NEW TRENDS ON YEAST AUTOLYSIS AND WINE AGEING ON LEES: A BIBLIOGRAPHIC REVIEW

ÉTAT DES CONNAISSANCES SCIENTIFIQUES ACTUELLES SUR LE PHÉNOMÈNE D'AUTOLYSE DES LEVURES ET L'ÉLEVAGE DES VINS SUR LIES

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Abstract: In enology, «grands crus» white wines are traditionally aged by the «sur lies» method, which consists of keeping the aging wine in contact with the lees (yeasts and organic residues). The lees can come either from the first or second fermentation and can be used for both white and red wines. This practice is still in the experimental stage. We reviewed scientific studies carried out on wine lees to determine the current situation in enology. We also provide some technological information relevant to such a practice.
The first part of this paper provides a clear definition of wine lees from a legal and technological point of view. The second part describes the mechanisms of autolysis and focuses on each class of autolysis product. Many scientific studies have discussed the phenomenon of yeast autolysis during wine ageing. Most of these studies simply identified the yeast macromolecules released into the wine during autolysis. However, the experimental methods used vary and it is difficult to extrapolate most of results to the process of wine ageing on lees. Only a few studies have dealt with the physicochemical properties of lees during autolysis, especially concerning oxygen, polyphenols and other wine compounds. We then summarize the recent data obtained on these topics. Finally, we discuss the technical effects of aging wine on lees.

Résumé : En œnologie, le vin peut être maintenu lors de l'élevage en présence de ses lies (levures et débris végétaux) qu'elles soient issues de la première ou de la seconde fermentation. Bien que, dans la pratique, peu de règles soient bien établies pour la mise en place de tels élevages, de nombreux travaux scientifiques ont pourtant eu lieu sur les nombreuses facettes de cette opération technologique. Toutefois, la multiplicité des modèles expérimentaux utilisés dans ces études ne rend pas forcément facile une appréciation globale des divers phénomènes biologiques mis en jeu. Cette revue bibliographique a été entreprise dans le but de détailler de façon la plus exhaustive possible la majorité des travaux scientifiques réalisés sur les lies de vin, en insistant notamment sur les macromolécules directement relarguées au cours de l'autolyse des levures, mais également en présentant les aspects techniques d'une telle pratique.

Key words: yeast, autolysis, *Saccharomyces cerevisiae*, lees, wine aging on lees.
Mots clés : levures, autolyse, *Saccharomyces cerevisiae*, élevage sur lies.

INTRODUCTION

This literature review aims to present current scientific knowledge concerning the autolysis of lees yeasts and to investigate the effect of «sur lies» wine aging on the changes in the physiological state of the lees and on the organoleptic and technological changes that occur during autolysis.

«Grands crus» white wines are traditionally aged «sur lies». However, this technique increased in popularity for the aging of red wines in recent years. This method involves aging wines in the presence of their lees (yeasts from alcoholic fermentation and organic residue from the must). The benefits of this method have mostly been studied in terms of interactions between the lees and their environment, notably with the

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macromolecules released from yeasts. Nonetheless, this practice is still in the experimental stage and has no solid scientific foundation.

WINE LEES

I - LEGAL DEFINITION OF WINE LEES

Wine lees are defined by EEC regulation n°337/79 as «the residue that forms at the bottom of recipients containing wine, after fermentation, during storage or after authorized treatments, as well as the residue obtained following the filtration or centrifugation of this product.» Three categories of lees, obtained after the end of alcoholic fermentation, were distinguished by the Direction Générale des Impôts (The French Tax and Excise Service) in 1978 (Direction Générale des Impôts, 1978) (figure 1).

- The clear lees, or «wine deposits», obtained after clarification, are products that do not dry out completely and that still contain a certain amount of wine. The «crude lees» (or «virgin lees») are followed by the «fine lees», which are followed by «false clears» (or cloudy wines) and by clear wines.

- The «fatty lees», which contain less wine than the clear lees.

- The «dry lees», from which no wine can be extracted.

The European Community prohibits the pressing of wine lees, which can be used to extract the remaining wine. However, according to this law, treatments such as «the filtration or centrifugation of wine lees are not considered to be forms of pressing, if the products obtained are healthy, reliable and of saleable quality, and when the lees treated in this way are not totally dried».

II - DEFINITION OF SUR LIES WINES

A wine is called «sur lies» if no clarification is performed once alcoholic fermentation has finished. EEC regulation n°822/87 authorizes the use of up to 5 p. cent fresh, healthy, non-diluted lees, containing yeasts from the recent production of dry wines, in wine-making (RENOUIL and FERET, 1988). Thus, the wines contain either their own lees or lees from other alcoholic fermentations.

The «sur lies» method of wine aging is used in certain wine-growing regions, notably in the Nantes, Savoie and Burgundy regions of France. The term «sur lies» is issued by production decree. This is particularly the case of Muscadet wines, wines from the Nantes region (Gros Plant Nantais), wines from the golfe du Lion region (Vin de Pays des Sables du golfe du Lion) and wines from the pays d'Oc region. The texts of the decrees take all of the production conditions into account, specifying in particular that the wines must still be on fine lees when they are bottled.

Wines aged according to the Burgundy method are generally kept in 228 liter barrels, in which they are regularly «stirred»: the wine is periodically stirred with a utensil (a special stick or other implement) to resuspend the lees. This stirring procedure is not subject to precise rules; the frequency can vary considerably depending on the practice in each wine production plant.

The bottling of wines aged «sur lies», corresponding specifically to the production of certain dry white wines from healthy grapes, must take place in the wine-making plant before July 1st in the year following the harvest or after the 15th October. The wines must be labeled with the vintage.

The aging of wines on lees is also common in other countries, such as Switzerland and Australia, where the wines spend less time on lees (2 to 6 months versus 12 to 15 months in France) (STUCKEY *et al.*, 1991), and in a more anecdotal manner in Japan (ARIZUMI *et al.*, 1994).

III - THE USES OF WINE LEES

1) Non-sparkling «sur lies» wines (lees from the first fermentation)

After alcoholic fermentation, the wine-producer can maintain wines «sur lies» in several different ways. The traditional method is to clarify the wines at a late stage (March) or to keep them on lees throughout the entire aging process (8 to 10 months), with periodic stirring. Another, more recent method involves the early clarification of the wines (November or December) followed by storage on fine lees. The lees used for aging wines corresponds to only a small proportion of the total quantity produced during wine production.

2) Sparkling wines (lees from second fermentation)

The production of sparkling wines, in particular those made by the Champagne method, requires the use of lees from the second alcoholic fermentation (RIBÉREAU-GAYON *et al.*, 1998a). The wines can also be kept in contact with the lees for several months to several years.

3) Lees for distillation

The remaining lees are generally sent to distilleries. In fact, «no wines or drinks for direct human consumption (except alcohols, spirits and low quality wines)»

can be made from the lees of wines from the European Community (RENOUIL and FERET, 1988).

All wine producers must send all of the by-products of their wine production, or failing that, some of their own wine, for distillation. Depending on the zone in which the wine was produced, the lees must contain a mean of 3 to 4 liters of pure alcohol per metric deciton, with a humidity of 45 p. cent (EEC regulation n°822/87, articles 5 and 6). If the lees obtained do not meet these minimum quality criteria, they cannot be used and are either sent for transformation other than distillation or destroyed under controlled conditions (FRÉDÉRIC, 1991).

IV - COMPOSITION OF FIRST FERMENTATION LEES

1) General features

The cloudy liquid or «fresh lees» obtained after the various clarification steps contain between 2 and 4 p. cent of the total volume of the wine (RENOUIL and FERET, 1988). They contain approximately 25 p.cent dry matter, which in turn consists of :

- 25 to 35 p. cent tartaric salts,

- 35 to 45 p. cent microorganisms (predominantly yeasts),

- 30 to 40 p. cent organic residues.

2) Microbiological composition of «sur lies» wines

The lees are, by definition, a reservoir of microorganisms. The most common microorganisms are the yeast that carried out the fermentation, but bacteria may also be present if malolactic fermentation took place. The current practice of adding dried active yeasts (DAY) to grape musts before the beginning of alcoholic fermentation favors the establishment of the yeast as the predominant species in the lees. *Saccharomyces cerevisiae* is the usual choice. However, other species, from the grape microflora, the soil and the harvesting equipment, may also be present. Notable examples include *Kloeckera apiculata*, *Metschnikowia pulcherrima*, *Candida stellata* and *Pichia membranefaciens* (RIBÉREAU-GAYON *et al.*, 1998a).

