meyenhorf. For that reason, it is also called the Embdem-Meyerhoff pathway in their honour although, regrettably, this name excludes other important contributors such as Gerti and Karl Cori, Carl Neuberg, Jacob Parnas, Hans von Euler and Otto Warburg (Kresge et al. 2005).

Yeasts use glycolysis as the main pathway for sugar catabolism (Gancedo 1988). The pentose pathway, which is used by some organisms such as acetic acid bacteria as the major pathway for sugar catabolism, is only used by yeast as a source of ribose and NADPH (Schaaf-Gersteenschaläger and Miosga 1996; Horecker 2002). Ribose is necessary for synthesizing nucleotides and nucleic acids whereas NADPH is required for some metabolic processes such as the lipid synthesis. Therefore yeasts use the pentose pathway not to obtain energy but rather to provide themselves with some of the substances indispensable for cell multiplication.

Glycolysis involves a sequence of 11 chemical reactions for breaking down hexoses and releasing energy in the chemical form of ATP (Barnett 2003). Figure 1.2 shows all the reactions in the glycolytic pathway.

Initially, hexoses are transported inside the cell by facilitated diffusion (Lagunas 1993). As the inner sugar concentration is lower than the external sugar concentration, no energy is necessary for this process.

The first step in glycolysis is the phosphorylation of glucose and fructose by a family of enzymes called hexokinases to form glucose 6-phosphate and fructose-6-phosphate (Gancedo 1988). This reaction consumes ATP, but it keeps the intracellular hexose concentration low and thus favours the continuous transport of sugars into the cell through the plasma membrane transporters. After this, phosphoglucose isomerase converts glucose-6-phosphate into fructose-6-phosphate.

Besides being intermediaries of glycolysis, glucose-6-phosphate and fructose-6-phosphate are also essential substrates for secondary metabolism. In fact, both hexose-phosphates are needed to synthesize the polysaccharides used to construct the cell wall (Cabib et al. 1982).

In the following stage, fructose-6-phosphate is phosphorylated again by the action of phosphofructokinase to form fructose-1,6-diphosphate. This reaction also consumes ATP. Later, the enzyme aldolase cleaves to fructose-6-phosphate. As a result of this reaction two triose phosphates are formed: dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. This reaction produces a much greater proportion of dihydroxyacetone phosphate (96%), which is rapidly transformed into glyceraldehyde-3-phosphate by triose phosphate isomerase (Heinisch and Rodicio 1996).

Afterwards, the enzyme glyceraldehyde-3-phosphate dehydrogenase transforms glyceraldehyde-3-phosphate into 1,3-diphosphoglycerate. This reaction involves the oxidation of the molecule that is linked to reducing NAD^+ to NADH in order to redress the redox balance. Simultaneously, a substrate level phosphorylation takes

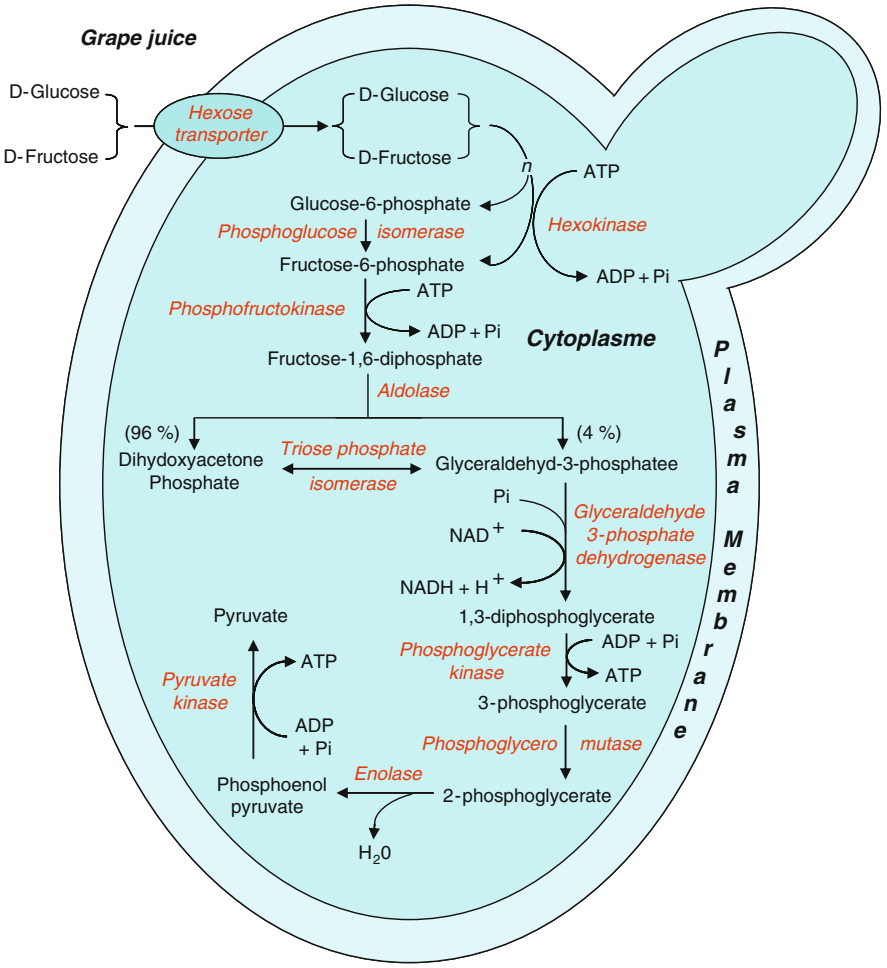

Fig. 1.2 Biochemical mechanism of glycolysis

place forming an energy rich bond between the oxidized carbon group and inorganic phosphate.

