153 Scholten, G., Woller, R., and Holbach, B., L'analyse automatisée des vins. Wein-Wiss. *37* (1982) 36-47.
154 Scholten, G., Woller, R., and Steinmetz, E., L'analyse automatisée des vins 2ème communication. Wein-Wiss. *38* (1983) 397-426.
155 Schreier, P., Flavor composition of wines: a review. Crit. Rev. Food Sci. Nutr. *12* (1979) 59–111.
156 Silva, A., Application de la spectrophotométrie d'absorption atomique à l'analyse de quelques composés minéraux des vins. Industria Bev. No. 10 (1980) 55-61.
157 Simon, H., and Rauschenbach, P., Différenciation de l'alcool de synthèse et de l'alcool de fermentation. Feuill. verts O.I.V. No. 541 (1975).
158 Soulis, T., Voulgaropoulos, A., and Kofidou, T., Teneurs de certains vins grecs du commerce en Pb, Cu, Zn, Ni et Co. Conn. Vigne Vin *18* (1984) 177–184.
159 Stackler, B., and Christensen, E.N., Quantitative determination of ethanol in wine by gas chromatography. Am. J. Enol. Vitic. *25* (1974) 202–207.
160 Stella, C., and Sabatelli, M.P., La statistica come complemento utile all'interpretazione dei resultati dell'analisi chimico-oenologica. Industria Bev. No. *1* (1980) 62–65.
161 Suarez, A., and Llaguno, C., Dosage du glycérol dans les vins et les vinaigres par voie enzymatique. Feuill. verts O.I.V. No. 582 (1975).
162 Sudraud, P., and Koziet, J., Recherche de nouveaux critères analytiques de charactérisation des vins. Annls Nutr. Aliment. *32* (1978) 1063–1072.
163 Symonds, P., Application de la chromatographie liquide haute performance au dosage de quelques acides organiques du vin. Annls Nutr. Aliment. *32* (1978) 957–968.
164 Symonds, P., and Cantagrel, E., Application de l'analyse discriminante à la différenciation des vins. Annls Falsif. Expert chim. *75* (1982) 63–74.
165 Tep, Y., Tep, A., and Brun, S., A propos du dosage du plomb dans les vins par colorimétrie du complexe plomb-dithizone. Trav. Soc. Pharm. Montpellier (1973) 65–72 and Feuill. verts O.I.V. No. 410 (1973).
166 Tep, Y., Brun, S., Leboeuf, J.P., and Bard, M., Détermination automatique du titre alcoométrique des vins secs. Annls Nutr. Aliment. *32* (1978) 899–905.
167 Tep, Y., Cabanis, M.T., and Raffy, J., Determinazione del titulo alcolometrico con bilancia idrostatica a correzione automatica dell'influenza della temperature. Industria Bev. *70* (1984) 84–86.
168 Tercero, C., and Sanchez, O., Proposition de modifications à apporter à la méthode du Recueil (A 21) pour le dosage du glycérol et du 2, 3-butanédiol. Feuill. verts O.I.V. No. 651 (1977).
169 Tricard, Ch., and Sudraud, P., Dosage semi-quantitatif de quelques amines biogènes dans les vins. Feuill. verts O.I.V. Nos 764 and 765 (1982).
170 Tricard, Ch., Dosage de l'histamine par spectrofluorimétrie. Feuill. verts O.I.V. No. 767 (1983).
171 Triquet-Pissard, R., Etude des polyols et acides fixes du vin par CPG. Thèse Docteur en Oenologie, Université de Bordeaux II, Bordeaux 1979.
172 Tusseau, D., and Bouniol, C., Détermination des glucides dans les moûts, vins et champagnes par HPLC (comparaison avec la méthode enzymatique). Feuill. verts O.I.V. No. 776 (1984).
173 Usseglio-Tomasset, L., Astegiano, V., and Matta, M., Il linalolo composto responsabile dell'aroma delle uve e dei vini aromatici. Industria Agrar. *4* (1966).
174 Usseglio-Tomasset, L., and Di Stefano, R., Osservazioni sui constituenti terpenici dell uve e dei vini aromatici. Vignevini *10* (1979) 33–38.
175 Van den Driessche, S., and Thys, L., Dosage enzymatique des acides citrique, lactique et malique, libres et liés dans les vins. Feuill. verts O.I.V. No. 755 (1982).
176 Vanlaethem-Meuré, N., and Gérard, P., Determination du cadmium et du plomb dans les vins. Feuill. verts O.I.V. No. 759 (1982).
177 Vialatte, C., Dosage du glycérol et du butanediol 2, 3 par chromatographie en phase gazeuse. Feuill. verts O.I.V. No. 588 (1976).
178 Webb, A.D., Application de la chromatographie en phase gazeuse au contrôle analytique des vins. Annls Technol. agric. *27* (1978) 411-419.
179 Williams, P.J., Strauss, C.R., and Wilson, B., Classification of the monoterpenoid composition of muscat grapes. Am. J. Enol. Vitic. *32* (1981) 230–235.
180 Woidich, H., and Phannhauser, W., Quantitative gaschromatographische Bestimmung von Aethanol in Weinen und Branntweinen. Mitt. Klosterneuburg, Sér. A No. 6 (1974) 431–436.
181 Wulf, L.W., and Nagel, C.W., High-pressure liquid chromatographic separation of anthocyanins of *Vitis vinifera*. Am. J. Enol. Vitic. *29* (1978) 42–49.
182 Zapysavigna, R., Brambatti, E., and Cerutti, G., Ricerca e determinazione delle ammine non volatili in vini, succhi, birra, aceto. Riv. Vitic. Enol. *27* (1974) 285.
183 Zee, J.A., Szogmy, I.M., Simard, R.E., and Tremblay, J., Elemental analysis of Canadian, European and American wines by photon-induced W ray fluoresence. Am. J. Enol. Vitic. *34* (1983) 152–156.