3) Chemical composition of the lees

Few studies have described the exact composition of wine lees. In fact, the compositions of lees are not definitive, given the diversity of wines and their res-

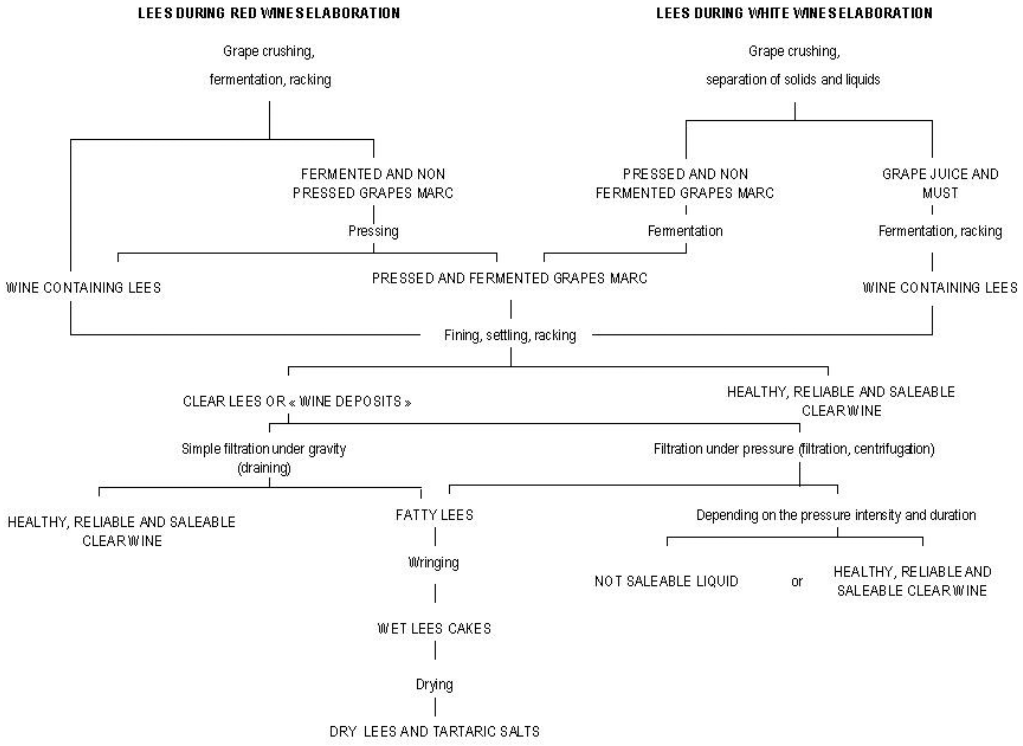

Fig. 1 - Summary of the utilization of lees in white and red wines elaboration processes (from Bulletin Officiel des Impôts, 1987)

Les lies dans la vinification « en rouge » et dans la vinification « en blanc » (d'après le Bulletin Officiel des Impôts, 1987)

pective lees, and given the way in which the product changes over time. A few studies have provided some information on wine lees, notably that by FRÉDÉRIC (1991), on lees from champagne that had been aged for 1 or 8 months, that by SCIANCAPELORE *et al.* (1983), on the lees from Italian red wines from which alcohol and tartrates were removed with the aim of improving wine residues, and that of FERRARI and FEUILLAT (1988), who studied the lipid and nitrogenous fractions of lees from white wines.

SCIANCAPELORE *et al.* (1983) used the Kjedahl method to show that the total nitrogen content was 4.2 p. cent dry weight and that the soluble nitrogen content was 0.16 p. cent dry weight. FERRARI *et al.* (1988) used the same method on lees from white Burgundy wines. Total nitrogen content varied between 1.55 p. cent dry weight at the beginning of the aging process and 1.15 p. cent dry weight after 5 months of aging with stirring.

SCIANCAPELORE *et al.* (1983) also determined the most common amino acids in the lees from red wines. They were, in descending order of molecular weight: glutamic acid, aspartic acid, lysine, valine and leucine. The study by FRÉDÉRIC (1991) concentrated on certain essential amino acids in the lees of white wines and found that there were large amounts of tryptophan and lysine in lees that had been aged for 8 months, whereas tryptophan was not even detected in the lees from red wines studied by SCIANCAPELORE *et al.* (1983). A study of the amino acids present in wine lees during the aging process showed that it is difficult to make generalizations concerning the presence of a given amino acid in the medium and in the yeast, and that the initial composition of the medium has a fundamental effect.

The total fatty acid content of lees was analyzed in controlled laboratory conditions, by various methods. The contents ranged from 1 to 6 p. cent total dry weight of the biomass (FORNAIRON-BONNEFOND, 2000). SCIANCAPELORE *et al.* (1983) showed that palmitic acid (C16:0) and linoleic acid (C18:2) are the most abundant fatty acids in the lees from Italian red wines, accounting for 29 and 28 p. cent of the lipid fraction of the lees residue, respectively. The next most abundant were oleic acid (C18:1) (15.3 p. cent), steric acid (C18:0) (10 p. cent) and linolenic acid (C18:3) (9.2 p. cent). The ratio of unsaturated to saturated fatty acids was 1.3.

Vitamins and oligoelements have been detected in Champagne lees (FRÉDÉRIC, 1991). Analysis of the cations and anions in these lees and in lees from non-sparkling white wines, showed that they contained high levels of potassium.

THE CONSEQUENCES OF «SUR LIES» WINE AGING

The chemical composition of lees changes during the storage of wines «sur lies». Autolysis, the biological phenomenon that leads to these changes, will be explained in detail in this chapter, as will the typical properties of the lees, which affect the characteristics of «sur lies» wines.

I - AUTOLYSIS OF YEASTS IN ENOLOGY

SALKOWSKI first used the term «autolysis» in 1875 (FEUILLAT, 1998) and the first studies explaining the biochemical bases of the autolysis of bakers' yeast were published in the 1950s by VOSTI and JOSLYN (1954).

1) Definition and description of autolysis

Autolysis is the term used to describe the reactions that occur in the non-viable yeasts (yeasts that have lost their ability to multiply) present in lees. The autolysis of yeasts is defined as the hydrolysis of intracellular biopolymers by the endohydrolases induced by cell death, followed by the formation of low molecular weight products (BABAYAN *et al.*, 1981).

These authors divide the mechanism of autolysis into three distinct steps:

- The disturbance of intracellular structures (cytoplasmic membrane and lysosome) leads to the release of hydrolytic enzymes and their substrates.

- Once these enzymes are activated they interact with intracellular polymers, leading to the accumulation of hydrolysed products in the periplasmic space defined by the yeast wall.

- Finally, the molecular mass of the hydrolysis products decreases, such that these products (proteins, polysaccharides) are small enough to diffuse out of the cell into the extracellular environment.

The wall components (gluccans and mannoproteins) are also degraded in parallel, which increases pore size, thereby facilitating the release of the products of autolysis from the wall into the extracellular environment.

In enology, an understanding of autolysis and its effects are especially important for wines that are aged in the presence of their fermentation yeasts for long periods of time. This includes sparkling wines, such as champagnes, which must remain in contact with their lees for at least one year if they are to be called «vin de Champagne». Champagne wines can also be kept in

contact with their lees for up to ten years, or even more in the case of particular vintages produced before stirring and extraction of the sediment-coated cork. This is also the case for white wines aged on lees (about one year of contact with the lees to earn this title). Autolysis occurs as wines age, and the molecules released may increase or improve the qualities and potentialities of certain wines. However, in enology, autolysis typically occurs slowly. For example, in certain sparkling wines made by the Champagne method, autolysis only occurs after a latent period (after the second alcoholic fermentation), which can last for 6 to 12 months, the time required for complete cell death and activation of the autolytic system (FEUILLAT and CHARPENTIER, 1982; TODD, 1995).

2) The by-products of autolysis used in enology

This natural process is slow and expensive, both in terms of the equipment required (the barrels and vats required during the aging process are often costly) and in terms of the labor involved (stirring time, tasting to test for the development of any aftertastes, etc.). To cope with these economic constraints, additives for use in enology, prepared by autolysis, have been developed by a number of teams (FEUILLAT, 1986; FEUILLAT and CHARPENTIER, 1998; LAFON-LAFOURCADE *et al.*, 1977; MOINE-LEDOUX *et al.*, 1997; TRIOLI and DULAU, 1995) to make it possible to take advantage of the benefits of autolysis without its disadvantages (FEUILLAT, 2000). These yeast autolysates are used to activate alcoholic and malolactic fermentation. Their use leads to optimal growth, more rapid fermentation and a high percentage viability (FEUILLAT and GUERREAU, 1996; TRIOLI and DULAU, 1995). This is also the case for cytoplasm-free yeast cell envelopes prepared by autolysis, which can be added to musts as survival factors. In other words, they do not affect growth, but prolong the viability of cells that are not multiplying (LAFON-LAFOURCADE *et al.*, 1977), increasing the amount of sugar that can be fermented. These yeast envelopes not only have nutritive value in themselves, they also act by preferentially adsorbing certain components or substances that are toxic to the yeast, such as intermediate-length fatty acids (particularly decanoic acid and its esters), without modifying the aromatic properties of the wines (LARUE *et al.*, 1984).

