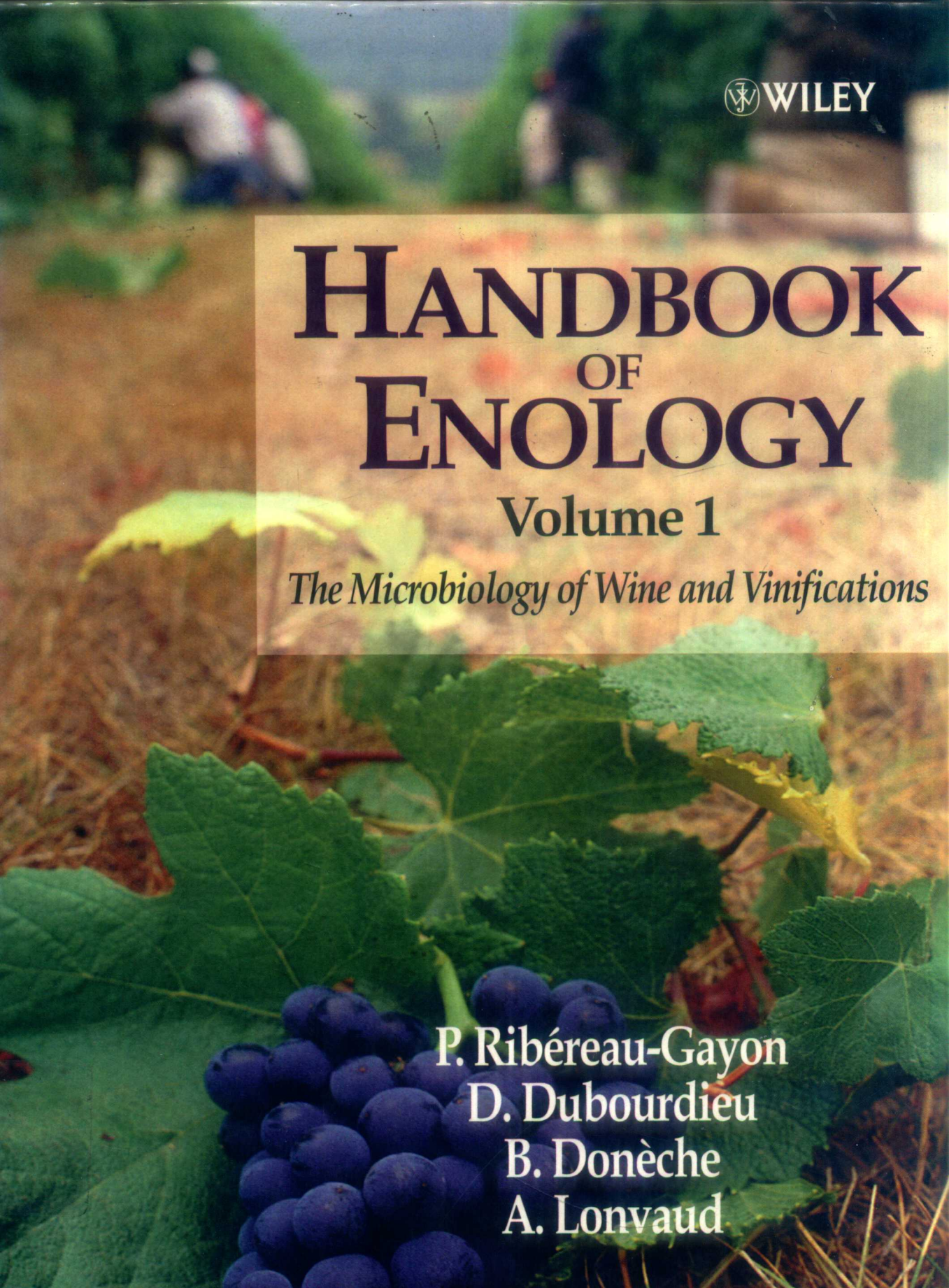
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Volume 1
The Microbiology of Wine and Vinifications
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The Microbiology of Wine and Vinifications

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Handbook of Enology
Volume 1
The Microbiology of Wine and Vinifications

Remarks concerning the expression of certain parameters of must and wine composition

UNITS

Metric system units of length (m), volume (l) and weight (g) are exclusively used. The conversion of metric units into Imperial units (inches, feet, gallons, pounds, etc.) can be found in the following enological work: *Principles and practices of wine-making*, R.B. Boulton, V.L. Singleton, L.F. Bisson and R.E. Kunkee, 1995, The Chapman & Hall Enology Library, New York.

EXPRESSION OF TOTAL ACIDITY AND VOLATILE ACIDITY

Although EC regulations recommend the expression of total acidity in the equivalent weight of tartaric acid, the French custom is to give this expression in the equivalent weight of sulfuric acid. The more correct expression in milliequivalents per liter has not been embraced in France. The expression of total and volatile acidity in the equivalent weight of sulfuric acid has been used predominantly throughout these works. In certain cases, the corresponding weight in tartaric acid, often used in other countries, has been given.