The next stage in glycolysis consists of transforming 1,3-diphosphoglycerate into 3-phosphoglycerate. This reaction, which is catalyzed by phosphoglycerate kinase, releases all the energy contained in the previously formed energy-rich bond, which the cell uses to phosphorylate one molecule of ADP into ATP.

After this, phosphoglycero mutase converts 3-phosphoglycerate into 2-phosphoglycerate, which is then dehydrated in phosphoenol pyruvate by the enzyme enolase. Phosphoenol pyruvate contains an energy-rich bond that is used by the enzyme pyruvate kinase to phosphorylate ADP into ATP. This reaction generates pyruvate, which is the final product of glycolysis.

As a consequence of glycolysis, each molecule of hexose generates two molecules of pyruvate, four of ATP and one of NADH. Since two molecules of ATP were

consumed previously during the phosphorylation of the hexoses, the net energy gain for the cell is two ATPs per hexose.

Pyruvate produced by glycolysis can be used by yeasts for several metabolic pathways. However, yeasts must regenerate NAD^+ from the NADH to re-establish the oxydoreduction potential of the cell. This can be done by fermentation or respiration.

1.4 Fermentation and Respiration

Yeasts are facultative anaerobic microorganisms because they possess the genetic equipment for metabolizing sugars aerobically or anaerobically (Boulton et al. 1996). Therefore, yeasts can consume sugars using two different metabolic pathways: respiration and fermentation (Racker 1974). Figure 1.3 illustrates these biochemical pathways.

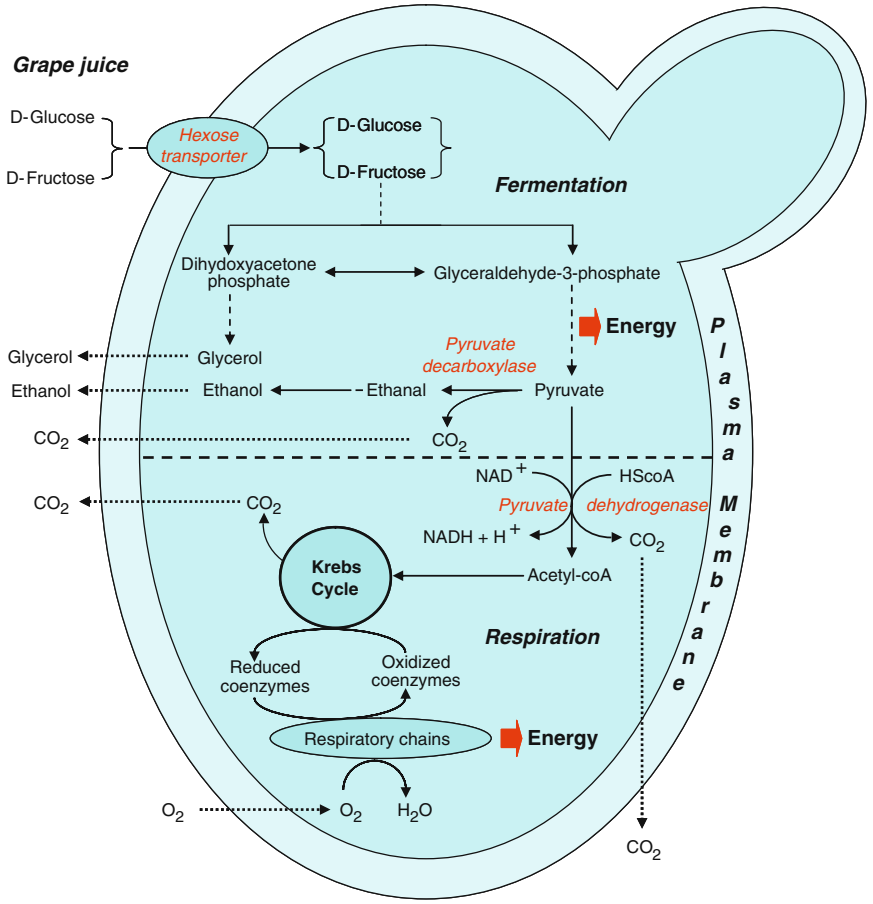

Fig. 1.3 Fermentation and respiration

Both pathways begin with glycolysis (as described above), which generates pyruvate as a final product. Pyruvate can be transformed into ethanal and carbon dioxide by the enzyme pyruvate decarboxylase and after ethanal can be reduced to ethanol. This process, named alcoholic fermentation, takes place within the cytoplasm. Alcoholic fermentation regenerates the NAD^+ consumed during glycolysis and gives yeast an energy gain of only two ATP molecules by metabolized hexose (Barnett and Entian 2005).