However, these additives are also used with the aim of improving the physicochemical stability of wines (tartaric salts, proteins and polyphenols). The wines prepared in this way are rich in total nitrogen (proteins and amino acids) and more generally in macromolecules. Thus, the additives have a positive effect on the sensory properties of the wine. However, these properties can currently only be used experimentally (FEUILLAT, 2000).

3) External factors that affect autolysis

The natural autolysis of yeasts, which occurs due to catabolism and the decrease in cellular viability, is different from «induced» autolysis, which can be triggered deliberately by increasing the temperature, by adding plasmolysis agents or other factors that decrease the integrity of cytoplasmic membrane or by activating lytic enzymes.

Only the natural autolysis of yeast occurs in enology. Nevertheless, some factors can affect the amplitude of this natural autolysis: temperature, pH, etc. The total amount of nitrogen released is normally used as a control when comparing the effects of these treatments on autolysis.

a) Temperature

Autolysis is activated by an increase in temperature. The activities of the enzymes involved in this process increase with temperature, the critical limit being the temperature at which the enzymes are denatured. Differences in operating conditions probably account for the apparently contradictory results reported concerning the effect of temperature on autolysis.

It has been shown that, during the induction of autolysis in a synthetic medium, the destruction of cellular endostructures and the activation of lytic enzymes depend on temperature. The optimal temperature is 60°C for proteases and 70°C for nucleases (BABAYAN *et al.*, 1981). These optimal temperatures are reduced in the presence of plasmolytic agents (ethanol, ether acetate, lecithin etc.). BABAYAN *et al.* (1981) claimed that the optimal temperature for autolysis was between 45 and 60°C at pH 5. However, these conditions do not reflect those used in enology.

FEUILLAT *et al.* (1982) studied the effect of temperature on autolysis by monitoring the quantity of nitrogen released by the yeast over time, in a solution buffered at pH 5. The higher the temperature, the more nitrogen was released. However, after heating for 4 hours at 55°C, no further nitrogen was released, whereas at lower temperatures, nitrogen was released for longer periods of time. This is due to inhibition of the enzymatic process at high temperatures.

MOLNAR *et al.* (1980) found that the rate of autolysis was generally linear between 4 and 40°C in acidic conditions, and that a 10°C increase in temperature led to a 6 to 7 p. cent increase in the rate of autolysis. The optimal temperature for proteolytic activity in sparkling wines is 10 to 12°C. Beyond this tempera-

ture range (4-40°C), the autolytic activity of the intracellular proteases stops. If autolysis proceeds too rapidly (for example at high temperatures), the components released cannot undergo certain secondary chemical reactions that favor the formation of the bouquet and may even develop an unpleasant «yeasty» taste in the mouth (KELLY-TREADWELL, 1988).

b) Other factors

Chemical factors may also accelerate autolysis. For example:

- Changes in ionic power;

- The pH: if the pH is lower than that of the wine, the intracellular components are released more rapidly (BREDDAM and BEENFELDT, 1991; FEUILLAT and CHARPENTIER, 1982; MIGUEL-GORDILLO *et al*., 1990),

- The ionic composition of the medium: calcium and magnesium favor autolysis at 30°C (HOUGH and MADDOX, 1970),

- The ethanol content of the medium: more proteolysis occurs in the presence of 10 p. cent ethanol (v/v) than in the presence of 12 p. cent ethanol (MIGUEL-GORDILLO *et al.*, 1990).

Finally, too much aeration and a lack of nitrogen or energy in the medium can also interfere with both structural and functional cellular processes and accelerate autolysis (BABAYAN and BEZRUKOV, 1985).

4) Mechanisms and consequences of autolysis in enology

After the drop in cellular viability, the autolysis of yeast leads to the release of cellular proteins, nucleic acids, lipids and polysaccharides, and is correlated with a decrease in overall biomass.

a) Proteolysis

One of the most obvious consequences of autolysis is the hydrolysis of proteins, increasing the amount of nitrogenous metabolites in the wine. The studies by LURTON (1988) and LURTON *et al.* (1989), which were confirmed by that of SATO *et al.* (1997), established that proteases are involved in the exchanges that occur between the yeast and the wine during autolysis. *S. cerevisiae* has highly diversified enzymatic equipment (ACHSTETTER and WOLF, 1985). Most of these enzymes are probably involved in the proteolysis of wine yeasts. However, the enological conditions used do not favor the action of all of these proteases, due to the low pH (between pH 3 and 4) and the temperature (between 10 and 15°C). According to LURTON (1988) and LURTON *et al.* (1989) the use of specific inhibitors for the four classes of protease found in *S. cerevisiae* (serine proteases, thiol group proteases, acid proteases and metalloproteases), suggests that protease A plays a predominant role in the proteolytic process. However, recent works revealed that the activity of protease A is detected at the end of alcoholic fermentation well before the start of autolysis, and was not the preponderant protease activity during autolysis (ALEXANDRE *et al*., 2001). At the pH of wine, this acid protease releases a very large number of peptides. It also activates other proteases, such as carboxypeptidase Y. The activity of the extracellular form of this carboxypeptidase, which is 25 times weaker than that of protease A, has only been detected in wine at the beginning of the aging «sur lies» process. Its intracellular form was detected for at least 6 months (SATO *et al.*, 1997). The possibility that synergistic acid proteases other than protease A are involved in the increase in amino acids in wines aged on lees cannot be ruled out (LURTON, 1988).

The intensity of protease activities varies considerably as a function of the strain of yeast. LEROY *et al.* (1990) showed considerable variation in activity during the second alcoholic fermentation of champagne wines made with two traditional strains of *S. cerevisiae*. Such variability was also observed by ARIZUMI *et al.* (1994), during their study of three strains of yeast (2 *S. cerevisiae* and 1 *S. bayanus*), and by SUZZI (1990), who studied the release of amino acids by ten strains of yeast. SUZZI (1990) did not precisely quantify the protease activities of the strains of yeast, but the significant differences in the amounts of amino acids detected may be attributed to different protease activities.

These protease activities were still detected after 5 to 7 months in storage, depending on the type of wine: white wines from the Burgundy region (FERRARI and FEUILLAT, 1988) and Koshu wines (ARIZUMI *et al.*, 1994). It has also been shown that the protease activities of champagne wines change with time. Proteolytic activities appear to decrease for several months after the end of the second fermentation. They then increase regularly for several years and reach a maximum at about 6 years (FEUILLAT and CHARPENTIER, 1982; LEROY *et al.*, 1990).

b) The autolytic capacity of yeasts

For comparative purposes, certain authors have tried to develop and to standardize a definition for the «autolytic capacity of yeasts» based on their proteolytic capacities. CHARPENTIER *et al.* (1986) defined this capacity as «the amount of soluble nitrogen released per gram of yeast in dry weight per unit time».

LEROY *et al.* (1990), refined this definition by stating that autolysis is carried out in a hydroalcoholic medium at pH 3.5 and at 37°C for 48 hours. SUZZI (1990) determined only the maximum amount of amino acids released by different strains of *S. cerevisiae*, following fermentation in a given medium for 10 days, at various temperatures. Given the variability in the autolytic behavior of yeasts, this author suggested that the autolytic capacity of *S. cerevisiae* yeasts may depend on the strain selected. HERNAWAN *et al.* (1995) studied that autolytic capacity of other species of yeast such as *Kloeckera apiculata* and *Candida stellata*, which may also potentially be used for fermentation.

c) Degradation of the cell wall

One direct effect of proteolysis is that the rigid structure of the cell wall is changed during autolysis (FEUILLAT, 1998). Electron microscopy has shown that crests form during autolysis in hydroalcoholic environments (CHARPENTIER *et al.*, 1986). PITON *et al.* (1988) also confirmed that the cell wall changes during the aging of champagne. Transformation of the cell wall starts during the first six months of aging, with the disappearance of the internal layer of the wall. The polysaccharides in the outer layer only change at a much later stage, between 8 and 11 years (PITON *et al.*, 1988).

The walls of *S. cerevisiae* are essentially composed of polysaccharides (90 p. cent). The remaining 10 p. cent consists of lipids and proteins (CHARPENTIER and FEUILLAT, 1992). The polysaccharide fraction is composed of branched glucans (essentially chains of β-(1→3)-D glucose, but also chains of β-(1→6)-glucose), mannoproteins (proteoglycans) and chitin (1 p. cent of total polysaccharides) (MANNERS *et al.*, 1973a; MANNERS *et al.*, 1973b). The changes in the wall depend on the initial growth conditions of the yeast (synthetic medium or natural must). These changes include:

- A 20 to 50 p. cent reduction in the thickness of the wall during growth in a synthetic medium (HERNAWAN and FLEET, 1995; CHARPENTIER *et al.*, 1986),

- A 10 p. cent increase in the thickness of the wall during growth on 10 p. cent (v/v) ethanol, accompanied by a 26% increase in the thickness of the polysaccharide layer (CHARPENTIER *et al.*, 1986).