0014-4754/86/080893-12$1.50 + 0.20/0

Applied microbiology

by S. Lafon-Lafourcade

Institut d'Oenologie, Université de Bordeaux II, and INRA, 351 Cours de la Libération, F-33405 Talence Cedex (France)

Key words. Fermentation, alcoholic, yeast ghosts; fermentation, malolactic; spoilage; lactic acid bacteria; acetic acid bacteria.

1. Introduction

Wine fermentation may be seen as an ancient biotechnology, even older than that of bread-making. However, the quality of wines has not ceased to improve and diversify, attesting to the progress arising from an increasing knowledge of microbiology.

Traditionally the transformation of must into wine remains a 'spontaneous phenomenon'. Two principal fermentations participate successively in the production of wines. First, yeasts transform sugar into ethanol, then lactic acid bacteria transform malic acid into lactic acid; the malolactic fermentation is general for red wines but occasional for white. Acetic acid bacteria, which are responsible for the vinegary spoilage of wines, must be inhibited.

During the vinification the microbiological selection that occurs is dictated by the composition of the media[55, 56]. The high concentration of sugar and acid pH of the must affect the growth of yeasts[33]. In such conditions the yeasts follow a specific cycle of growth and metabolism[38]. Fermentation activity is progressively inhibited by the products of sugar metabolism. The fermentation may be modified by the presence and secretions of other microor-

ganisms that have developed on the grape and equally by the constraints of the technology used to produce a quality product. At present, a major microbiological problem is the premature cessation of the alcoholic fermentation before all of the must sugar is exhausted. Research has focussed on some factors that affect yeast survival, and indicates new solutions to this problem.

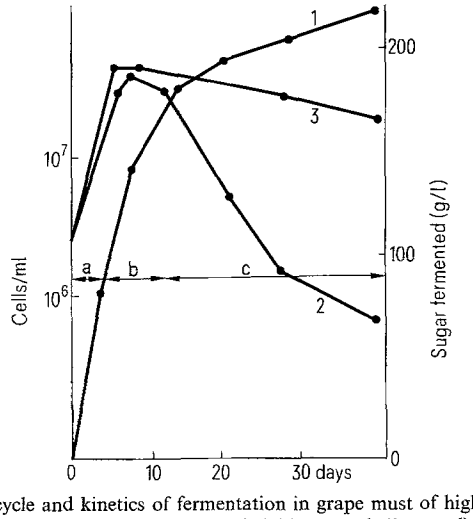
The malolactic fermentation is based on bacterial growth. But in wines all nutritional and physico-chemical factors are at the limit of tolerance of these microorganisms[55]. The metabolism of other substrates such as sugars, organic acids and glycerol leads to spoilage. Consequently, problems concerning the control of bacterial growth and the nature of the degradable substrate have not yet been solved completely. Research has focussed on some factors that facilitate the implantation of selected lactic acid bacteria in the wine.

2. *Alcoholic fermentation of grape must*

Yeasts, growth cycle and kinetics of fermentation

Musts are fermented 'naturally'. Fermentation is characterized by an initial rapid growth and death of *K. apiculata/Hanseniaspora uvarum*, followed by a predominant growth of *S. cerevisiae*[55]. It has been shown that this species is the principal agent that 'makes' the wine. The species *S. bayanus* is found mainly towards the end of the fermentation; it is the 'finishing' yeast. *S. rosei* produces only a small concentration of acetic acid during the fermentation. This property can be used to lower the volatile acidity of wines from grape musts too acid or too rich in sugar[33]. *Schizosaccharomyces* ferments high concentrations of malic acid to ethanol; this property can be used for the deacidification of acid musts[55]. *Saccharomycodes ludwigii, Brettanomyces vini, Zygosaccharomyces, Hansenula, Pichia* can predominate accidentally. They produce such substances as acetic acid, acetaldehyde, and ethyl acetate in excessive amounts. Their development leads to a lower organoleptic quality. It must be avoided.

The growth cycle of the yeast in the must is representative of the classical phases of microbial growth. However several variations may be noted[38] (fig.); 1) the duration is particularly long; 2) the total growth is limited; 3) the cessation of growth does not arise from the exhaustion of sugar in the must; 4) a disproportion exists between the principal phases: the decline phase can last 3 or 4 times longer than the phase of multiplication.



Growth cycle and kinetics of fermentation in grape must of high sugar concentration. *1* Fermentation curve; *2* viable yeasts (cells per ml); *3* total yeasts; *a* exponential phase; *b* quasistationary phase; *c* decline phase. (Lafon-Lafourcade et al.[38]; used here with the permission from the publishers).

The kinetics of fermentation are directly related to the growth cycle. For the fermentation to be rapid and complete (that is utilization of all sugar), it must take place entirely during the proliferation and stationary phases. This is generally realized in musts containing less than 200 g/l of sugar. For musts with more elevated levels of sugar, the final part of the fermentation is conducted by cells in the decline phase, where their metabolic activity has decreased. This can lead to a premature cessation of the fermentation.

Inhibition of the yeasts in the must

The spontaneous arrest of alcoholic grape must fermentation, before the complete transformation of sugar or before the wine contains an appropriate level of alcohol, remains one of the major problems of modern wine-making. This is commonly known as leading to 'stuck wine'[2]. Apart from the above technical problems, sticking may give rise to undesirable development of lactic acid bacteria and spoilage.