Nevertheless, pyruvate can also be transformed into acetyl-coA and carbon dioxide by pyruvate dehydrogenase. This reaction reduces NAD^+ to NADH and must incorporate the coenzyme A. Acetyl-coA can then be incorporated to the Krebs cycle, being transformed into carbon dioxide and producing several molecules of reduced coenzymes (NADH and $FADH_2$). The reduced coenzymes produced by the Krebs cycle, and also by glycolysis, are later reoxidized in the respiratory chains, reducing molecular oxygen to water (Barnett and Entian 2005). This process, known as respiration, yields an overall energy gain of 36–38 ATP molecules per metabolized hexose. Consequently, this process is much more beneficial to yeast than fermentation, in terms of energy. However, it needs oxygen as a substrate and it is inhibited by high sugar concentration (Crabtree 1929).

The transformation of pyruvate into ethanal or acetyl-coA is therefore a key point for regulating yeast metabolism (Ribéreau-Gayon et al. 2000c).

1.5 Regulation Between Respiration and Fermentation: Pasteur and Crabtree Effects

Louis Pasteur found that aeration increases biomass production and decreases the kinetics of sugar consumption and ethanol production (Pasteur 1861). He, therefore, concluded that aeration inhibits alcoholic fermentation (Racker 1974).

This phenomenon, which is known as the Pasteur effect, has been attributed to several mechanisms (Barnett and Entian 2005). Respiration needs very high amounts of ADP inside the mitochondria as a subtract for oxidative phosphorylation. Therefore, when respiration takes place, the cytoplasm lacks ADP and inorganic phosphate (Lagunas and Gancedo 1983), which in turn decreases the sugar transport inside the cell (Lagunas et al. 1982). These mechanisms explain how aeration inhibits the alcoholic fermentation.

Evidently, once the yeast starts to consume sugars, large quantities of carbon dioxide are produced. The release of carbon dioxide displaces the oxygen and creates semianaerobic conditions that favour fermentation. However, even in the presence of oxygen, *Saccharomyces cerevisiae* will not ferment if the sugar concentration is higher than 9 g/l. Crabtree first described this phenomenon in 1929 that is known by different names: the Crabtree effect, catabolic repression by glucose or the Pasteur contrary effect (Meijer et al. 1998; Ribéreau-Gayon et al. 2000c).

When *Saccharomyces cerevisiae* grow in a high sugar concentration, as is found in grape juice, their mitochondria degenerate. Simultaneously, the enzymes of the

Krebs cycle and the constituents of respiratory chains are repressed (Gancedo 1992; Polakis et al. 1965; Barnett and Entian 2005). Therefore, under wine fermentation conditions, *Saccharomyces cerevisiae* can only ferment sugars. *Saccharomyces cerevisiae* can only use respiration when the sugar concentration is really low and when oxygen is present in the medium. These conditions are used for the industrial production of selected dry yeast.

1.6 Alcoholic Fermentation

As was quoted above, when fermenting grape juice, *Saccharomyces cerevisiae* mainly directs the pyruvate to produce of ethanol in order to regenerate the NAD^+ consumed by glycolysis. This process, called alcoholic fermentation, is shown in Fig. 1.4.