Regardless of the nature of the medium, the cell wall conserves its integrity. An increase in the mannose/glucose ratio is also observed, indicating an overall decrease in glucan content. The decrease in the polysaccharide content of the walls (polysaccharides confer shape and rigidity), coupled with a loss of amino acids, accounts for the structural collapse of the wall (FREYSSINER *et al.*, 1989). The β-(1→3)-D glucanases, located in the wall and still present after 4 months of aging on lees, have been reported to be responsible for these phenomena (CHARPENTIER and FREYSSINET, 1989; FEUILLAT *et al.*, 1989). The weak mannosidase activity detected in the walls plays a much more minor role than the glucanase activities detected (FREYSSINET *et al*, 1989). The study by FREYSSINET *et al.* (1989) on isolated walls, under conditions similar to those encountered in wine, led to the description of three phases during the degradation of the wall:

- The hydrolysis of glucans under the action of β-glucanases leads to the release of mannoproteins covalently bonded to glucans,

- The glucans are then hydrolyzed by a soluble β-glucanase present in the medium,

- Finally, the mannoproteins are hydrolyzed by an β-mannosidase or by the proteases released during proteolysis.

Commercial enzyme preparations have been developed with the aim of optimizing and accelerating autolysis during the aging of wines on lees. These products essentially consist of mixtures of pectinases and glucanases. They considerably increase the amount of mannoproteins released into the medium, for both white and red wines (PELLERIN *et al.*, 2001; TRIONE *et al.*, 2001).

d) Reduction of dry weight

One of the consequences of the release of proteins and of the destruction of cell walls during yeast autolysis is a decrease in the total amount of dry matter of the lees with time. This decrease has been observed in numerous experiments carried out in a variety of conditions:

- After 25 months in contact with the wine, the dry weight of champagne lees is only 50 p. cent of that at the beginning of the aging process, it then decreases to 30 p. cent after 18 years of aging (LEROY *et al.*, 1990),

- FERRARI and FEUILLAT (1988) showed that after aging a non-sparkling Burgundy wine on lees for 5 months, the content of the dry extract decreased from 32 to 22 p. cent, this decrease being accompanied by a decrease in dry weight,

- In model conditions, FORNAIRON-BONNEFOND (2000) observed that following 21 days

of autolysis at 28°C the dry weight of the lees decreased by between 17 and 19 p. cent,

- HOUGH and MADDOX (1970) showed that during the autolysis of brewers' yeasts for 14 days at 45°C, pH 5-6, dry weight decreased by at least 20 percent,

- HERNAWAN and FLEET (1995) also reported a decrease in biomass from initial levels during the autolysis of three different strains of yeast. The biomass decreased by between 26 and 33 p. cent after 10 days at 45°C, pH 4.5,

- Finally, PUEYO *et al.* (2000) suspended three strains of yeast in a model wine medium and cultured them at 30°C, pH 3 for 12 days with constant shaking. After two days these authors noted a rapid decrease (over 30 p. cent) in dry weight. The dry weight stabilized after 6 days, at just 40 p. cent of the initial dry weight.

5) The products of autolysis

During autolysis, due mainly to the hydrolytic activities described above, the medium becomes enriched in compounds, principally of cell wall origin, released by the yeast. These compounds are essentially nitrogenous substances such as amino acids, oligopeptides and polypeptides, but also polysaccharides, lipids and nucleic acids. The release of these compounds during aging contributes to the organoleptic and physicochemical properties of wines. More precisely, the products of autolysis, present in wine-making, may be of two types: 1) the primary products of autolysis, which act directly on the wine, 2) the products of secondary reactions between the products of autolysis and other wine components (TODD, 1995).

a) Nitrogenous substances

Nitrogenous substances are generally considered to be the major products of lees autolysis. About 50 p. cent of all the nitrogen in the yeast can diffuse into the extracellular medium at optimum pH (pH 5 to 6) (BABAYAN and BEZRUKOV, 1985). The study of these substances during autolysis showed that proteins and peptides are released, and that these compounds are then hydrolyzed extracellularly to give free amino acids (BABAYAN and BEZRUKOV, 1985; MARTINEZ-RODRIGUEZ and POLO, 2000; MORENO-ARRIBAS *et al.*, 1996; MORENO-ARRIBAS *et al.*, 1998). This process ends in an increase in hydrolytic activity, leading to the autodigestion of the enzymes themselves (BABAYAN and BEZRUKOV, 1985). According to FEUILLAT (1998), amino acids are not particularly good markers of autolysis, as those related to autolysis only appear after the release of peptides and proteins into the medium. Indeed, it is difficult to distinguish between the various stages of amino acid release, which must not be confused with autolysis. The amino acids are first assimilated by the yeast, during alcoholic fermentation, and are then used for cellular multiplication or stored, notably in vacuoles. After sugar depletion, the yeast uses its own reserves to maintain its metabolic activity. The cell then degenerates and the cellular pool of amino acids is very rapidly, passively released by exsorption (this step is not affected by protease inhibitors consisting primarily of glutamic acid and alanine). This phenomenon must not be confused with strict autolysis. There is then a short latency period, during which the amount of amino acids does not appear to change. Further amino acids are then released, but much more slowly than before, due to strict autolysis (FEUILLAT and CHARPENTIER, 1982; KELLY-TREADWELL, 1988).

The release of nitrogenous substances is correlated with intracellular proteolytic activity, which, as indicated above, depends on the strain of yeast and the growth medium (CHARPENTIER *et al.*, 1986). HERNAWAN and FLEET (1995) thus considered the protein, peptide and amino acid composition of the autolysates to be only relative results that cannot be compared easily, because proteases and peptidases are also released into the extracellular medium after a certain amount of time, and remain active. Thus, these authors state that it is difficult to use the exact protein and peptide compositions of autolysates as a marker of autolysis, as concentrations change with time and the presence of amino acids in the medium does not depend on autolysis alone.

However, several authors have estimated and analyzed amino acid and protein release during the aging of wines on lees (ARIZUMI *et al.*, 1994; MORENO-ARRIBAS *et al.*, 1998). The amounts of individual amino acids are difficult to measure, because they vary enormously from year to year, from strain to strain (FERRARI and FEUILLAT, 1988) and from wine to wine. As a general rule, regardless of the medium (synthetic medium or wine), the amount of amino acids present in the yeast decreases during autolysis and increases in the medium. However, changes in the amounts of individual amino acids do not seem to show that autolysis preferentially favors the rupture of certain peptide bonds or acts specifically on certain cell wall proteins (CHARPENTIER *et al.*, 1986). Table I summarizes some of the results obtained in various conditions concerning the release of nitrogenous substances.

TABLEAU I

Exemples non exhaustifs de l'évolution des acides aminés libres libérés par les lies au cours de différentes conditions d'autolyse.

Examples of amino-acids release from yeast according to different autolysis conditions

Autolysis conditions						[free amino acids released (mg l^{-1})] Main amino acids relased during autolysis (mg l^{-1})	References
Autolysis medium	Yeast strain	Biomass	Temperature	Duration	Stirring or «batonnage»		
Synthetic medium	laboratory X2180a	6,4 g l^{-1}	45°C	10 days	continuous (100 rpm)	[750] Glu (94,1) Phe (89,6) Leu (77,5) Ala (58,2) Arg (54,4)	HERNAWAN et FLEET, 1995
Koshu wine	laboratory Y378	n.d. end of alcoholic fermentation	15°C	4 months	without	[107] Pro (60) Leu (5,5) His (5,5) Lys (4,7) Glu (4,6) Thr (3,8)	ARIZUMI *et al.*, 1994
	enological Lalvin 71B (Lallemand)					[140] Pro (87) Leu (6,2) Phe (6,2) His (5,5) Glu (5,4) Thr (5,0)	
	œnologique 8130 (Lallemand)					[144] Pro (80) Leu (7,0) Glu (6,3) Lys (5,8) Phe (5,4) His (5,4)	
Chardonnay wine	enological n.p.*	n.d. end of alcoholic fermentation	n.p., de cave	5 months	once a week	[40] Ala (10,0) Arg (4,0) Gly (0,35) His (0,4)	STUCKEY *et al.*, 1991
					without	[60] Ala (20,0) Arg (4,8) Gly (0,25) His (0,25)	
Chardonnay wine «champenoise» method	enological n.p . (*Saccharomyces cerevisiae*)	n.d. from second fermentation	n.p., de cave	18 months	without	[80] Ala (12,6) Pro (9,1) Ile (8,1) Orn (6,9) Trp (4,3) Lys (2,5) Phe (2,0) Thr (1,1) Gln (0,6)	MORENO-ARRIBAS *et al.*, 1998
Champagne wine	enological n.p . (*Saccharomyces cerevisiae*)	n.d. from second fermentation	n.p., de cave	4 years	without	[122] Glu (80) Phe (60) Leu (45) His (40) Thr (10) Gly (10) Met (7) Ser (5)	FEUILLAT et CHARPENTIER, 1982
Bourgogne white wine	enological n.p . (*Saccharomyces cerevisiae*)	n.d. end of alcoholic fermentation	n.p., de cave	6 months	once a week	[83,4] Lys (26,0) Arg (18,3) His (11,5) Asp (11,4) Leu (10,3) Phe (5,9) Glu (1,6)	FERRARI et FEUILLAT, 1988
					without	[84,7] Leu (13,1) Ala (12,7) Glu (12,5) Lys (10,7) Ser (9,8) His (7,8) Arg (6,8)	

* n.p. : non précisé.