Certain technological constraints can cause such difficulties during the fermentation[33]:

- anaerobic conditions due to very large volumes of must in large fermenters[55, 62, 68];
- high fermentation temperature (above 35 °C);
- high concentration of sugars either naturally or after addition of saccharose[33, 64];
- high concentration of ethanol produced by fermentation of sugars[64, 55];
- insufficient inoculum, qualitatively or quantitatively;
- clarification of musts before fermentation for white wine production[25, 35, 57, 61];
- presence in the must of pesticide residues resulting from vineyard operations[23, 60];
- phenomena of microbial antagonism.

These phenomena consist of a) inhibition of yeasts by the substances secreted by other yeasts or by other microorganisms; b) competition between yeasts or yeasts and lactic acid bacteria.

One mechanism of antagonism between yeasts involves the production of killer toxin[5, 6, 46, 53]. Despite unfavorable factors related to high concentrations of sugars, low pH and the presence of polyphenols, a 'killer' effect has been shown in grape must[5]. Some yeasts of the genera *Saccharomyces, Debaryomyces, Torulopsis, Candida* and *Pichia* are able to produce killer toxins. The killer phenomenon permits an explanation of fermentation difficulties encountered with the inoculation of musts with certain active dried yeast preparations.

Yeasts occur on grapes with other fungi, lactic and acetic bacteria, in quantities which are more or less significant according to the degree of sanitation existing during vintage. The incidence of *Botrytis cinerea* has been the best studied. Its development on the grape berry during its maturation conveys to the berry the condition of 'noble rot' upon which the production of certain prestigious white wines exists (Sauternes)[58].

Experientia 42 (1986), Birkhäuser Verlag, CH-4010 Basel/Switzerland

When a fermentation is carried out with different mixtures of must from healthy grapes and grapes infected by *B. cinerea*, all taken to the same pH and sugar concentration, fermentation is slowest in the mixtures containing the highest proportion of botrytized grapes. When antifungal substances are precipitated by 80% ethanol, the precipitate exerts on the alcoholic fermentation an inhibitory action clearly stronger than that of the alcohol it contains[58]. The formation of acetic acid and glycerol (normal products of sugar fermentation) are strongly increased. The precipitate contains 5% of proteins, but is essentially composed of polysaccharides rich in mannose. On grapes that have undergone ordinary rot (pourriture vulgaire), microbial species other than *Botrytis cinerea* are found; these include *Aspergillus, Penicillium, Mucor, Alternaria* and the acetic acid bacteria *Acetobacter* and *Gluconobacter*. Their intervention has the effect of retarding the commencement of fermentation, diminishing the activity of fermentation and reducing the biomass formed[27].

The lactic acid bacteria are not generally found on grapes, and sometimes these bacteria are added to the must to stimulate the malolactic fermentation[22, 31, 69, 70]. When a large inoculation of bacteria is made into a weak alcoholic fermentation there may be a partial inhibition of the yeasts and a premature arrest of the fermentation, leaving residual sugar. Thus, in general wine-making practice, it is imperative to inoculate lactic acid bacteria only after all the must sugar has been utilized[34, 55].

However, given optimal nutritional and physicochemical conditions, wine fermentation comes to a natural end as a consequence of the yeast growth cycle. The final stages are characterized by a long declining phase, the length of which depends to some extent on the initial concentration of sugar[33]. In this case, the fermentative activity of the yeast cells decreases and finally stops. The inhibition here is caused by modifications in medium composition due to yeast metabolism; these modifications act on the cellular structure itself.

Identification of substances inhibiting yeast fermentation

It is well-known that ethanol accumulation is a major inhibitory factor. To investigate the role of ethanol in inhibiting yeast fermentation, viable cells of *S. cerevisiae* were inoculated (10^7 cells per ml) into the following complete nutritional media: 1) prefermented media 1, 2, and 3, which contained 1.7, 7, and 9.5% alcohol by volume and 160, 65, and 23 g of sugar per liter, respectively; fermentation was conducted by *S. cerevisiae*, and yeast samples were removed by centrifugation at the proliferation, stationary, and decline phases, respectively; 2) control, unfermented media 1, 2, and 3 that had been adjusted, by the addition of ethanol and sugar, to give alcohol and sugar concentrations similar to those of the prefermented media[20, 36].

Table 1. Yeast growth or survival in prefermented media and control media adjusted to similar alcohol and sugar concentrations

Medium[a]	Viable yeasts (10^6 cells per ml) at			
	Day 0	Day 1	Day 3	Day 6
Control 1	8	70	100	ND[b]
Prefermented 1	8	32	54	62
Control 2	10	100	120	120
Prefermented 2	10	21	25	25
Control 3	10	ND	14	18
Prefermented 3	10	5.2	1	0.047

[a] Media 1, 2 and 3 contained 1.7, 7, and 9.5% ethanol by volume and 160, 65, and 23 g of sugar per liter, respectively. [b] ND, not done.

Table 2. Effect of various organic acids and esters on growth or survival of yeast in alcohol-adjusted medium

Medium[a]	Viable yeasts (10^6 cells per ml) at				
	Day 0	Day 1	Day 3	Day 6	Day 9
Control 3	7	6	8	12	20
Prefermented 3	7	0.09	0.007	0.009	0.05
Prefermented 3 + charcoal (2 g/l)	7	1.6	3.6	6	8.3
Control 3 + ethyl octanoate (10 mg/l) + ethyl decanoate (10 mg/l)	7	1.5	1.5	4.2	5.5
Control 3 + octanoic acid (3 mg/l) + decanoic acid (3 mg/l)	7	0.35	0.05	0.03	0.13

[a] Media 3 contained 10.5% ethanol by volume and 25 g of sugar per liter.