Using the weight of the milliequivalent of the various acids, the table below permits the conversion from one expression to another.

More particularly, to convert from total acidity expressed in H_2SO_4 to its expression in tartaric acid, add half of the value to the original value (4g/l H_2SO_4 → 6 g/l tartaric acid). In the other direction a third of the value must be subtracted.

The French also continue to express volatile acidity in equivalent weight of sulfuric acid. More generally, in other countries, volatile acidity is expressed in acetic acid. It is rarely expressed in milliequivalents per liter. The below table also allows simple conversion from one expression to another.

The expression in acetic acid is approximately 20% higher than in sulfuric acid.

	Desired Expression			
Known Expression	*meq/l*	*g/l* H_2SO_4	*g/l tartaric acid*	*g/l acetic acid*
meq/l	1.00	0.049	0.075	0.060
g/l H_2SO_4	20.40	1.00	1.53	1.22
g/l tartaric acid	13.33	0.65	1.00	
g/l acetic acid	16.67	0.82		1.00

Multiplier to pass from one expression of total or volatile acidity to another

EVALUATING THE SUGAR CONCENTRATION OF MUSTS

This measurement is important for tracking grape maturation, fermentation kinetic and if necessary determining the eventual need for chaptalization.

This measurement is always determined by physical, densimetric or refractometric analysis. The expression of the results can be given according to several scales: some are rarely used, i.e. degree Baumé and degree Oeschle. Presently, two systems exist (10.4.3):

1. The potential alcohol content (titre alcoométraque potentiel or TAP, in French) of musts can be read directly on equipment, which is graduated using a scale corresponding to 17.5 or 17 g/l of sugar for 1% volume of alcohol. Today, the EC recommends using 16.83 g/l as the conversion factor. The 'mustimeter' is a hydrometer containing two graduated scales: one expresses density and the other gives a direct reading of the TAP. Different methods varying in precision exist to calculate the TAP from a density reading. These methods take various elements of must composition into account (Boulton *et al.*, 1995).
2. Degree Brix expresses the percentage of sugar in weight. By multiplying degree Brix by 10, the weight of sugar in 1 kg, or slightly less than 1 liter, of must is obtained. A conversion table between degree Brix and TAP exists in section 10.4.3 of this book. 17 degrees Brix correspond to an approximate TAP of 10% and 20 degrees Brix correspond to a TAP of about 12%. Within the alcohol range most relevant to enology, degree Brix can be multiplied by 10 and then divided by 17 to obtain a fairly good approximation of the TAP.

In any case, the determination of the Brix or TAP of a must is approximate. First of all, it is not always possible to obtain a representative grape or must sample for analysis. Secondly, although physical, densimetric or refractometric measurements are extremely precise and rigorously express the sugar concentration of a sugar and water mixture, these measurements are affected by other substances released into the sample from the grape and other sources. Furthermore, the concentrations of these substances are different for every grape or grape must sample. Finally, the conversion rate of sugar into alcohol (approximately 17 to 18 g/l) varies and depends on fermentation conditions and yeast properties. The widespread use of selected yeast strains has lowered the sugar conversion rate.

Measurements using visible and ultraviolet spectrometry

The measurement of optic density, absorbance, is widely used to determine wine color (Volume 2, section 6.4.5) and total phenolic compounds concentration (Volume 2, section 6.4.1). In these works, the optic density is noted as OD, OD420 (yellow), OD520 (red), OD620 (blue) or OD280 (absorption in ultraviolet spectrum) to indicate the optic density at the indicated wavelengths.

Wine color intensity is expressed as:

$$CI = OD420 + OD520 + OD620,$$

Or is sometimes expressed in a more simplified form: CI = OD420 + OD520.

Tint is expressed as:

$$T = \frac{OD420}{OD520}$$

The total phenolic compound concentration is expressed by OD280.

The analysis methods are described in Chapter 6 of *Handbook of Enology Volume 2, The Chemistry of Wine*.

Preface

Wine has probably inspired more research and publications than any other beverage or food. In fact, through their passion for wine, great scientists have not only contributed to the development of practical enology but have also made discoveries in the general field of science.

A forerunner of modern enology, Louis Pasteur developed simplified contagious infection models for humans and animals based on his observations of wine spoilage. The following quote clearly expresses his theory in his own words: 'when profound alterations of beer and wine are observed because these liquids have given refuge to microscopic organisms, introduced invisibly and accidentally into the medium where they then proliferate, how can one not be obsessed by the thought that a similar phenomenon can and must sometimes occur in humans and animals.'

Since the 19th century, our understanding of wine, wine composition and wine transformations has greatly evolved in function of advances in relevant scientific fields i.e. chemistry, biochemistry, microbiology. Each applied development has lead to better control of winemaking and aging conditions and of course wine quality. In order to continue this approach, researchers and winemakers must strive to remain up to date with the latest scientific and technical developments in enology.