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The distribution of free amino acids is different to that in peptides and proteins. The overall nitrogen content of wines increases before the increase in amino nitrogen. This confirms that during autolysis, the peptides and/or proteins are released first and partially degraded into amino acids, the distribution of which becomes more and more similar during the aging process (MORENO-ARRIBAS *et al.*, 1998). During aging, proteins are broken down into smaller and smaller compounds and the total amount of protein decreases (LUGUERA *et al.*, 1997). The nature of the amino acids released depends predominantly on the peptide and protein compositions of the wine, and the time for which the wine is in contact with the yeasts (MORENO-ARRIBAS *et al.*, 1998).

Thus, there are always more peptides in champagne and sparkling wines than in the corresponding musts, because these peptides are derived from the yeast (CARNEVILLIER, 1999; MORENO-ARRIBAS *et al*., 1996). Once the second fermentation has finished the amount of peptides increases, reaching a maximum after 12 to 15 months, and decreasing thereafter (MORENO-ARRIBAS *et al*., 1996). This is consistent with the slow degradation of the peptides over time. The ratio of hydrophobic to hydrophilic peptides increases during the aging process. The hydrophobicity of the released peptides may affect the quality of bubbles, thus the observed improvement in this quality with age may be related to the increase in the amount of these peptides.

b) Polysaccharides

The polysaccharides released following the action of β-(1→3) glucanases are also major components released during autolysis. Numerous analytical methods have been used to quantify polysaccharides, giving results differing by a factor of 20 (between 100 and 2230 mg/l (FEUILLAT *et al.*, 1989; LLAUBERES and DUBOURDIEU, 1987). Very little glucose or reducing sugar is found in autolysates, confirming that the molecules released are polysaccharides (HERNAWAN and FLEET, 1995). FEUILLAT *et al.* (1989) showed that much more glucose than mannose was released; the mannans in the cell wall are released without hydrolysis whereas the glucans are hydrolyzed to give short chains or monomers. The action of β-mannosidase on the cell wall, which was characterized by FLEET and MANNERS (1977) is minimal (FREYSSINET *et al.*, 1989).

LLAUBERES and DUBOURDIEU (1987) showed that mannoproteins are the major components of the extracellular polysaccharides in yeasts, although they vary in quantity according to strain, fermentation temperature and the contact time for the lees and medium: the quantity of mannoproteins released is even higher if «sur lies» white wines are stirred. CHARPENTIER and FEUILLAT (1992) found that the mannoproteins and peptidomannan released during autolysis have a significant effect on the sensory qualities of wines, particularly the size and persistence of the bubbles, which are very important in sparkling wines. Furthermore, the release of mannoproteins has been shown to have a positive effect on the stability of wines. This will be discussed later. Finally, a maximal release of neutral polysaccharides by the yeast during autolysis in Chardonnay wines has been shown to result in good quality bubbles (ANDRÉS-LACUEVA *et al.*, 1997).

c) RNAs

The release of ribonucleic acid (RNA) into the aging medium is another reaction characteristic of yeast autolysis. The release of these compounds, which are known to function as flavor enhancers in food products, is even more important in non-sparkling «sur lies» wines, which contain more lees than sparkling wines made by the Champagne method (COURTIS *et al.,* 1998).

LEROY *et al.* (1990) noted a very good correlation between the RNase activity of cells and the release of nucleic acids in champagne. RNase activity, which occurs during fermentation in bottles, stops after two years of aging. Between 400 and 480 mg/l of nucleic acids are released over the two years and the amount of nucleic acids with respect to that in the initial biomass at the beginning of the aging process decreases by 50 p. cent. If autolysis is carried out in a phosphate buffer at 45°C and pH 4.5, more than 85 p. cent of the RNA present in the yeast disappears (6 to 8 p. cent of the dry weight in *S. cerevisiae*) (HERNAWAN and FLEET, 1995). Only some (15 p. cent) of the products of RNA degradation (probably nucleotides, nucleosides, purines and pyrimidines) can be recovered in the autolysates (HERNAWAN and FLEET, 1995; HOUGH and MADDOX, 1970). Finally, in model media simulating non-sparkling « sur lies » wines, preliminary experiments have shown that in 80 days, the amount of nucleic acids present in the wine increases by 160 p. cent, from 130 to 340 mg/l. After this period, very little transfer occurs (4p. cent during the next 50 days) (COURTIS *et al*., 1998).

d) Lipids and sterols

(1) Lipid content of a yeast

In fermentation conditions, the lipid fraction, which is mainly associated with the cell membranes, is essentially present in the form of triglycerides constituing reserve lipids, and in the form of membrane phospho-

glycerides and sterols. The lipids from *S. cerevisiae* constitute a class of minor compounds, accounting for 37 to 147 mg/g dry matter (between 3 and 15 p. cent) (RATTRAY, 1988). The amount of sterols is also very low, accounting for only 0.03 to 4.3 p. cent of the dry weight of the yeast (GIOVANELLI *et al*., 1996). Compared to other yeasts, *S. cerevisiae* is particularly rich in sterols.

Triglycerides and phospholipids are formed by a large range of fatty acids. The most common are palmitoleic acid (C16:1) and its esters and oleic acid (C18:1). However, the amount of fatty acids depends on the availability of molecular oxygen. Again, it is difficult to give exact values because the culture conditions (growth temperature, type of culture medium, etc.) affect the amount and quality of lipids in the yeast (SLAUGHTER and MINABE, 1994).

(2) Autolysis and the lipid content of wines

It is generally accepted that wines naturally contain small amounts of lipids, due to clarification processes, which cause lipid depletion. In enology, the autolysis that occurs during aging on lees leads to an increase in the lipid content of wines. However, the transfer of lipids from yeast into wine is less marked than that of other compounds released by the yeast during autolysis.

The phenomenon of lipid release depends on the conditions during autolysis and it is difficult to generalize all of the data on the subject. According to BABAYAN and BEZRUKOV (1985), the lipids tend to aggregate and to rest within the cell if the pH is between 5 and 6: only 3 p. cent of the initial lipids are released from the cell. During the *in vitro* production of autolysates, FERRARI *et al*. (1987) showed that few lipids were released into the supernatant and that most of the lipids remained in the cell envelopes in acid conditions (pH 3.5). Conversely, the study by CHEN *et al.* (1980) of the release of fatty acids by brewers' yeasts, showed that free fatty acids are good markers of yeast autolysis. These authors induced autolysis by various means, such as heating, changing the pH and adding alcohols. The most commonly obtained fatty acids were short-chain fatty acids, such as decanoic acid (C10:0) and octanoic acid (C8:0), which are the main components of what is known as the «caprylic» odour (also called «yeast» or «fat» odour). Nevertheless, in typical brewing conditions, autolysis cannot occur during the time in which the beer is in contact with the yeast and is indeed not desirable, apart from in certain speciality beers. Only changes in storage temperature or prolonged contact between the beer and the yeast may trigger autolysis.

The lipids released during autolysis cannot be ignored because they may be involved in the formation of certain volatile components, such as esters, ketones and aldehydes (CHARPENTIER and FEUILLAT, 1992; PUEYO *et al*., 2000). Information concerning the lipids released is of greatest importance in the manufacture of sparkling wines (champagne). Most groups have essentially looked at the effect of lipids on the amount of bubbles, one of the major sensory attributes of sparkling wines. In fact, PUEYO *et al.* (1995) reported a positive relationship between bubble stability and the presence of linolenic acid (C18:3). They also found that the presence of palmitic acid (C16:0) has a beneficial effect on the size of the bubbles. However, brewing studies have shown that lipids have a negative effect on bubbles in beers. DUSSAUD *et al.* (1994) have suggested that these differences are due to the different alcohol contents of these two types of fermented drink.

The most common free fatty acids in «sur lies» wines are palmitic acid (C16:0), stearic acid (C18:0) and palmitoleic acid (C16:1) (FERRARI *et al*., 1987). FERRARI *et al.* showed that yeasts contain considerably more saturated fatty acids than unsaturated fatty acids (only 13 p. cent of total fatty acids) if kept in contact with wine for over eight months. Conversely, unsaturated fatty acids accounted for over 60 p. cent of total fatty acids in dried activated yeasts (FERRARI *et al.*, 1987).

Similarly, the addition of yeast envelopes or fresh lees generally leads to a transient increase in the amount of free long-chain fatty acids in the wine, followed by a long-lasting decrease. Indeed, after a certain time, the free fatty acids are adsorbed by the lees or the yeast envelopes (ANCIN *et al*., 1998; FERRARI and FEUILLAT, 1988; FERRARI *et al*., 1987; SOUFLEROS and BERTRAND, 1988), although no mechanism has been put forward to account for this observation. HERRAIZ *et al.* (1990) suggested that the yeasts may have residual enzyme activity.