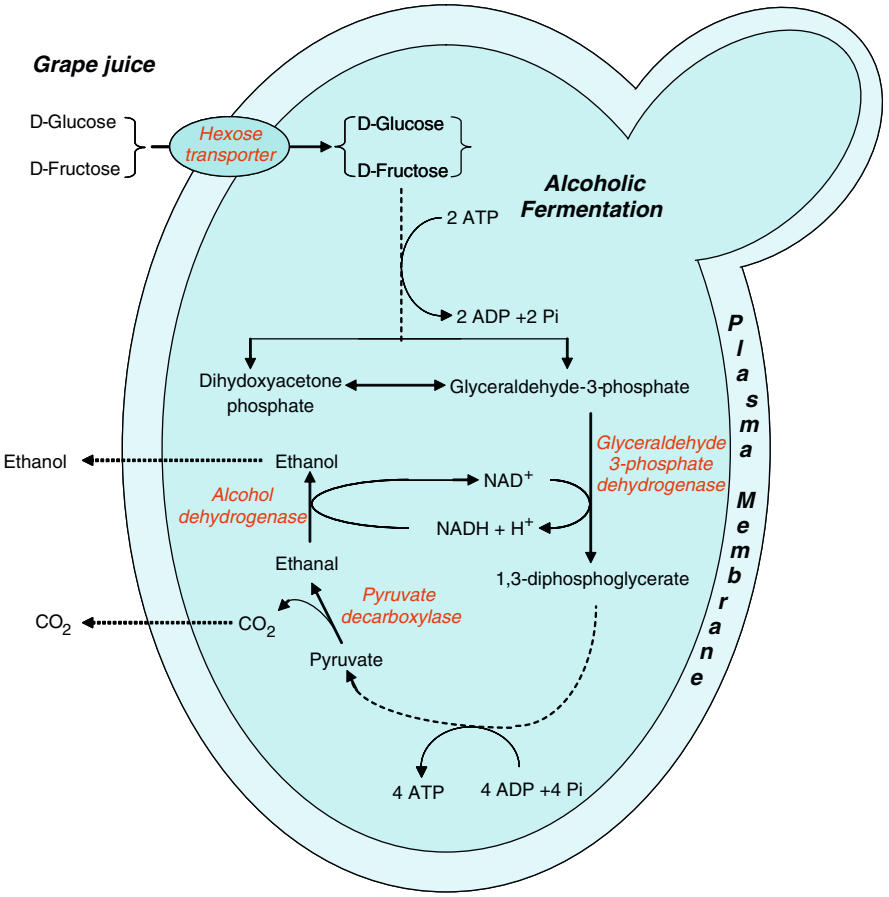

Fig. 1.4 Alcoholic fermentation

Pyruvate is initially decarboxylated into ethanal by pyruvate decarboxylase. This enzyme needs magnesium and thiamine pyrophosphate as cofactors (Hohmann 1996). Thereafter, alcohol dehydrogenase reduces ethanal to ethanol, recycling the NADH to NAD^+. There are three isoenzymes of alcohol dehydrogenase in *Saccharomyces cerevisiae*, but isoenzyme I is chiefly responsible for converting ethanal into ethanol (Gancedo 1988). Alcohol dehydrogenase uses zinc as cofactor (Ciriacy 1996).

Both final products of alcoholic fermentation, ethanol and carbon dioxide, are transported outside the cell by simple diffusion.

1.7 Glyceropyruvic Fermentation

Although the production of ethanol is the most important pathway to regenerate NAD^+, there is an alternative pathway for this purpose. This pathway, called glyceropyruvic fermentation, generates glycerol as its final product (Prior and Hohmann 1996). Figure 1.5 shows the biochemical mechanism of glyceropyruvic fermentation.

The first evidence of this pathway was found by Neuberg (1946). He discovered that the fermentation of glucose by yeast in the presence of sulphite produced a lot of glycerol. Sulphite combines with ethanal which then prevents NAD^+ from regenerating via alcohol dehydrogenase. Under these conditions, the yeasts need to oxide NADH through an alternative pathway in order to compensate for the NAD^+ deficit and the only way to do this is by producing glycerol.

Dyhroxyacetone phosphate, the main product of aldolase reaction, can be oxidized to glycerol-3-phosphate by the enzyme glycerol-3-phosphate dehydrogenase. This reaction is coupled to the oxidation of a molecule of NADH to NAD^+. Then, glycerol-3-phosphate phosphatase catalyzes the production of glycerol by dephosphorylating glycerol-3-phosphate. The production of glycerol consumes ATP but it is necessary to compensating for the redox imbalance in the cell (Barre et al. 1998).

Although glyceropyruvic fermentation was first described through the effect of sulphites, it can also be active in other situations. At the beginning of winemaking, yeasts need a lot of substrates to grow. Cell multiplication implies a very active biosynthesis of proteins, lipids, nucleotides, etc., and most of these biomolecules are synthesised using pyruvate as a substrate. Each time a molecule of pyruvate is used anabolically, a NAD+ deficit is produced which must be recovered through the glyceropyruvic pathway. For this reason, glycerol is mainly produced during the first steps of alcoholic fermentation, when yeasts are growing and they need a large proportion of pyruvate to increase their biomass (Ribéreau-Gayon et al. 2000c). Furthermore, yeasts produce glycerol as a protector against high osmotic pressures (Prior and Hohmann 1996).

For these reasons, glycerol is the third major component of dry wines (after water and ethanol). Its concentration is usually between 6 and 10 g/l and it improves wine quality because it confers sweet and mouthfeel sensations.