For a long time, the Bordeaux school of enology was largely responsible for the communication of progress in enology through the publication of numerous works (Béranger Publications and later Dunod Publications):

Wine Analysis U. Gayon and J. Laborde (1912); *Treatise on Enology* J. Ribéreau-Gayon (1949); *Wine Analysis* J. Ribéreau-Gayon and E. Peynaud (1947 and 1958); *Treatise on Enology* (2 Volumes) J. Ribéreau-Gayon and E. Peynaud (1960 and 1961); *Wine and Winemaking* E. Peynaud (1971 and 1981); *Wine Science and Technology* (4 volumes) J. Ribéreau-Gayon, E. Peynaud, P. Ribéreau-Gayon and P. Sudraud (1975-1982).

For an understanding of current advances in enology, the authors propose this book *Handbook of Enology Volume 1: The Microbiology of Wine and Vinifications* and the second volume of the *Handbook of Enology Volume 2: The Chemistry of Wine: Stabilization and Treatments.*

Although written by researchers, the two volumes are not specifically addressed to this group. Young researchers may, however, find these books useful to help situate their research within a particular field of enology. Today, the complexity of modern enology does not permit a sole researcher to explore the entire field.

These volumes are also of use to students and professionals. Theoretical interpretations as well as solutions are presented to resolve the problems encountered most often at wineries. The authors have adapted these solutions to many different situations and winemaking methods. In order to make the best use of the information contained in these works, enologists should have a broad understanding of general scientific knowledge. For example, the understanding and application of molecular biology and genetic engineering have become indispensable in the field of wine microbiology. Similarly, structural and quantitative physiochemical analysis methods such as chromatography, NMR and mass spectrometry

must now be mastered in order to explore wine chemistry.

The goal of these two works was not to create an exhaustive bibliography of each subject. The authors strove to choose only the most relevant and significant publications to their particular field of research. A large number of references to French enological research has been included in these works in order to make this information available to a larger non-French-speaking audience.

In addition, the authors have tried to convey a French and more particularly a Bordeaux perspective of enology and the art of winemaking. The objective of this perspective is to maximize the potential quality of grape crops based on the specific natural conditions that constitute their 'terroir'. The role of enology is to express the characteristics of the grape specific not only to variety and vineyard practices but also maturation conditions, which are dictated by soil and climate.

It would, however, be an error to think that the world's greatest wines are exclusively a result of tradition, established by exceptional natural conditions, and that only the most ordinary wines, produced in giant processing facilities, can benefit from scientific and technological progress. Certainly, these facilities do benefit the most from high performance installations and automation of operations. Yet, history has unequivocally shown that the most important enological developments in wine quality (for example, malolactic fermentation) have been discovered in ultra premium wines. The corresponding techniques were then applied to less prestigious products.

High performance technology is indispensable for the production of great wines, since a lack of control of winemaking parameters can easily compromise their quality, which would be less of a problem with lower quality wines.

The word 'vinification' has been used in this work and is part of the technical language of the French tradition of winemaking. Vinification describes the first phase of winemaking. It comprises all technical aspects from grape maturity and harvest to the end of alcoholic and sometimes malolactic fermentation. The second phase of winemaking 'winematuration, stabilization and treatments' is completed when the wine is bottled. Aging specifically refers to the transformation of bottled wine.

This distinction of two phases is certainly the result of commercial practices. Traditionally in France, a vine grower farmed the vineyard and transformed grapes into an unfinished wine. The wine merchant transferred the bulk wine to his cellars, finished the wine and marketed the product, preferentially before bottling. Even though most wines are now bottled at the winery, these longstanding practices have maintained a distinction between 'wine grower enology' and 'wine merchant enology'. In countries with a more recent viticultural history, generally English speaking, the vine grower is responsible for winemaking and wine sales. For this reason, the Anglo-Saxon tradition speaks of winemaking, which covers all operations from harvest reception to bottling.

In these works, the distinction between 'vinification' and 'stabilization and treatments' has been maintained, since the first phase primarily concerns microbiology and the second chemistry. In this manner, the individual operations could be linked to their particular sciences. There are of course limits to this approach. Chemical phenomena occur during vinification; the stabilization of wines during storage includes the prevention of microbial contamination.

Consequently, the description of the different steps of enology does not always obey logic as precise as the titles of these works may lead to believe. For example, microbial contamination during aging and storage are covered in Volume 1. The antiseptic properties of SO_2 incited the description of its use in the same volume. This line of reasoning lead to the description of the antioxidant related chemical properties of this compound in the same chapter as well as an explanation of adjuvants to sulfur dioxide: sorbic acid (antiseptic) and ascorbic acid (antioxidant). In addition, the on lees aging of white wines and the resulting chemical transformations cannot be separated from vinification and are therefore

also covered in Volume 1. Finally, our understanding of phenolic compounds in red wine is based on complex chemistry. All aspects related to the nature of the corresponding substances, their properties and their evolution during grape maturation, vinification and aging are therefore covered in Volume 2.

These works only discuss the principles of equipment used for various enological operations and their effect on product quality. For example, temperature control systems, destemmers, crushers and presses as well as filters, inverse osmosis machines and ion exchangers are not described in detail. Bottling is not addressed at all. An in-depth description of enological equipment would merit a detailed work dedicated to the subject.