In sparkling wines, the amount of polar lipids decreases drastically and the amount of neutral lipids increases in parallel (table II).

The neutral monoglycerides and diglycerides seem to be converted into triglycerides. These neutral lipids are predominant, accounting for more than 90 p. cent of all the lipids determined. However, although SLAUGHTER *et al.* (1994) reported an increase in the amount of triglycerides, they did not observe an increase in the amounts of mono- and diglycerides. They suggested that the fatty acids and acyl-glycerols were rapidly re-esterified. They also observed the appea-

rance of waxes in the culture medium when the cells were no longer viable.

More recently, the amount of lipids released by three strains of yeasts in a model system was monitored for 12 days during autolysis at 30°C with constant stirring (PUEYO *et al*., 2000). Triglycerides, 1-3 diglycerides, 2-monoglycerides and free fatty acids were released from day 2 onwards, a period corresponding to the maximum loss of viability and dry matter in yeast. The general decrease in the amount of lipids in the medium can be accounted for by the action of hydrolytic enzymes, which are released into the medium during modification of the cell wall. Between the 8th and 10th days, further lipids are released, and cell viability increases again. No phospholipids or 1-monoglycerides were found in the corresponding autolysates, even though they are integral parts of the constitutive yeast lipids. Phospholipid degradation was initially noted by HERNAWAN and FLEET (1995) and by SLAUGHTER and MINABE (1994), who found no traces of phosholipids in the supernatant. This degradation is described as the most significant change in lipids during the autolysis of yeasts, especially in baking (WATANABE *et al.*, 1983).

It has also been shown that at the end of advanced autolysis, the amounts of di-glycerides decrease due to enzyme action (probably lipases). These compounds are subsequently found in the form of free fatty acids (palmitoleic, palmitic, stearic and oleic acids, and glycerol (HERNAWAN and FLEET, 1995).

TROTON *et al.* (1989) attempted to identify the mechanisms underlying changes in the amounts of lipids during the second alcoholic fermentation. However, it was PITON *et al.* (1988) who studied the changes in lipid content of champagne yeasts during aging on the lees in bottles. Microscopy identified numerous heterogeneous vesicles in yeasts, the smallest of which seemed to be of lipid origin. After the second fermentation had been completed, microscopy suggested that the yeast had undergone plasmolysis. However, the interior of the yeasts seemed to show that certain membranes had been degraded and that neutral lipids had accumulated in the growing lipid vesicles. After the dysfunction of all of the yeast structures, only a very thin wall (0.1 µm thick), a double outer membrane and the lipid vesicles remain after 15 years of aging (PITON *et al.*, 1988).

Sterol analysis in a model medium has also been used to estimate the amount of sterols released during autolysis (LE FUR *et al.*, 1999). Sterols are known to affect protein/phospholipid interactions, and therefore also the fluidity of the membrane and the activities of membrane enzymes. In this study, ten sterols were separated and identified as being either esterified or non-esterified during the accelerated autolysis of a yeast in a model acidic medium. Autolysis seems to induce a reduction in the amount of esterified sterols in yeast, particularly those in the form of the first intermediates resulting from the synthesis of ergosterol, whereas the amounts of non-esterified forms do not change. Sterol contents changed from 0.92 to 0.43 p. cent (with respect to the dry weight of the yeast). Conversely, the release of sterols into the medium was negligible, because it only accounted for 0.015 p. cent of the total amount of sterols in the biomass.

The study by PUEYO *et al.* (2000) also showed that sterol esters and sterols were released from the second day of autolysis onwards. Fornairon-Bonnefond (2000) used yeast that had been used for fermentation before the onset of autolysis as a model for studying the differences between aging in the presence and absence of oxygen. They found that the only difference between the two processes was the amount of sterols, ergosterol in particular, the concentration of which decreased by over 80 p. cent during aging in the presence of oxygen.

e) Vitamins

Only CHEN *et al.* (1980) have found that certain vitamins, such as thiamine (vitamin B1), niacin (nicotinic acid) and biotin are produced during autolysis in brewing.

II – THE PHYSICOCHEMICAL PROPERTIES OF LEES

TABLEAU II

Distribution (expressed in lipid percent) of different lipids (sterols excluded) of *S. cerevisiae* during champagne wine ageing.

Distribution (exprimée en p.cent des lipides) des différentes classes de lipides (stérols exclus) chez *S. cerevisiae* pendant le vieillissement du Champagne (PITON *et al.*, 1988).

Time	Triglycerides	Diglyceides	Monoglycerides	Free fatty acids	Polar lipids
0	8.7	3.1	0.5	23.9	63.7
6 weeks	28.6	33.9	5.7	4.3	27.5
19 years	53.0	17.8	0.3	19.0	10.0

1) Redox phenomena associated with the presence of lees

One of the main properties of lees is their strong reductive power. Thus, certain wine-growers think twice about aging on lees. Indeed, if a wine is stored on total biomass, unpleasant smells soon appear. These smells are inevitable during the first month of aging on lees. As a result the wine must be clarified rapidly to prevent these « reduced » odours from becoming worse. Only aging in barrels, which allows only minimal contact with oxygen, makes it possible to store wines on total lees for prolonged periods (CHATONNET, 1991), provided that the must is carefully clarified and the wine treated with sulfite appropriately. Recent studies have shown that yeast lees can consume significant amounts of oxygen after alcoholic fermentation, for periods of aging of up to three and a half years at 14°C (FORNAIRON *et al*., 1999; SALMON *et al*., 2000). The ability of lees to consume oxygen is not uniquely due to residual cell viability. It decreases during the « sur lies » aging process. After the decrease in the viability of the yeast, oxygen is still consumed, probably for chemical reasons. The maximum amount of oxygen consumed by lees has been estimated to be 4 mg/l under specific operating conditions (specific strain, medium and temperature) simulating the average composition of a wine containing about 4 p. cent lees. These results show that the consumption of oxygen by the lees is probably due to preferential attack of the lipids in the cell membranes by oxygen (SALMON *et al.*, 2000), which is known to alter the structure of cell membranes. The ability of lees to consume oxygen seems to be related to the composition of cells in terms of certain sterol fractions in particular (FORNAIRON-BONNEFOND, 2000). However, this interaction between the lees and oxygen does not seem to affect the autolysis of the lees (FORNAIRON-BONNEFOND, 2000). This potential interaction between oxygen and the lees has never been taken into account, either in terms of its effect on the integrity of the lees or on possible production of final products that may affect the final quality of the wines aged.

2) Interactions between lees and polyphenols

a) Removal of unwanted color from musts by the lees

VASSEROT *et al.* (1997) showed that yeasts can adsorb anthocyanins. This adsorption depends on the structure of the anthocyanins, the presence of â-glucosidase activity in the yeast and certain environmental factors, such as the ethanol concentration, temperature, pH and the concentration of SO_2 in the wine. Thus, the lees can fix anthocyanin molecules resulting from too strong an extraction during the pressing of red grapes with white juice. This phenomenon leads to removal of unwanted color from wines, as observed by VASSEROT and MAUJEAN (1998) during experiments on « stained » champagne Pinot noir musts. As a result, wine lees can be used as an alternative for the widely used organic carbons, which may alter the organoleptic content and sparkling and bubbling properties of white wines. Thus, despite the fact that they are less efficient and more costly than organic carbons they can be used in smaller amounts to remove unwanted color from slightly or moderately « stained» wines.

b) Reactivity with polyphenol compounds in the wine

Various studies have recently tried to determine the precise nature of the potential interactions between yeast lees and polyphenols during the aging of wines on lees. Firstly, VIVAS and SAINT-CRICQ de GAULEJAC (2000) found that the oxidation potential of red wines aged on lees was lower than that of a control wine, and that the components released into the wine during autolysis considerably slowed the oxidation of several purified phenolic compounds. According to FEUILLAT *et al.* (2001), who studied a number of wines aged on lees, aging does not seem to have a major effect on the anthocyanin content of aged wines. Conversely, after stirring, the total anthocyanins fraction seemed to increase considerably. The same study also showed that the amount of colloidal tannins also seems to increase in stirred wines, indicating that the tannins are less active against proteins. In a model medium, yeast lees were found to be highly reactive with wine polyphenols if the yeast had been cultured in the absence of polyphenols (SALMON *et al.*, 2001). This type of interaction leads to a strong decrease in the amount of free polyphenols in the wine, similarly to that observed for free anthocyanins in musts (VASSEROT *et al*., 1997). However, the reactivity of the lees and the wine polyphenols towards oxygen seems to depend on the duration of the interaction: the fraction of free polyphenols in the wine seems to become more reactive with oxygen, whereas the lees that adsorb wine polyphenols are only slightly reactive with oxygen (SALMON *et al.*, 2001).