The six media were incubated at 25 °C, and yeast counts were measured as a function of time for up to 6 days after incubation.

Table 1 shows a circa 12-fold increase in yeast levels in control media 1 and 2 after 6 days of incubation and a 2-fold increase of inoculated cell number in control medium 3. However, increases in the corresponding prefermented media 1 and 2 were much less, and, particularly, yeast cells in prefermented medium 3 showed a reduction in cell number from circa 10^7 to 10^4 cells per ml after day 6 of incubation.

Since ethanol concentrations in prefermented medium 3 and control medium 3 were the same, factors other than ethanol were responsible for the inhibition of yeast multiplication and the loss of yeast viability. Nutritional deficiencies were not responsible, since the addition of vitamins or other growth activators to the prefermented medium 3 did not prevent the above effect. It seemed, therefore, that the inhibitory phenomenon was due to the presence of toxic substances produced by yeast fermentation. Such substances were probably not proteins, since heating the prefermented media at 65 °C for 15 min or the addition of bentonite (700 mg/l) did not overcome the inhibition or destruction of inoculated yeast cells. However, these effects were relieved upon treatment of the prefermented media with activated charcoal (2 g/l) (table 2), suggesting that some low-molecular-weight toxic substances can be adsorbed to and removed by this material.

In an attempt to identify inhibitory or toxic substances among the secondary products of yeast metabolism, various higher alcohols, esters, and fatty acids were added to the control media, and their effects on yeast growth were recorded (table 2). Higher alcohols (per liter: 1-propanol, 30 mg; methanol, 100 mg; 2-methyl-1-propanol, 60 mg; 2-phenylethanol, 100 mg; 3-methyl-1-butanol, 100 mg; 1-pentanol, 3 mg; and 1-butanol, 3 mg) were without effect on yeast growth or survival. However, yeast growth and survival were affected by the addition of octanoic and decanoic acids, as cell numbers decreased from 7×10^6 to 1.3×10^5 cells per ml when these were added to control medium 3.

These acids were added at concentrations similar to those which occur in wines (3 mg/l). Ethyl esters were not as inhibitory as the free acids, but growth was still only one-fourth that of the control.

Fundamental research work has been conducted at the cellular and molecular level. Navarro and Durand[49,50] found that inhibition occurs when ethanol accumulates in the cell because it is excreted too slowly. But, under the conditions of vinification one observes[45]: 1) a relatively weak variation in the endocellular concentration of ethanol; 2) in the presence of steroids (ergosterol, oleanolic acid), a concentration of cellular ethanol two times higher, with a higher concentration of sugar fermented; 3) an absence of cellular pyruvic acid and acetaldehyde which testifies to the activity of carboxylase and alcohol dehydrogenase.

Another possibility is that the enzymes of sugar metabolism are inhibited by products that develop during the fermentation. Nagodawithana et al.[48] reported that ethanol had an inhibitory effect on hexokinase activity and, consequently, the functioning of the glycolysis pathway. Millar et al.[47] have found that 12% w/v of intracellular ethanol is required to cause the inhibition of hexokinase (HK) and alcohol-dehydrogenase (ADH). But under conditions of vinification the intracellular concentration of ethanol at the arrest of the fermentation is only about 0.7%. Larue[42] observed that both HK and ADH remained active after fermentation had ceased. These activities were also present in cells grown in a medium of low sugar concentration supplemented with ethanol and fatty acids, conditions that simulate a stuck fermentation.

On the other hand, it is known that sterols, notably ergosterol, are indispensable to the yeast; they function as growth factors under strict anaerobiosis. They intervene in the regulation of the physiological state of the cell membrane, affecting the nature of the phospholipids which contribute to membrane structure[15]. During fermentation of the must, the concentration of sterols in the yeast population decreases rapidly during the phase of proliferation and then more slowly (table 3). The cessation of growth and the slowing of the fermentation coincides with the depletion of cellular sterols. The addition of sterols to the must (table 3) increases the concentration of sterols in the cells and increases the viability of the population and the speed of fermentation[44]. The deficiency in cellular sterols would be an index of membrane alteration and would account for the difficulties in exchange between the cell and the medium.

Moreover, the cellular concentration of glucose in the population does not cease to decrease during the proliferation phase (table 4); it is relatively stable during the stationary phase; then it decreases during the decline phase, falling to zero at day 17, when the viable population, of the order of 4×10^6 cells/l, will be able to ferment 5 g/l of sugar[45]. The concentrations account for the non-phosphorylated sugars waiting to be metabolized. The decrease in sugar content observed during the phase of proliferation can be explained by the development of enzymatic activities; but it is not very probable that these same systems would become more active with the aging of the population. However, at the end of the decline phase, the transformation of must sugar to ethanol attests to their functioning, when the absence of endocellular glucose shows that the penetration of sugar is just sufficient to cover the activity of these enzymes. According to these results, under the conditions of vinification the slowing of the fermentation would be due, essentially, to a difficulty of sugar penetration[45].

On the other hand, the mechanisms of toxicity of the fatty acids and esters[24,28,43,51,52,59,67] are not yet clear. New results have shown that they are accumulated into the cell walls of viable cells during fermentation and so introduce problems of cellular exchange.

All these results tend to suggest that inhibition of yeasts in the must is due mainly to a membrane alteration phenomenon.