Wine tasting, another essential role of the winemaker, is not addressed in these works. Many related publications are, however, readily available. Finally, wine analysis is an essential tool that a winemaker should master. It is, however, not covered in these works except in a few particular cases i.e. phenolic compounds, whose different families are often defined by analytical criteria.

The authors thank the following people who have contributed to the creation of this work: J.F. Casas Lucas, Chapter 14, Sherry; A. Brugirard, Chapter 14, Sweet wines; J.N. de Almeida, Chapter 14, Port wines; A. Maujean, Chapter 14, Champagne; C. Poupot for the preparation of material in Chapters 1, 2 and 13; Miss F. Luye-Tanet for her help with typing.

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Pascal Ribéreau-Gayon
Bordeaux

Contents

1

Cytology, Taxonomy and Ecology of Grape and Wine Yeasts

1.1 INTRODUCTION

Man has been making bread and fermented beverages since the beginning of recorded history. Yet the role of yeasts in alcoholic fermentation, particularly in the transformation of grapes into wine, was only clearly established in the middle of the nineteenth century. The ancients explained the boiling during fermentation (from the Latin *fervere*, to boil) as a reaction between substances that come into contact with each other during crushing. In 1680, a Dutch cloth merchant, Antonie van Leeuwenhoek, first observed yeasts in beer wort using a microscope that he designed and produced. He did not, however, establish a relationship between these corpuscles and alcoholic fermentation. It was not until the end of the eighteenth century that Lavoisier began the chemical study of alcoholic fermentation. Gay-Lussac continued Lavoisier's research into the next century. In 1837, a French physicist named Charles Cagnard de La Tour proved for the first time that the yeast was a living organism. According to his findings, it was capable of multiplying

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and belonged to the plant kingdom; its vital activities were at the base of the fermentation of sugar-containing liquids. The German naturalist Schwann confirmed his theory and demonstrated that heat and certain chemical products were capable of stopping alcoholic fermentation. He named the beer yeast *zuckerpilz*, which means sugar fungus—*Saccharomyces* in Latin. In 1838, Meyen used this nomenclature for the first time.

This vitalist or biological viewpoint of the role of yeasts in alcoholic fermentation, obvious to us today, was not readily supported. Liebig and certain other organic chemists were convinced that chemical reactions, not living cellular activity, were responsible for the fermentation of sugar. In his famous studies on wine (1866) and beer (1876), Louis Pasteur gave definitive credibility to the vitalist viewpoint of alcoholic fermentation. He demonstrated that the yeasts responsible for spontaneous fermentation of grape must or crushed grapes came from the surface of the grape; he isolated several races and species. He even conceived the notion that the nature of the yeast carrying out the alcoholic fermentation could influence the gustatory characteristics of wine. He also demonstrated the effect of oxygen on the assimilation of sugar by yeasts. Louis Pasteur proved that the yeast produced secondary products such as glycerol in addition to alcohol and carbon dioxide.

Since Pasteur, yeasts and alcoholic fermentation have incited a considerable amount of research, making use of progress in microbiology, biochemistry and now genetics and molecular biology.

In taxonomy, scientists define yeasts as unicellular fungi that reproduce by budding and binary fission. Certain pluricellular fungi have a unicellular stage and are also grouped with yeasts. Yeasts form a complex and heterogeneous group found in three classes of fungi, characterized by their reproduction mode: the sac fungi (Ascomycetes), the club fungi (Basidiomycetes), and the imperfect fungi (Deuteromycetes). The yeasts found on the surface of the grape and in wine belong to Ascomycetes and Deuteromycetes. The haploid spores or ascospores of the Ascomycetes class are contained in the ascus, a type of sac made from vegetative cells. Asporiferous yeasts, incapable of sexual reproduction, are classified with the imperfect fungi.

In this first chapter, the morphology, reproduction, taxonomy and ecology of grape and wine yeasts will be discussed. Cytology is the morphological and functional study of the structural components of the cell (Rose and Harrison, 1991).

Yeasts are the most simple of the eucaryotes. The yeast cell contains cellular envelopes, a cytoplasm with various organelles, and a nucleus surrounded by a membrane and enclosing the chromosomes. (Figure 1.1). Like all plant cells, the yeast cell has two cellular envelopes: the cell wall and the membrane. The periplasmic space is the space between the cell wall and the membrane. The cytoplasm and the membrane make up the protoplasm. The term protoplast or sphaeroplast designates a cell whose cell wall has been artificially removed. Yeast cellular envelopes play an essential role: they contribute to a successful alcoholic fermentation and release certain constituents which add to the resulting wine's composition. In order to take advantage of these properties, the winemaker