3) Oxidative pinking

The presence of lees during the aging of white wines also helps to prevent the oxidative pinking of wines (RIBÉREAU-GAYON *et al*., 1998b). Oxidative pinking, which turns certain white wines a pink-gray color as a result of slight oxidation, has been long described in the literature (SIMPSON, 1977). DUBOURDIEU (1995) confirmed that the lees can adsorb the precur-

sors of the molecules responsible for pinking, unlike other inefficient treatments (binding to casein, use of polyvinylpolypyrrolidone or use of SO_2), and they also reduce the likelihood of pinking.

4) Adsorption of thiols on the walls of yeast lees

Wine lees can completely eliminate certain volatile thiols from wines (LAVIGNE and DUBOURDIEU, 1996). Experiments carried out with fresh lees or the walls of yeasts in the presence of model solutions containing thiols (methane thiol and ethane thiol) showed that yeast lees can completely eliminate these two compounds. LAVIGNE and DUBOURDIEU (1996) suggested that disulfide bridges are created between the constitutive cysteine of mannoproteins (localized in the external layer of the yeast wall) and the SH groups of volatile thiols, enabling the yeast to fix the thiols.

5) Role of the lees in tartaric and protein stability

After being in contact with lees, wines are more stable with respect to tartaric precipitations and cloudiness due to proteins. Indeed, the stability of proteins in white wines systematically improves if they are aged on lees in barrels. When new wines are kept on lees, they are less likely to turn cloudy due to heat (MOINE-LEDOUX and DUBOURDIEU, 1988). Similarly, only small amounts of bentonite (protein precipitation stabilizer) are required to maintain the stability of proteins. However, the grape proteins responsible for this effect in white wines (six main protein fractions) are not digested or adsorbed by yeast lees during aging. They become thermostable in the presence of certain colloids derived from yeast walls (LEDOUX *et al.*, 1992). The stability of the proteins in wines aged on lees is due to the presence of a seventh protein fraction, which only appears during aging on lees. This fraction contains a 32 kDa mannoprotein, MP32, which is a fragment of the wall invertase of S. cerevisiae. This protein has been characterized and clearly described as thermostable and a thermostabilizer of proteins in wines aged on lees. Following the autolysis of yeasts during aging, this protein fragment is released from the yeast wall by the joint action of vacuolar glucanases and proteases. This mannoprotein has been obtained in vitro from the walls of yeast digested enzymatically with glucanases (Glucanex™ preparation) and may soon be an integral part of a new product to replace bentonite, at least in part (DUBOURDIEU and MOINE-LEDOUX, 1996).

Similarly, other highly glycosylated mannoproteins of about 40 kDa, obtained by the digestion of yeast walls, inhibit the tartaric crystallization of white, red and rosé wines (MOINE-LEDOUX *et al.*, 1997; RIBÉREAU-GAYON *et al.*, 1998b). Based on these data, a product has been registered under the name of Mannostab™ for the prevention of tartaric precipitation in wines. However, its use remains experimental.

Recently, DUPIN *et al.* (2000a and b) proposed a mechanism of action for the *S. cerevisiae* mannoproteins, which are protective following the breakdown of proteins in wines. They suggest that this phenomenon is due to competition between the protective mannoproteins and the wine proteins for an unknown compound present in wine, which is necessary for the formation of insoluble aggregates.

III – ORGANOLEPTIC VALUE OF «SUR LIES» WINE AGING

The autolysis of yeasts during aging on lees (a slow and expensive process in enology) is essentially sought because it improves the aromatic balance of wine. It favors the release of certain components (lipids, nitrogenous substances, ribonucleic acids, vitamins and polysaccharides), some of which can be considered to be precursors of aromas, which may therefore affect the final bouquet of wines aged on lees.

In wine-making language, the lees associated with wines are commonly reputed to add «body» and «roundness» to the bouquet of wines, and potentially to confer better keeping qualities. Although these enological terms are a bit enigmatic and subjective, certain studies described below have described and analyzed these taste variations. In general, yeast macromolecules affect aromatic quality when released into the wine. For example, if clarification is too harsh (30 p. cent reduction in the amount of macromolecules) it can cause enological defects, such as a decrease in the persistence of the aroma. Conversely, wines left in contact with the lees for several months contain more yeast macromolecules, which have a positive effect of the sensory qualities of wines (FEUILLAT, 1997). This phenomenon has been attributed to the existence of interactions between yeast macromolecules and the aromatic compounds present in wines. However, the many experiments carried out with isolated and purified polysaccharides from wine (mannoproteins derived from fermentation and autolysis) have shown no significant effect on the sensory qualities of the wine (WILL *et al.*, 1991; SCHOBINGER *et al.*, 1992; SAMSON, personal communication).

In a model medium, the interactions between volatile compounds and wine macromolecules depend on the hydrophobic nature of the aromatic substance and the protein part of the macromolecule (LUBBERS *et al.*, 1994). These macromolecules do not interfere in a unilateral manner because they can increase or decrease the relative volatility of the aromatic sub-

stances, these two phenomena occurring to about the same extent (FEUILLAT *et al.*, 1998). For example, the most non-polar molecules are the most frequently fixed by macromolecules.

CHUNG (1986) used another model medium to monitor changes in the amounts of volatile compounds released by the yeast during simulated autolysis. Most of the volatile compounds were present at higher concentrations if storage time was longer. However, an increase in initial biomass does not systematically lead to a proportional increase in the amounts of volatile compounds.

MOLNAR *et al.* (1981) studied changes in the composition of aromatic substances in sparkling wines, using whole and disintegrated yeasts. Temperature was found to affect the concentrations of certain components, such as those with a high boiling point (certain ethyl esters and fatty acids). Furthermore, the concentrations of volatile compounds may be two to five times higher for sparkling wines aged in the presence of ground yeast than for wines aged with whole yeasts. However, not all of the volatile compounds released favor the organoleptic properties of sparkling wines.

According to ZOECKLEIN *et al.* (1997), the aging of Riesling white wines on lees (10 °C for 45 days) reduces the amounts of non-volatile aromatic precursors, glycosylated compounds, by 52 p. cent with respect to wines that are not aged. These glycosylated components are degraded by glycosidases, which are present in the periplasmic spaces of the yeast and are released into the medium during autolysis.

CHATONNET's (1991) experiments in barrels with real medium showed that the intensity and apparent rate of diffusion of aromatic substances diffusing out of the wooden vats depended on whether the wines were kept on fine lees or total lees. The biomass probably has two roles: (1) the polysaccharide yeast walls can refix certain aromatic components (volatile phenols, β-methyl-β-octalactones, etc.) and (2) certain yeast enzyme systems are still active and can limit the intensity of the «woody» trait by transforming certain molecules, such as vanillin and furanic aldehydes into less odorant or less «woody» products.

Finally, autolysis of yeast lees may favor the growth of *Brettanomyces* species, by the low amount of glucose liberated during cell wall autolysis. (< 150 mg/l). Such yeast contaminations are frequently associated to the production of volatile phenols (4-ethyl phenol et 4-ethyl guaiacol) (GUILLOUX-BENATIER *et al*, 2001).

A number of authors have expressed different opinions about whether wines aged on lees were better than the control:

- STUCKEY *et al.* (1991) stated that the addition of lees to a Chardonnay wine added a «new dimension». With stirring, the wine was also better balanced in terms of fruity, woody and yeasty odors. The author seemed to find a relationship between positive properties of these wines and the release of amino acids into the medium after 5 months of aging. We know that amino acids can also undergo chemical reactions, leading to the formation of certain aromatic substances (FEUILLAT and CHARPENTIER, 1982). This is the case for:

- The deamination of threonine into β-ketobutyric acid, which is in turn transformed into 3-hydroxy-4,5-dimethyl-2(5H)-furanone, which is also known as sotolon. This lactone smells of green walnuts.

- The formation of vitispirane, a typical component of old wines with floral aromas, which is formed from methionine.

- FERRARI and FEUILLAT (1988) showed that Burgundy wines clarified at the end of alcoholic fermentation are preferable to those aged on lees, but the latter are still better at the end of the aging period. However, with champagnes to which supernatants or autolysate products were added, the wines obtained receive good marks for the intensity and perfume of their aroma, and for the typicality of their aroma. Conversely, after two years of storage, the control was preferred. Therefore, the absence of autolysates seems to be beneficial after a year, but a disadvantage thereafter (CHUNG, 1986).

- The method of aging wines on lees is used virtually systematically when making Koshu wine in Japan, to counter the initial flatness of the grapes. Analyses of the sensory qualities of wines, evaluating the optimum period of contact between the wine and the lees, showed that the best wines were those that remained on lees for 4 months when stored in vats, and five to six months when stored in bottles (ARIZUMI *et al.*, 1994).

- Finally, LLAUBERES (1987) showed that stirring the lees increases the amount of polysaccharides released into the medium, but that this difference could not be detected upon tasting. Conversely, the wines aged on lees were systematically preferred to control wines. However, the direct organoleptic effect of the polysaccharides on the roundness, on the «ease of drinking» and on the mellowness of white wines aged on

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lees has never been shown conclusively (RIBÉREAU-GAYON *et al.*, 1998b).