Stimulation of the alcoholic fermentation

The addition of yeasts to the must augments the cellular concentration and accelerates the speed of the fermentation at the beginning. But the principal reason for doing this is the possibility of increasing the total quantity of

Table 3. Effect of anaerobic and semi-aerobic conditions on the sterol content of the yeast cells and on fermentation

	Fermented sugar (g/l)		Sterols (% dry yeast weight)	
	Anaerobic	Semi-aerobic	Anaerobic	Semi-aerobic
Control grape must				
Day 2	37	30	1.6	2.7
Day 9	164	187	0.4	1.2
Day 10	170	246	0.3	1.0
Addition of ergosterol (50 mg/l)				
Day 2	36	27	1.4	2.8
Day 9	169	175	1.0	1.1
Day 10	199	234	0.6	0.8

Table 4. Evolution of hexoses and endocellular ethanol in *Saccharomyces cerevisiae* during the fermentation of grape must

Day		Control	+ ergosterol (50 mg/l)
1	Hexoses (g/l)	35	
	Ethanol (g/l)	2	1.9
	Viable cells (10^6/ml)	1.0	1.4
2	Hexoses (g/l)	22.5	30
	Ethanol (g/l)	3.9	6.0
	Viables cells (10^6/ml)	3.5	3
3	Hexoses (g/l)	14.5	14.9
	Ethanol (g/l)	6.8	12.9
	Viable cells (10^6/ml)	24	26
6	Hexoses (g/l)	14	14.2
	Ethanol (g/l)	6.8	14.4
	Viable cells (10^6/ml)	25	26
10	Hexoses (g/l)	5.7	4.6
	Ethanol (g/l)	4	7.8
	Viable cells (10^6/ml)	9.4	15.9
14	Hexoses (g/l)	3.1	1.8
	Ethanol (g/l)	4.3	8.3
	Viable cells (10^6/ml)	5	8.6
17	Hexoses (g/l)	0	0
	Ethanol (g/l)	4.0	8.7
	Viable cells (10^6/ml)	4.0	9
23	Hexoses (g/l)	0	0
	Ethanol (g/l)	4.5	8.5
	Viable cells (10^6/ml)	1.2	6

The results are expressed in g per liter of intracellular water. Initial content sugar: 260 g/l; active dry *Saccharomyces cerevisiae;* intracellular content hexoses: 0; initial population: 5.6×10^6 cells/ml; temperature of fermentation: 25 °C.

Table 5. Variations in sterol concentrations of yeasts during the alcoholic fermentation of grape must

Conditions	Permanent aeration			Strict anaerobiosis		
	C	+E	+OA	C	+E	+OA
2nd day						
Sugar fermented (g/l)	30	27	24	37	36	23
Sterols (% of dry weight)	2.7	2.8	2.3	1.6	1.4	1.7
Viable cells (10^6/ml)	12	16	21	22	20	17
5th day						
Sugar fermented (g/l)	116	101	95	113	111	105
Sterols (% of dry weight)	1.9	1.9	1.5	0.6	1.1	0.4
Viable cells (10^6/ml)	21	16	20	13	10	12
9th day						
Sugar fermented (g/l)	187	175	155	164	169	154
Sterols (% of dry weight)	1.2	1.1	0.7	0.4	1.0	0.3
Viable cells (10^6/ml)	20	16	20	5	7	5
End of fermentation						
Sugar fermented (g/l)	256	234	211	170	199	185
Sterols (% of dry weight)	1.0	0.8	0.4	0.3	0.6	0.2
Viable cells (10^6/ml)	18	12	16	0.05	0.5	0.1

C, Control must; +E, ergosterol (25 mg/l); +OA, oleanic acid (50 mg/l). Must sugar after enrichment: 250 g/l; yeast: active dry *Saccharomyces cerevisiae;* initial cell population: 2.2×10^6/ml.

sugar fermented by using a selected yeast strain which tolerates a high concentration of ethanol[39]. This is well known to be the case for *S. bayanus,* and also for particular strains of *S. cerevisiae.* This property is increased by the mode of preculture of the yeast. This is demonstrated by inoculating a must with the same yeast *S. cerevisiae,* one part of which was precultivated aerobically and another part precultivated anaerobically[41]. Must inoculated with the aerobically cultivated yeast fermented much more rapidly and completely. This observation can be explained by the role of oxygen in producing yeast sterols[29], and the need for these in anaerobic yeast growth during fermentation. These qualities are associated in the industrial preparations of dried *S. cerevisiae* and *S. bayanus.*

New perspectives are introduced by the notion of 'survival factors' which include the specific action of steroids in vinification. The steroids have been described as factors necessary for the anaerobic growth of yeasts[3,11]. However, in vinification, the must is aerated during its extraction and it is further inoculated, by contact with winery materials, with indigenous yeasts, which have themselves developed aerobically; finally the fermentation takes place anaerobically. In these conditions the addition of ergosterol does not augment the maximum population (table 5). In contrast, the viability of the yeasts during the decline phase is raised 2–10 times; a greater quantity of sugar is then degraded. In this case the steroids function not as factors of growth but as factors contributing to yeast survival[37,38,44]. But if the fermentation develops aerobically, it is spontaneously rapid; the ergosterol behaves as an inhibitor. The interest in these survival factors comes from their action on the final grams of sugar, and they may serve to prevent premature cessation of the fermentation.