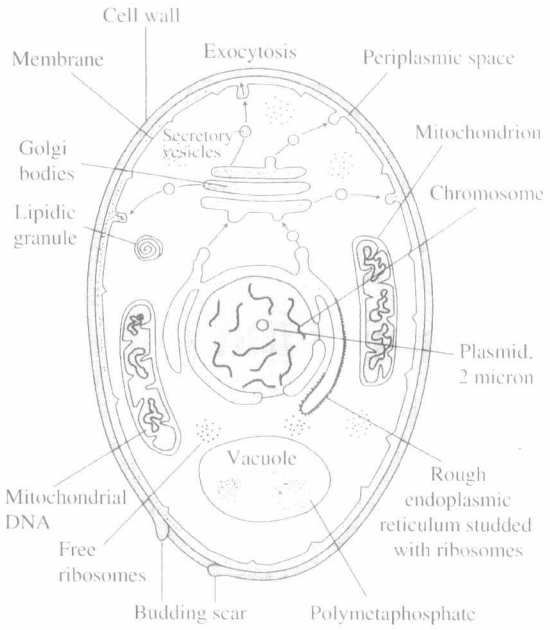


Fig. 1.1. A yeast cell (Gaillardin and Heslot, 1987; Rose and Harrisson, 1969)

or enologist must have a profound knowledge of these organelles.

1.2 THE CELL WALL

1.2.1 The General Role of the Cell Wall

During the last 20 years, researchers (Fleet, 1991; Klis, 1994; Stratford, 1994) have greatly expanded our knowledge of the yeast cell wall, which represents 15–25% of the dry weight of the cell. It essentially consists of polysaccharides. It is a rigid envelope, yet endowed with a certain elasticity.

Its first function is to protect the cell. Without its wall, the cell would burst under the internal osmotic pressure, determined by the composition of the cell's environment. Protoplasts placed in pure water are immediately lysed in this manner. Cell wall elasticity can be demonstrated by placing yeasts, taken during their log phase, in a hypertonic (NaCl) solution. Their cellular volume decreases by approximately 50%. The cell wall appears thicker and is almost in contact with the membrane. The cells regain their initial form after being placed back into an isotonic medium.

Yet the cell wall cannot be considered an inert, semi-rigid 'armor'. On the contrary, it is a dynamic and multifunctional organelle. Its composition and functions evolve during the life of the cell, in response to environmental factors. In addition to its protective role, the cell wall gives the cell its particular shape through its macromolecular organization. It is also the site of molecules which determine certain cellular interactions such as sexual union, flocculation, and the killer factor, which will be examined in detail later in this chapter (Section 1.7). Finally, a number of enzymes, generally hydrolases, are connected to the cell wall or situated in the periplasmic space. Their substrates are nutritive substances of the environment and the macromolecules of the cell wall itself, which is constantly reshaped during cellular morphogenesis.

1.2.2 The Chemical Structure and Function of the Parietal Constituents

The yeast cell wall is made up of two principal constituents: β-glucans and mannoproteins. Chitin represents a minute part of its composition. The most detailed work on the yeast cell wall has been carried out on *Saccharomyces cerevisiae* — the principal yeast responsible for the alcoholic fermentation of grape must.

Glucan represents about 60% of the dry weight of the cell wall of *S. cerevisiae*. It can be chemically fractionated into three categories:

1. Fibrous β-1,3 glucan is insoluble in water, acetic acid and alkali. It has very few branches. The branch points involved are β-1,6 linkages. Its degree of polymerization is 1500. Under the electron microscope, this glucan appears fibrous. It ensures the shape and the rigidity of the cell wall. It is always connected to chitin.
2. Amorphous β-1,3 glucan, with about 1500 glucose units, is insoluble in water but soluble in alkalis. It has very few branches, like the preceding glucan. In addition to these few branches, it is made up of a small number of β-1,6 glycosidic linkages. It has an amorphous aspect under the electron microscope. It gives the cell wall its elasticity and acts as an anchor for the mannoproteins. It can also constitute an extraprotoplasmic reserve substance.
3. The β-1,6 glucan is obtained from alkali-insoluble glucans by extraction in acetic acid. The resulting product is amorphous, water soluble, and extensively ramified by β-1,3 glycosidic linkages. Its degree of polymerization is 140. It links the different constituents of the cell wall together. It is also a receptor site for the killer factor.

The fibrous β-1,3 glucan (alkali-insoluble) probably results from the incorporation of chitin on the amorphous β-1,3 glucan.

Mannoproteins constitute 25–50% of the cell wall of *S. cerevisiae*. They can be extracted from the whole cell or from the isolated cell wall

by chemical and enzymatic methods. Chemical methods make use of autoclaving in the presence of alkali or a citrate buffer solution at pH 7. The enzymatic method frees the mannoproteins by digesting the glucan. This method does not denature the structure of the mannoproteins as much as chemical methods. Zymolyase, obtained from the bacterium *Arthrobacter luteus*, is the enzymatic preparation most often used to extract the parietal mannoproteins of *S. cerevisiae*. This enzymatic complex is effective primarily because of its β-1,3 glucanase activity. The action of protease contaminants in the zymolyase combine, with the aforementioned activity to liberate the mannoproteins. Glucanex, another industrial preparation of the β-glucanase, produced by a fungus (*Trichoderma harzianum*), has been recently demonstrated to possess endo- and exo-β-1,3 and endo-β-1,6-glucanase activities (Dubourdieu and Moine, 1995). These activities also facilitate the extraction of the cell wall mannoproteins of the *S. cerevisiae* cell.