TECHNOLOGICAL CONSEQUENCES OF THE PRESENCE OF LEES

The process of aging on lees has been studied in detail in terms of the macromolecular nitrogenous constituents, polysaccharides and lipids released during the autolysis of yeasts. CHATONNET (1991) extended these studies by associating complementary notions, such as the effect of the aging recipients. In fact, from a technological point of view, the type of storage recipient, as well as the nature of the wine (red or white), has a direct effect on the oxidation of the wine and therefore on the way in which it ages.

I – AGING WINES IN WOODEN CASKS

The aging of wines in oak barrels is an ancient practice that both refines the wine and confers typical characteristics. The typicality of wines is partly due to compounds entering the wine from the wood (GLORIES, 1987). This mode of storage also allows oxidation, which the author described as mild and permanent, or «managed», of the wine over time.

1) White wines, wood, lees and oxygen

The unique feature of the traditional Burgundy method for aging white wines in barrels is that the barrel is the site of both alcoholic fermentation and aging. Aging is always carried out on lees, without clarification. This initiates the exchange between the original wine constituents, those of the yeast and those of the oak barrel (RIBÉREAU-GAYON *et al.*, 1998b). These mechanisms are favored by the regular resuspension of the lees (stirring). Contact with the oxygen in the air generally reduces the quality of white wines. Thus, ullaging is carried out to reduce the surface exchange between the air and the contents of the barrel as much as possible. This process involves injecting neutral gas to eliminate the oxygen (DELTEIL *et al.*, 1998), or adding SO_2 as an anti-oxidant. These practices prevent the development of exogenous microorganisms in the wines once alcoholic fermentation has finished. Indeed, the development of oxidative «velum forming» or «flor» yeasts or acetic bacteria is not desirable during classical wine making, due to their negative and contaminating effects on the quality of biological aging (CHARPENTIER, 1990).

Wood is a porous material, through which a certain amount of oxygen can diffuse depending on the age of the barrel. CHATONNET (1991) measured the redox potential of this type of barrel and showed that it favors oxidation, whereas wine in stainless steel vats tends to remain more highly reduced (RIBÉREAU-GAYON *et al.*, 1998b). The potential within the barrel decreases with increasing distance from the surface of the wine and decreasing distance to the lees (CHATONNET, 1991). Stirring re-equilibrates the redox potential of the barrel by dissolving oxygen. Thus, the reduction of the lees, like the oxidation of the wine at the surface of the barrel, is prevented.

However, CHATONNET suggested that the successful aging in the presence of total biomass without the development of sulfurous odors cannot be accounted for solely by the diffusion of oxygen in the barrel. A catalyst seems to be necessary to make these revolting volatile sulfur smells disappear totally or to prevent them from forming in the first place. However, although oak is rich in ellagitanins, wines aged in barrels only contain small amounts of these compounds, by comparison with the phenolic compound content of the wine (MOUTOUNET *et al.*, 1989; VIVAS *et al.*, 1996). These low concentrations of ellagitanins are essentially due to the thermosensitivity of these molecules during the processes of cintrage and during the heating of the barrels (MOUTOUNET *et al.*, 1992).

The wood retrocedes aromatic substances into the wine, which react with the lees and with the constituents of the wine. The compounds present in the wood that become soluble during aging, can be divided into two classes: substances retroceded into the wine without being transformed, such as whisky-lactones (notably α-methyl-octalactone) and substances from the wood that are transformed during alcoholic and malolactic fermentations, such as volatile phenols (FEUILLAT, 1992). The presence of the lees has been shown to decrease the accumulation of polyphenols from the wood in the wine (CHATONNET, 1991). The mannoproteins from yeast walls that are released into the medium seem to be able to combine and to fix polyphenols (DUBOURDIEU, 1992). Thus, the total amount of polyphenols and the yellow color of the wine are reduced during «sur lies» aging in barrels (CHATONNET *et al.*, 1992).

2) «Sur lies» aging of red wines in large barrels

The Burgundy white wines undergo alcoholic fermentation and are aged in large barrels in the presence of their lees. Conversely, red wines are not kept in contact with wood after their alcoholic fermentation has ended and are generally not aged on lees. However, in recent years, it has become more common to age red wines on lees, and even in barrels. The practices used are still experimental. The wines are generally crudely separated from their lees once fermentation is complete. The wine and the lees are then put into barrels and classical aging is carried out, involving the resus-

pension of the lees by regular stirring, as in the aging of white wines on lees (BIOTEAU, 1998).

II – AGING WINES IN VATS

Not all «sur lies» wines are aged in barrels. Barrels are onerous to maintain and their regular replacement or maintenance to maintain a certain level of quality are not always assured. Wines can also be aged in vats, as long as certain precautions, related to the nature of the contents, are taken.

1) Aging of white wines

Due to the large volumes involved, it is a lot more difficult to age white wines in vats than in barrels (RIBÉREAU-GAYON *et al.*, 1998b). In fact, problems related to reduction are more difficult to handle: the reducing nature of the lees is not counterbalanced by the presence of oxygen diffusing through the recipient and there is no longer a redox equilibrium. If a wine presents no reduction problems, it is aged on fine lees, after early clarification (November - December) (CHATONNET, 1991). Otherwise, the wine is aged in the absence of lees (RIBÉREAU-GAYON *et al*., 1998b).

Often the reduced taste of white wines that have been treated with a sulfite in large vats are not eliminated by clarification with aeration. They reappear as soon as the lees settle. According to LAVIGNE *et al.* (1996), this is due to the pressure exerted on the lees by the top of the vat. The development of these reduced flavors is partly due to the sulfur-reductase activity of the yeast catalyzing the transformation of SO_2 into H_2S. To overcome this constraint, LAVIGNE *et al.* (1996) suggested that the wine be temporarily separated from the lees after the addition of sulfite. The lees are stored in large barrels so that they lose their reducing activity (for about one month). They are then added back to the wine. At this stage, they no longer generate sulfur-containing compounds and their addition leads to a dramatic decrease in the concentration of certain thiols, as discussed above (RIBÉREAU-GAYON *et al.*, 1998b).

2) Aging of red wines

Red wines are often aged in inert vats, generally without lees. However, lees can be included, and some cuvees are aged on lees. Oxygenation is assured by the various fortifying processes or by using a «micro-oxygenation» system, which adds the desired quantities of oxygen at regular intervals, such that it does not accumulate in the wines (MOUTOUNET and MAZAURIC, 2000).

The presence of lees during the aging of red wines limits the increase in redox potential observed when the wines are stored without lees (VIVAS and SAINT CRICQ de GAULEJAC, 2000). These authors suggested that the lees act as antioxidants, even in red wines.

CONCLUSIONS

The aging of wines on lees involves a process called autolysis. When considering yeast autolysis, it is important to remember the diversity of experimental conditions used, all of which are known as autolysis. As a result, it is necessary to add a relative dimension to the comparison of a certain number of values provided in the scientific literature. Indeed, the quality of the primary matter (the yeasts) and the medium (fermentation medium and autolysis medium) is not identical in all cases. Some authors have studied the effect of aging white wines on lees (yeasts that have only undergone one fermentation on grape musts), whereas others have looked at sparkling wines (yeasts undergoing their second fermentation process on wine and not on grape musts), and others have used dried activated yeasts that have been rehydrated and placed directly in a model medium (hydro-alcoholic medium, mimicking the composition of a wine).

Furthermore, the markers of autolysis studied are not always the same; some authors consider that autolysis only occurs after the passive release of amino acids into the medium, whereas others consider that an increase in the nitrogen content of the medium is synonymous with autolysis. Finally, other authors consider that autolysis may take a long time to occur (6 to 18 months before the beneficial effects appear in certain champagnes), whereas Burgundy-style white wines are often aged for a maximum of 9 months before being bottled to prevent the development of too strong a «woody» flavor. It is difficult to compare the effects of autolysis that have been «induced» by very high temperatures and the effects of autolysis in prestigious champagnes, which takes years to become apparent.

The wide variety of experimental models used to study the autolysis of yeasts during the aging of wines on lees makes it difficult to draw any categorical conclusions about the release of the various yeast components into the wine.

However, scientific studies of physicochemical properties are undeniably the least subject to interpretation errors, although only a few studies have been carried out in this domain. These studies clearly show the importance of the environment in which the wine is aged : the nature of the primary matter (polyphenol and anthocyanin contents) and the type of equipment

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and technology used (vats, barrels, oxygenation, etc.) have a major effect on the organoleptic quality of the wine in question.

Moreover, few scientific studies have adequately explained the beneficial organoleptic effects of aging the wine in the presence of lees. The number of molecules released during autolysis, and the strong interaction between these molecules, or even the lees, with compounds present in the wine, may account for this deficiency, resulting from a lack of clear research targets. Future studies on wine lees should probably focus on this issue.

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