Table 7. Stimulation of alcoholic fermentation of grape must containing inhibitory substances by addition of yeast ghosts

Condition	Weight of sugar consumed (g/l) when fermentation stops[a]		
	Must 1[b]	Must 2[c]	Must 3[d]
Control	191	201	192
Addition of yeast ghosts (g/l)			
0.2	232	218	200
0.5			237
1	247	243	

[a] Initial sugar concentrations: must 1 and 2: 250 g/l; must 3: 320 g/l. Initial viable yeast level: 10^6 cells per ml; dry yeast: *S. cerevisiae;* temperature of fermentation: 19 °C. [b] Containing Euparene (dichloromethyl thiodimethyl phenyl sulfamide (Bayer), 4 mg/l). [c] Containing Mikal (trio-*o*-ethylphosphonate-aluminium plus trichloromethylthio-iso-indolinedione (Rhône-Poulenc), 10 mg/l. [d] From grapes infected by *B. cinerea.*

On the other hand, the new results show that the inhibition of populations is linked to the production by yeasts themselves of toxic substances acting in synergy with ethanol[20]. These results modify the approach to the problems of grape must fermentation and the solutions resulting.

The ability of activated charcoal to stimulate must fermentations and thus overcome stuck fermentations has been known in enology for a long time[56], but its mechanism of action has not been explained. We have now shown that the addition of 2 g of activated charcoal per liter to fermentation medium containing 10 and 3 mg of octanoic and decanoic acids per liter, respectively, removed circa 97% of each of the acids after 1 h of contact. Consequently, the beneficial action of the charcoal in grape must fermentation might be explained by its ability to adsorb toxic fatty acids; however, its use for this purpose on a commercial basis is limited by legislation and the difficulty of its subsequent removal from the wine.

Preliminary observations in this laboratory indicated that yeast cells themselves adsorb ethyl esters when added to the medium, and it was thought that this property might be further exploited to overcome premature stoppage of fermentation or to induce a second fermentation in stuck wine.

On a laboratory scale, yeast ghosts were found to exhibit this adsorption property to overcome difficult fermentations (C. Geneix, S. Lafon-Lafourcade, and P. Ribéreau-Gayon, French patent 8309215, June 1983). This has now been explored in more detail with an industrial preparation of these ghosts[33]. The addition of 0.2 to 1 g of

Table 6. Stimulation of wine fermentation by addition of yeast ghosts to grape must[a] (results after fermentation)

Conditions	Addition of ghosts before fermentation			Addition of ghosts at day 5 of fermentation		
	Sugar consumed (g/l)	Total population (10^7 cells/ml)	Viable population (10^6 cells/ml)	Sugar consumed (g/l)	Total population (10^7 cells/ml)	Viable population (10^6 cells/ml)
Control (no addition)	206	9	3.5	206	9	3.5
Addition of yeast ghosts (g/l)						
0.2	247	11	10	239	9.8	16
1	257	14	26	254	8.2	29
Addition of $(NH_4)_2SO_4$ (0.2 g/l)	212	10	2.7	ND[b]	ND	ND

[a] Initial sugar concentration: 260 g/l; initial viable yeast level: 10^6 cells/ml; dry yeast: *S. cerevisiae;* temperature of fermentation: 19 °C.

yeast ghosts per liter to the must before fermentation or at day 5 of fermentation permits the fermentation of a greater quantity of sugar (table 6) and hence the avoidance of fermentation stoppages; at the end of fermentation, the total yeast populations were similar, but the viable populations were much higher in must with added yeast ghosts.

The addition of yeast ghosts is effective in the following cases: 1) must with an initial high concentration of sugar (table 6); 2) must containing residual chemical substances used to protect the vines (Mikal, Euparène) (table 7); 3) must infected by *B. cinerea* and 4) must fermenting at high temperature. In a must initially containing 220 g of sugar per liter incubated at 30 °C, 160 g of sugar per liter were consumed in the control; the addition of 0.2 and 1 g of yeast ghosts per liter permitted an increase in this value of 32 and 53 g/l respectively.

Table 8 shows the effect of adding yeast ghosts to two stuck wines. Wine 1 (residual sugar, 57.7 g/l; ethanol, 10.5% vol/vol) was fermented at 19 °C; wine 2 (residual sugar, 121 g/l; ethanol, 7% vol/vol) was fermented at 30 °C. In these cases, the initial fermentation stopped naturally, leaving residual sugar. Adding yeast ghosts to the stuck wines relieved the inhibited or restricted fermentation; the residual sugars decreased faster and to lower levels than those of the controls. All of these results have been confirmed by many other experiments.

Mechanism of action of yeast ghosts

The addition of yeast ghosts to fermenting musts decreases the loss of yeast viability during the latest stages of fermentation, and this seems to be related to their ability to adsorb toxic fatty acids and esters produced as end products of the fermentation. The adsorption of the main acids involved and their esters is shown in table 9. Various levels of yeast ghosts were added to a sterile medium containing ethanol, 10% (vol/vol) hexanoic acid, 6 mg; octanoic acid, 10 mg; decanoic acid, 3 mg; ethyl hexanoate, 1.01 mg; ethyl octanoic, 1.37 mg; and ethyl decanoate, 0.61 mg. After 24 h at 20 °C, without agitation, the medium was centrifuged at 7000 × g to sediment the yeast ghosts, and the levels of the various acids and esters in the supernatant were determined. The data in table 9 show a strong adsorption or removal of ethyl decanoate and ethyl octanoate by the yeast ghosts and a lesser adsorption of ethyl hexanoate. With the exception of decanoic acid, the other fatty acids were only weakly adsorbed. The extent of adsorption increased with the amount of yeast ghosts added, but prolonging the time of adsorption past 24 h was without increased effect.