The mannoproteins of *S. cerevisiae* have a molecular weight between 20 and 450 kDa. Their degree of glycosylation varies. Certain ones containing about 90% mannose and 10% peptides are hypermannosylated.

Four forms of glycosylation are described (Figure 1.2) but do not necessarily exist at the same time in all of the mannoproteins.

The mannose of the mannoproteins can constitute short, linear chains with one to five residues. They are linked to the peptide chain by *O*-glycosyl linkages on serine and threonine residues. These glycosidic side-chain linkages are α-1,2 and α-1,3.

The glucidic part of the mannoprotein can also be a polysaccharide. It is linked to an asparagine residue of the peptide chain by an *N*-glycosyl linkage. This linkage consists of a double unit of *N*-acetyl-glucosamine (chitin) linked in β-1,4. The

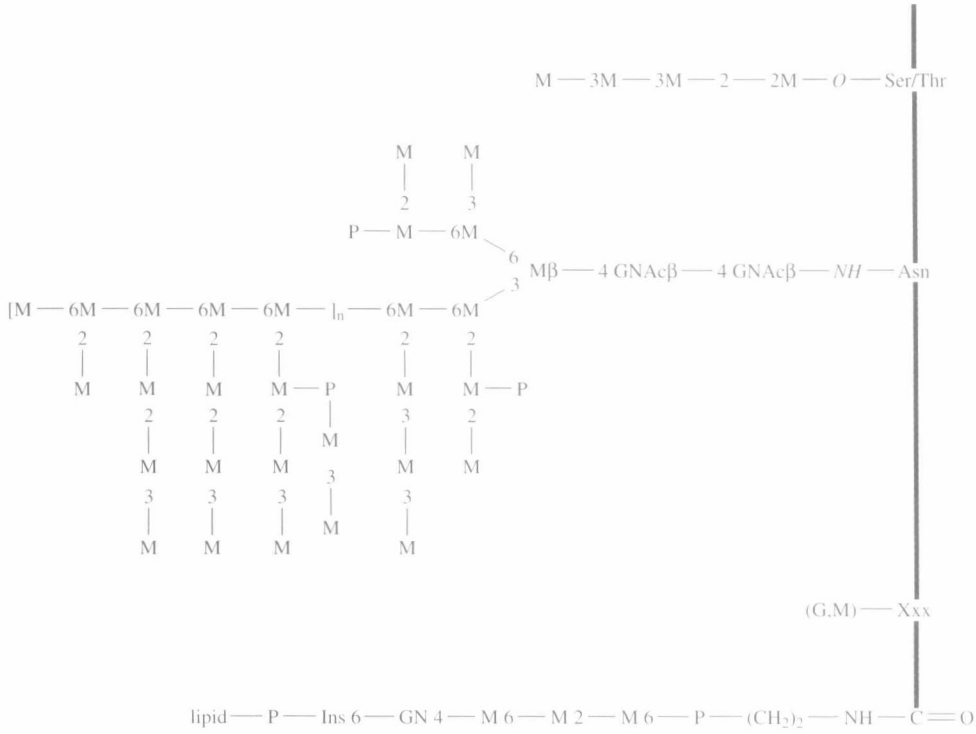


Fig. 1.2. The four types of glucosylation of parietal yeast mannoproteins (Klis, 1994). M = mannose; G = glucose; GN = glucosamine; GNAc = *N*-acetylglucosamine; Ins = inositol; Ser = Serine; Thr = threonine; Asn = asparagine; Xxx = the nature of the bond is not known

mannan linked in this manner to the asparagine includes an attachment region made up of a dozen mannose residues and a highly ramified outer chain consisting of 150 to 250 mannose units. The attachment region beyond the chitin residue consists of a mannose skeleton linked in α-1,6 with side branches possessing one, two or three mannose residues with α-1,2 and/or α-1,3 bonds. The outer chain is also made up of a skeleton of mannose units linked in α-1,6. This chain bears short side-chains constituted of mannose residues linked in α-1,2 and a terminal mannose in α-1,3. Some of these side-chains possess a branch attached by a phosphodiester bond.

A third type of glycosylation was described more recently. It can occur in mannoproteins, which make up the cell wall of the yeast. It consists of a glucomannan chain containing essentially mannose residues linked in α-1,6 and glucose residues linked in α-1,6. The nature of the glycan–peptide point of attachment is not yet clear, but it may be an asparaginyl–glucose bond. This type of glycosylation characterizes the proteins freed from the cell wall by the action of a β-1,3 glucanase. Therefore, *in vivo*, the glucomannan chain may also comprise glucose residues linked in β-1,3.

The fourth type of glycosylation of yeast mannoproteins is the glycosyl–phosphatidyl–inositol anchor (GPI). This attachment between the terminal carboxylic group of the peptide chain and a membrane phospholipid permits certain mannoproteins, which cross the cell wall, to anchor themselves in the plasmic membrane. The region of attachment is characterized by the following sequence (Figure 1.2): ethanolamine-phosphate-6-mannose-α-1,2-mannose-α-1,6-mannose-α-1,4-glucosamine-α-1,6-inositol-phospholipid. A C-phospholipase specific to phosphatidyl inositol and therefore capable of realizing this cleavage was demonstrated in the *S. cerevisiae* (Flick and Thorner, 1993). Several GPI-type anchor mannoproteins have been identified in the cell wall of *S. cerevisiae*.