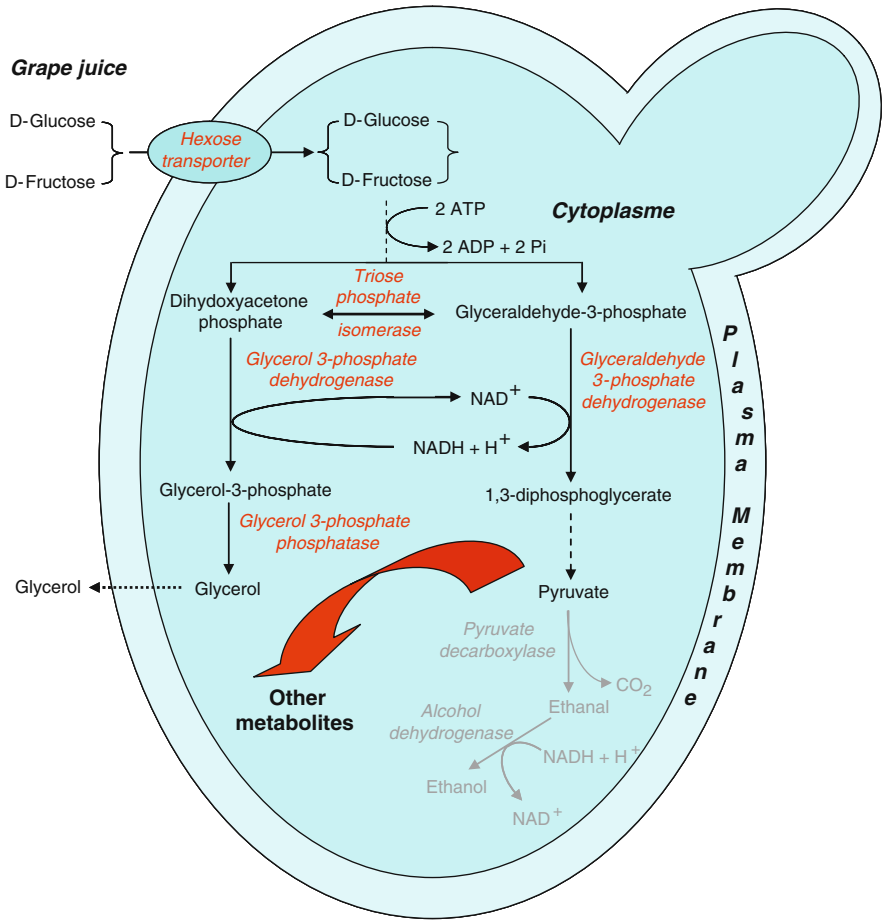

Fig. 1.5 Glyceropyruvic fermentation

1.8 Nitrogen Metabolism

When *Saccharomyces cerevisiae* grows in grape juice it needs significant amounts of assimilable nitrogen to synthesize biomass (Kunkee 1991). Grape juice contains a variety of nitrogen compounds such as ammonia, amino acids, peptides, proteins, etc., but only some of them can be assimilated by *Saccharomyces cerevisiae* (Hensche and Jiranek 1993). When fermenting grape juice fermentation, *Saccharomyces cerevisiae* can only use ammonia and amino acids, with the exception of proline, as an assimilable source of nitrogen (Barre et al. 1998). Proline can be assimilated by Saccharomyces cerevisiae but only under aerobic conditions (Boulton et al. 1996). For this reason, the term easily-assimilable nitrogen (EAN) has been proposed to describe collectively all the ammonia and amino acids, except

proline. This EAN can simply be determined using the formol index (Taylor 1957; Aerny 1997).

Grape juice is relatively poor in ammonia and amino acids (Kunkee 1991). Consequently, the uptake of these compounds by the yeasts may be a critical stage in some cases. At a low concentration of EAN the risk of stuck and sluggish fermentations is really high (Bisson 1999; Zamora 2004). For that reason, winemakers used to supplement grape juice with ammonium salts (Barre et al. 1998). The EAN requirement for a complete alcoholic fermentation depends on the yeast strain (Manginot et al. 1998) and the potential alcoholic degree (Bisson and Butzke 2000). Generally, it is considered that an EAN lower than 130 mg/l can seriously affect the correct development of alcoholic fermentation.In contrast, excessive nitrogen can lead to the presence of non-assimilated residual nitrogen in the wine, which is a factor in microbiological instability and can even favour the production of ethyl carbamate and biogenic amines (Ribéreau-Gayon et al. 2000b). For that reason, nitrogen must be supplemented carefully and taking into account the initial EAN concentration of grape juice and its potential alcoholic degree (Bisson and Butzke 2000).

The first step in nitrogen assimilation is its transport inside the cell. Figure 1.6 illustrates nitrogen uptake in yeasts.

Ammonium ion is transported inside the cell by facilitated diffusion. However, the intracellular pH causes ammonium ion to release a proton, which must be sent outside the cell via H^+-ATPase. Once inside the cytoplasm, ammonia

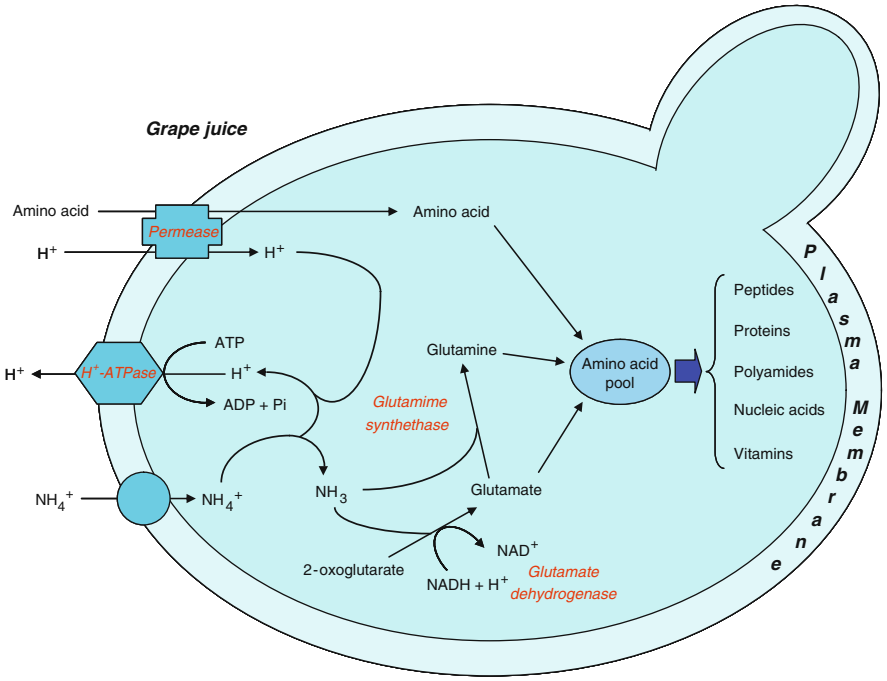

Fig. 1.6 Nitrogen metabolism

is incorporated to the amino acid pool via glutamate dehydrogenase or via glutamine synthetase, thus producing glutamate or glutamine respectively (Hensche and Jiranek 1993).