Table 10. Quantity of volatile substances in wine prepared with yeast ghosts[a]

Volatile substances	Amount (mg/l)		
	Control	Added yeasts ghosts (g/l)	
		0.2	0.5
Acetic acid	0.29	0.23	0.23
Hexanoic acid	8.00	7.20	6.45
Octanoic acid	4.96	4.40	4.13
Decanoic acid	2.03	1.70	1.25
Isoamyl acetate	1.84	2.13	2.52
Ethyl hexanoate	0.71	0.62	0.61
Hexyl acetate	0.15	0.11	0.11
Hexanol	0.58	0.57	0.57
Ethyl octanoate	0.70	0.66	0.63
Ethyl decanoate	0.49	0.48	0.39
Diethyl succinate	0.14	0.24	0.20
Phenyl ethyl acetate	0.18	0.24	0.26
Phenylethanol	23.90	39.40	35.00

[a] Initial sugar content: 250 g/l; temperature of fermentation: 19 °C.

The addition of yeast ghosts to grape must before the commencement of alcoholic fermentation decreased fatty acid content in wines but did not significantly affect the final concentration of ethyl esters (table 10). As a result, no significant changes were observed in the organoleptic characteristics of wines prepared with the addition of yeast ghosts. It is known that fatty acids and ethyl esters are important aroma compounds in wine.

In order to explain this paradoxical phenomenon Larue et al.[43] observed during the fermentation the evolution of fatty acids toxic to the yeast. Concentrations of organic acids and esters were determined in the cells and in the grape must. When yeast ghosts are added to must they have the effect of limiting endocellular and parietal accumulations of octanoic and decanoic acids. However, the concentration of these substances in the must is only slightly affected. The concentration of ethyl esters is unchanged. This suggests that as the yeast ghosts adsorb ethyl esters the fermenting yeasts in turn excrete additional esters to maintain a balance between endocellular and exocellular concentrations.

The ability of yeast ghosts to adsorb toxic substances from fermenting grape musts has substantial practical importance; addition during the stationary phase (day 5 of fermentation) is just as effective as in the must. For the

Table 8. Use of yeast ghosts to induce a second fermentation in stuck wines

Sample	Sugar content (g/l) after addition of yeast ghosts (0.5 g/l) and inoculation with *S. cerevisiae* (10^6 cells/ml) at fermentation day					
	0	9	12	16	27	36
Stuck wine 1	67.1	57.6	49	35.5	15.6	13.4
Stuck wine 1 + yeast ghosts added	67.1	53.3	37	23.6	2.8	1.4
Stuck wine 2	121	66.4	53	33	9.9	8.6
Stuck wine 2 + yeast ghosts added	121	40.1	27.6	12.9	0.6	

Table 9. Adsorption of fatty acids and their esters by yeast ghosts in noninoculated synthetic medium[a]

Weight of yeast ghosts (g/l)	Acids adsorbed (%)			Ethyl esters adsorbed (%)		
	Hexanoic	Octanoic	Decanoic	Hexanoic	Octanoic	Decanoic
0.2	0	1.2	20.2	25.8	58.2	88.8
0.5	0	4.5	40.7	36.2	73.8	94.6
1	0	7.2	54.5	36.8	77.7	94.6

[a] Numbers are the percentage of acids and esters adsorbed after 24 h of contact. Initial concentration of medium: hexanoic acid: 6 mg/l; octanoic acid: 10 mg/l; decanoic acid: 3 mg/l; ethyl hexanoate: 1.01 mg/l; ethyl octanoate: 1.37 mg/l; ethyl decanoate: 0.61 mg/l.

same reasons, the yeast ghosts allow an easier second fermentation in stuck wine.

The yeast ghosts act a little on the total population, but they permit the survival of a higher number of viable cells during the declining phase of the yeast population. Their mechanism of action corresponds precisely to the definition of survival factors[38]. It allows the yeast cells to fulfill their potential fermentation capacity better, and could hence be used to replace the inoculation of must with alcohologenic yeast cells or the addition of ammonium salts (table 6) and vitamins which are much less effective.

3. *Malolactic fermentation of wine*

The role of malolactic fermentation in vinification

Pasteur described the lactic acid bacteria as spoilage organisms. The works of L. Ferre (1922–1928) in Burgundy and of J. Ribéreau-Gayon and Peynaud in Bordeaux (1937–1946) established the beneficial role of the malolactic fermentation in wine. This phenomenon is desired for two main reasons; 1) it deacidifies the wine conveniently, 2) it provides a biological stability in wines conserved with a minimum of sulphurous anhydride. In northern viticultural regions, it occurs generally in red wines. In white wines it occurs less frequently. In warm regions musts have a relatively high pH. One tries to prevent the malolactic fermentation.

Lactic acid bacteria, growth cycle and kinetics of fermentation

Lactic acid bacteria are found on grapes and leaves but in smaller numbers than the yeasts or the acetic acid bacteria. These strains belong principally to the homofermentative lactobacilli[4], *Lactobacillus plantarum* and *Lactobacillus casei* or heterofermentative lactobacilli, *Lactobacillus hilgardii* and *Lactobacillus brevis*. All studies confirm the rarity of the occurrence of *Leuconostoc oenos* on grape. This species predominates in French wines at the end of the alcoholic fermentation[35]. It is often considered as the species responsible for the degradation of malic acid[12–14]. But it seems that homofermentative species can predominate in German wines and Australian wines[33].

This apparent divergence could be caused by the specific properties of the wine. Its composition and pH vary with the variety, the climate, the mode of vinification and conservation. It may also result from the isolation method.

In traditional vinification, malolactic fermentation is induced by lactic acid bacteria on grapes and winery equipment. But the grapes are sulfited to prevent bacterial growth and alteration of the wine as a result of sugar fermentation by the bacteria[55]. In a well-controlled vinification lactic acid bacteria complete their growth cycle in the wine[34].