Chitin is a linear polymer of *N*-acetylglucosamine linked in β-1,4 and is not generally found in large quantities in yeast cell walls. In *S. cerevisiae*, chitin constitutes 1–2% of the cell wall and is found for the most part (but not exclusively) in bud scar zones. These zones are a type of raised crater easily seen on the mother cell under the electron microscope (Figure 1.3). This chitinic scar is formed essentially to assure cell wall integrity and cell survival. Yeasts treated with D polyoxine, an antibiotic inhibiting the synthesis of chitin, are not viable; they burst after budding.

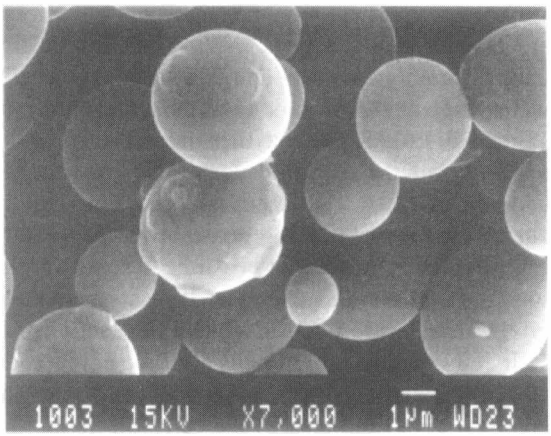


Fig. 1.3. Scanning electron microscope photograph of proliferating *Sacch. cerevisiae* cells. The budding scars on the mother cells can be observed

The presence of lipids in the cell wall has not been clearly demonstrated. It is true that cell walls prepared in the laboratory contain some lipids (2–15% for *S. cerevisiae*) but it is most likely contamination by the lipids of the cytoplasmic membrane, adsorbed by the cell wall during their isolation. The cell wall can also adsorb lipids from its external environment, especially the different fatty acids that activate and inhibit the fermentation (Chapter 3).

Several enzymes are connected to the cell wall or situated in the periplasmic space. One of the most characteristic enzymes is the invertase (β-fructofuranosidase). This enzyme catalyses the hydrolysis of saccharose into glucose and fructose. It is a thermostable mannoprotein anchored to a β-1,6 glucan of the cell wall. Its molecular weight is 270 000 Da. It contains approximately 50% mannose and 50% protein. The periplasmic acid phosphatase is equally a mannoprotein.

Other periplasmic enzymes that have been noted are β-glucosidase, α-galactosidase, melibiase, trehalase, aminopeptidase and esterase. Yeast cell walls also contain endo- and exo-β-glucanases (β-1,3 and β-1,6). These enzymes are involved in the reshaping of the cell wall during the growth and budding of cells. Their activity is at a maximum during the exponential log phase of the population and diminishes notably afterwards. Yet cells in the stationary phase and even dead yeasts contained in the lees still retain β-glucanases activity in their cell walls several months after the completion of fermentation. These endogenous enzymes are involved in the autolysis of the cell wall during the ageing of wines on lees. This ageing method will be covered in the chapter on white winemaking (Chapter 13).

1.2.3 General Organization of the Cell Wall and Factors Affecting its Composition

The cell wall of *S. cerevisiae* is made up of an outer layer of mannoproteins. These mannoproteins are connected to a matrix of amorphous β-1,3 glucan which covers an inner layer of fibrous β-1,3 glucan. The inner layer is connected to a small quantity of chitin (Figure 1.4). The β-1,6 glucan probably acts as a cement between the two layers. The rigidity and the shape of the cell wall are due to the internal framework of the β-1,3 fibrous glucan. Its elasticity is due to the outer amorphous layer. The intermolecular structure of the mannoproteins of the outer layer (hydrophobic linkages and disulfur bonds) equally determines cell wall porosity and impermeability to macromolecules (molecular weights less than 4500). This impermeability can be affected by treating the cell wall with certain chemical agents, such as β-mercaptoethanol. This substance provokes the rupture of the disulfur bonds, thus destroying the intermolecular network between the mannoprotein chains.

The composition of the cell wall is strongly influenced by nutritive conditions and cell age. The proportion of glucan in the cell wall increases with respect to the amount of sugar in the culture medium. Certain deficiencies (for example, in mesoinositol) also result in an increase in the proportion of glucan compared with mannoproteins. The cell walls of older cells are richer in glucans and in chitin and less furnished in mannoproteins. For this reason, they are more resistant to physical and enzymatic agents used to degrade them. Finally, the composition of the cell wall is profoundly modified by morphogenetic alterations (conjugation and sporulation).