On the other hand, amino acids are transported inside the cell by different transporters. To date,15 transport systems have been identified for amino acids in *Saccharomyces cerevisiae* (Barre et al. 1998) and all of them are symport systems coupled to the entry of a proton. This proton must also be sent outside the cell in order to maintain the cellular homeostasis. Therefore, the uptake of ammonium and amino acids must be considered as active transport because it consumes ATP via H^+-ATPase.

All amino acids, except proline, may be used by *Saccharomyces cerevisiae* in grape juice fermentation. Amino acids can be directly used to synthesize proteins. However, the amino acid composition of the grape juice is not necessarily similar to the needs of the cell. For that reason, yeasts must use the remaining amino acids to synthesize those which it lacks (Hensche and Jiranek 1993; Ribéreau-Gayon et al. 2000b). In this case, ammonia is incorporated into other amino acids whereas the carbon skeleton is metabolized by the cell.

For this reason, the lack of enough EAN can make the yeast use sulphur amino acids (cysteine and methionine), thus releasing hydrogen sulphite and mercaptans. Thus, supplementing with ammonium salts is recommended not only to avoid stuck and sluggish fermentations but also to prevent reduction off-odours (Jiranek et al. 1995).

Finally, the relationship between the amino acid composition of grapes and the final aromatic composition of wine has been recently described (Hernández-Orte et al. 2002, 2006). Therefore, it is possible that in the near future grape juice will be complemented with specific mixtures of amino acids in order to improve the aromatic quality of wine.

1.9 Oxygen and Lipid Biosynthesis

As discussed previously,*Saccharomyces cerevisiae* does not need oxygen to obtain energy when fermenting grape juice. However, there are some essential biosynthetic pathways that use oxygen as substrate. This is the case for the biosynthesis of sterols and unsaturated fatty acids (Ratledge and Evans 1989).

During the growth phase, while the cell multiplication is active, yeast needs to build new plasma membranes continually. For that reason, yeasts must synthesize great amounts of sterols, fatty acids and phospholipids during the first stages of alcoholic fermentation (Ribéreau-Gayon et al. 2000b).

Figure 1.7 illustrates the synthesis of sterols in yeasts. Basically, sterols are synthesised by the mevalonate pathway. The key stage in this pathway is, without any doubt, the reaction catalysed by squalene monooxygenase. This reaction, which uses oxygen as substrate, transforms squalene into squalene 2,3, epoxide. Later, squalene epoxide lanosterol cyclase catalyses the synthesis of the first sterol of the pathway,

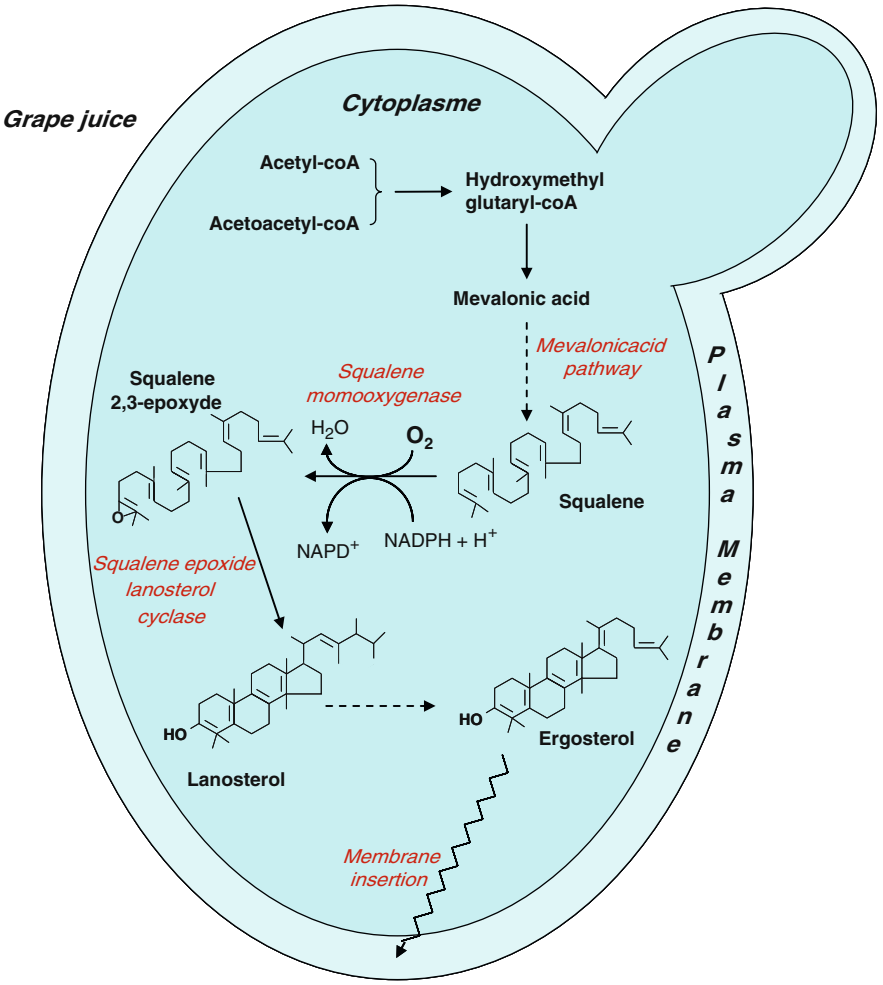

Fig. 1.7 Synthesis of ergosterol in yeasts

lanosterol. Afterwards, lanosterol is used to obtain ergosterol, which is the main sterol in *Saccharomyces cerevisiae* (Nes et al. 1993).