1.3 THE PLASMIC MEMBRANE

1.3.1 Chemical Composition and Organization

The plasmic membrane is a highly selective barrier controlling exchanges between the living cell and

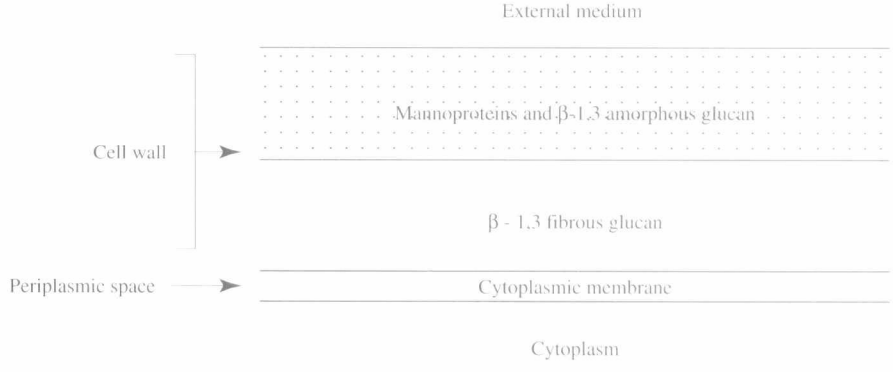
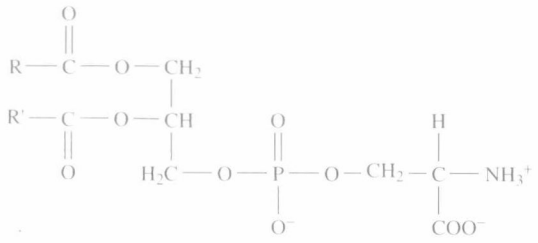
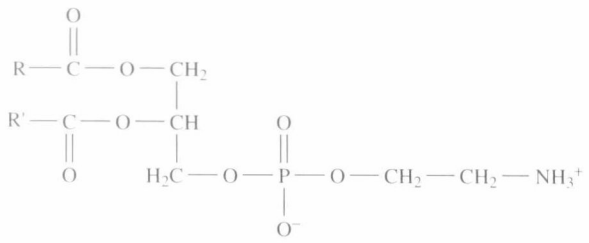


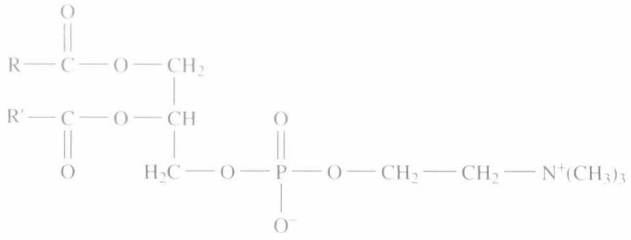
Fig. 1.4. Cellular organization of the cell wall of *Sacch. cerevisiae*



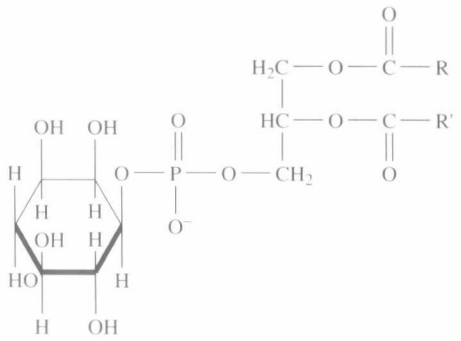
Phosphatidyl serine



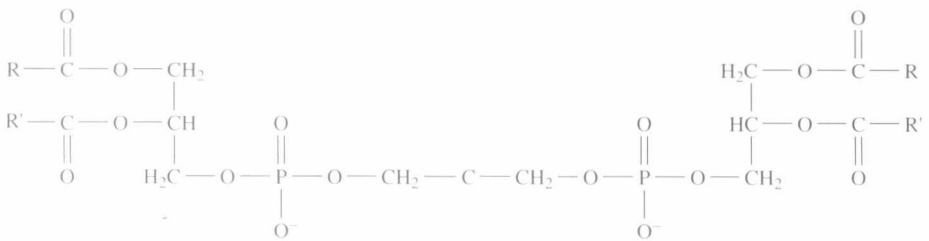
Phosphatidyl ethanolamine



Phosphatidyl choline



Phosphatidyl inositol



Diphosphatidyl glycerol (cardiolipin)

Fig. 1.5. Yeast membrane phospholipids

its external environment. This organelle is essential to the life of the yeast.

Like all biological membranes, the yeast plasmic membrane is principally made up of lipids and proteins. The plasmic membrane of *S. cerevisiae* contains about 40% lipids and 50% proteins. Glucans and mannans are only present in small quantities (several per cent).

The lipids of the membrane are essentially phospholipids and sterols. They are amphiphilic molecules, i.e. possessing a hydrophilic and a hydrophobic part.

The three principal phospholipids (Figure 1.5) of the plasmic membrane of yeast are phosphatidyl-ethanolamine (PE), phosphatidyl-choline (PC) and phosphatidyl-inositol (PI) which represent 70–85% of the total. Phosphatidyl-serine (PS) and diphosphatidyl-glycerol or cardiolipin (PG) are less prevalent. Free fatty acids and phosphatidic acid are frequently reported in plasmic membrane