Therefore, in the absence of oxygen, this pathway is completely blocked and its final yeast product, ergosterol, cannot be produced.

Figure 1.8 shows the synthesis of fatty acids. This complex process is catalysed by the multienzymatic complex, fatty acid synthetase. This enzyme uses as substrates acetyl-coA and malonyl-coA to produce palmitic acid. Afterwards, palmitic acid, a saturated fatty acid of 16 carbon atoms, can be used to produce other fatty acids (Ratledge and Evans 1989). Fatty acids with more carbon units, such as estearic acid, are obtained by elongation of palmitic acid.

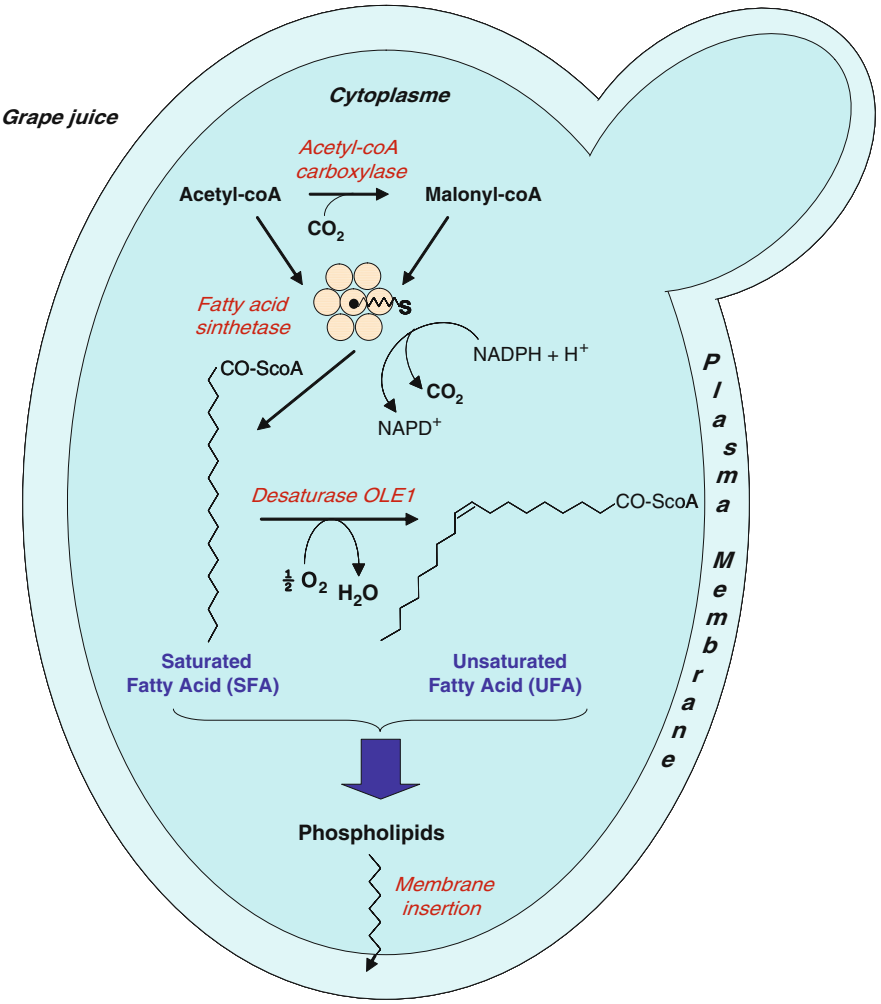

Fig. 1.8 Synthesis of fatty acids in yeasts

Nevertheless, unsaturated fatty acids (UFA) need oxygen to be synthesised (Alexandre et al. 1994). In *Saccharomyces cerevisiae*, UFA production is catalysed by the desaturase OLE1 (Kajiwara et al. 2000). This enzyme is activated by low temperatures and the presence of oxygen (Nakagawa et al. 2002). Afterwards, fatty acids are used to synthesize phospholipids, which are inserted into the plasmatic membranes.

Since oxygen is necessary to synthesize ergosterol and UFA, aeration during alcoholic fermentation limits the growth of *Saccharomyces cerevisiae* (Sablayrolles and Barre 1986; Ribéreau-Gayon et al. 2000b) and the lack of oxygen may sometimes cause stuck and sluggish fermentations (Sablayrolles et al. 1996; Bisson 1999;

Zamora 2004). For that reason, aerating the grape juice is recommended during the exponential growth phase of the fermentation in order to encourage yeast to build their membranes and avoid fermentation problems (Barre et al. 1998; Ribéreau-Gayon et al. 2000b).

Another aspect to take into account is that all microorganisms need to maintain adequate fluidity in their membranes (Rodríguez et al. 2007). Excessive rigidity can prevent cellular transport systems from functioning correctly (Los and Murata 2004).In contrast, excessive fluidity can alter the organization and the dynamic properties of phospholipidic bilayer (Laroche et al. 2001).

The fluidity of the plasmatic membrane is considerably affected by temperature (Rodríguez et al. 2007) and ethanol concentration (Jones and Greenfield 1987). Therefore, during alcoholic fermentation*Saccharomyces cerevisiae* must adapt the fluidity of the membrane to the changing environmental conditions. It should be emphasized that the temperature of fermentation and aeration depend on the type of winemaking. Usually, white wines are made at low temperatures (14–18 °C) and without aeration to conserve aromas whereas red wines are fermented at relatively high temperatures (28–30 °C) and are aerated in order to enhance colour extraction.

In white winemaking, *Saccharomyces cerevisiae* must develop at low temperature, which reduces membrane fluidity. To maintain adequate fluidity in their membranes, yeasts increase the proportion of UFA in the phospholipids (Thurston et al. 1981; Torrija et al. 2003). Phospholipids with unsaturated fatty acids have a lower melting point and more flexibility than phospholipids with saturated acyl chains (Rodríguez et al. 2007). Such adaptation involves inducing the fatty acid desaturase OLE1 which incorporate unsaturated bonds at defined positions in fatty acids (Nakagawa et al. 2002).

However, under normal white fermentation conditions, grape juice is very poor in fatty acids and fermentation is usually carried out under hypoxia conditions (Bertrand and Miele 1984). Under these conditions, yeasts cannot synthesize unsaturated fatty acids. Consequently, *Saccharomyces cerevisiae* need to use another strategy to fluidize their membranes and the only possibility is incorporating medium chain fatty acids (MCFA) within the phospholipids of the membrane (Rozès 1992). The effect of a short chain is similar to that of the double bond of a long chain (Quinn and Chapman 1980) and, therefore, the increased synthesis of MCFA could also modulate membrane fluidity.

Nevertheless, some of these MCFA can be released into the medium, reducing yeast viability and leading to stoppages in fermentation (Geneix et al. 1983). In standard red winemaking, this problem does not exist because fermentations are done at high temperatures and oxygen is introduced during the racking process.

Moreover, during alcoholic fermentation very important changes take place in the yeast's environment. Basically, the ethanol concentration increases progressively and yeasts need to adapt their plasmatic membranes to this aggressive new environment (Weber and Bont 1996). Apparently, the presence of ethanol in the medium alters drastically the fluidity of the membrane (Jones and Greenfield 1987). Under these conditions, *Saccharomyces cerevisiae* must increase its proportion of sterols and unsaturated fatty acids to compensate for this effect and consequently

enhance its tolerance to ethanol (Alexandre et al. 1994). In the particular case of red winemaking, these changes can be done without problems because oxygen is introduced during the racking process. Nevertheless, as mentioned previously, white wines are usually made without aeration, and in that case the lack of oxygen may make it very difficult for yeast to adapt to ethanol.

1.10 Stuck and Sluggish Fermentations: Causes and Solutions

Sometimes alcoholic fermentation becomes too slow towards the end of the process. Yeasts drastically reduce their sugar consumption and fermentation may even stop before all the fermentable sugars have been completely metabolised. When this happens, oenologists are faced with two problems. First, the wine is not finished and something has to be done to finish it. Second, the risk of bacterial spoilage is very high. Heterolactic acid bacteria can metabolize the sugars and produce high amounts of acetic acid (Ribéreau-Gayon et al. 2000b). Winemakers are well aware of this problem, and for that reason they are continually concerned about the chances of having stuck and sluggish fermentation.

The causes of stuck and sluggish fermentations have been the subject of several studies (Larue et al. 1982; Ingledew and Kunkee 1985; Alexandre and Charpentier 1998; Bisson 1999). Some of them have already been described in previous points. The following list summarizes the possible causes and solutions of stuck and sluggish fermentations.

1. *Very high sugar concentration*: Excessive sugar concentration may be a factor which inhibits yeasts. Moreover, during the latter stages of fermentation, an excessive concentration of ethanol can seriously complicate the full consumption of the sugars. This is an important problem nowadays, especially with red wines, because winemakers look for complete phenolic maturity in grapes, which is usually attained at very high sugar concentration. In this case, using yeast with high ethanol resistance is recommended.
2. *Temperature extremes*: Yeasts have problems growing when the temperature is too low. Therefore, too low temperature at the beginning of the process can lead to a deficient yeast population. On the other hand, if the temperature is too high (more than 30 °C) the fermentation is at considerable risk of stopping. For that reason, thermic control of fermentations is nowadays indispensable. Another aspect to take into account is that sudden changes of temperature may provoke serious problems in fermentation. As previously mentioned, yeasts adapt their membrane composition to maintain the correct fluidity. A drastic temperature decrease can provoke excessive rigidity in the membranes before yeasts can adapt to it.
3. *Complete anaerobiosis*: Oxygen is necessary to synthesize ergosterol and MCFA. Without oxygen, yeast will struggle to grow and adapt their membranes to the environmental conditions. For that reason, aeration is recommended, at least during the exponential growth phase.