During the alcoholic fermentation, the population of lactic acid bacteria decreases down to some hundred cells per ml. Separation of the wine from the maceration and transfer to barrels are accompagnied by an enrichment in the population.

Wine is a poor culture medium and the bacteria multiply under restricted nutritional, physical and chemical conditions[1, 18, 55]. It is known that the capacity of lactic acid bacteria to grow in wines is determined by temperature, wine pH, alcohol concentration, and concentration of sulphur dioxide in its free form as well as combined with aldehydes and ketones[10, 33] (table 11). It is quite possible that difficulties with growth in wine stem from the partial lack of nutrients[18]. The strain of yeast which carries out the alcoholic fermentation also affects malolactic fermentation[33]. The role of polyphenols and aeration are controversial. The interruption of malolactic fermentation can be due to an attack by a bacteriophage which lyses the bacteria[63].

Owing to these factors, the bacterial development is not facilitated. Total growth is limited to about 10^7 cells per ml, but sometimes it stops before this. The duration of the growth cycle can be particularly long. It may last several weeks or months. In order to improve growth some modifications have been suggested. These are the heating of the cellar to about 19–20 °C, chemical deacidification of the wine and moderate sulfiting of the grapes.

The kinetics of the malolactic fermentation is directly correlated with the bacterial biomass formed[34]. If it is considerable, the biochemical reaction is carried out by bacteria during the phase of growth, hence it is rapid. If it is smaller, the fermentation continues during the stationary phase or the declining phase; it is much slower.

After completion of the malolactic fermentation, during conservation, the population of lactic acid bacteria can remain viable. Temperature exerts a very important influence on this survival. At the higher temperatures and especially above 20 °C a rapid decline in viability was noted. Decrease in cell viability during conservation was

Table 11. Effects of SO_2 addition and temperature of storage on the development of lactic bacteria populations and the time for completion of malolactic fermentation

Vintage	SO_2 to must (mg/l)	SO_2 to wine (mg/l)	Temp. of storage (°C)	Maximum population (cells/ml)	Time of completion of malolactic fermentation days
1979	0	0	14	3×10^7	16
1979	50	0	14	1×10^7	21
1979	100	0	14	1×10^6	31
1979	0	50	14	1×10^4	–[a]
1979	50	50	14	5×10^4	–
1979	100	50	14	1×10^4	–
1979	0	0	19	1×10^8	10
1979	50	0	19	9×10^7	17
1979	100	0	19	4×10^7	19
1980	0	0	18	1×10^8	16
1980	50	0	18	8×10^7	17
1980	100	0	18	5×10^7	24

[a] –, No fermentation after 200 days.

also accelerated by the lowering of pH, increasing alcohol concentration and addition of SO_2[34].

Stimulation of the malolactic fermentation by inoculating the wine

For quite some time attempts have been made to induce malolactic fermentation by inoculating the wine with selected lactic acid bacteria[22, 31, 32, 65, 69, 70]. Several species have been proposed, but *L. oenos* is generally considered to be the most appropriate. Until now, the problem has been a lack of readily usable bacterial biomass in sufficient quantity. The desired physiological state of the bacteria in the inoculum and the proportions to be used are also not known.

However, lyophilized and frozen biomass preparations are now appearing on the market[33, 65]. Recently an industrial preparation of *L. oenos* had been tried. This strain is resistant to low pH (3.2), to high concentrations of alcohol (12%) and SO_2 (13 mg/l). However, after it has been thawed as specified by the manufacturer, the addition of such a preparation of *L. oenos* to the wine results in a significant loss of bacterial viability in red wines, and even more in white wines. Before use, these preparations have to be reactivated to establish a physiological state which permits the bacteria to resist the conditions prevailing in wine. An incubation of 24 h in grape must fortified with yeast extract is necessary. An inoculation with these bacteria of the order to 10^7 cells/ml can compensate for the inhibitory effect of ethanol, of nutritional deficiencies and of the presence of sulfur dioxide.

The time at which the wine has to be inoculated is still a matter of dispute. Some authors recommend inoculation during the alcoholic fermentation[30, 70]. Others are indifferent; according to them, bacteria may be added to the wine before or after the alcoholic fermentation. Ribéreau-Gayon et al.[55] recommend inoculation only after the sugars are completely exhausted.

Spoilage by lactic acid bacteria

In musts and wines the lactic acid bacteria also find substrates other than malic acid, such as sugars, amino acids, organic acids and glycerol[33, 54, 55]. The degradation of sugars leads to an increase in the fixed and volatile acidities of the wine; this is the 'piqûre lactique'. The oxidation of citric acid leads to the formation of acetic acid and pyruvic acid. During the oxidation of tartaric acid the fixed acidity increases. The odor resembles that of fermenting sauerkraut; this is the 'tourne'. The oxidation of glycerol leads to the formation of acrolein. This substance reacts chemically with the polyphenols of the wine to produce a bitter flavor; this is the 'amertume' spoilage.

Some strains of lactic acid bacteria can produce a film of carbohydrates and a gelatinuous texture. It gives the wine an oily aspect; this is the 'fatty' spoilage.

A toxic material, histamine, has been found in wines but generally in small concentrations largely below the threshold of toxicity. It stems in part from the grapes and in part from yeast and bacterial metabolism[33].

To avoid the 'piqûre lactique' it is necessary to prevent bacterial growth in the presence of sugar[9, 33, 55]. To avoid the other types of spoilage it is not sufficient to sulphur the wine. It is imperative from the termination of the malolactic fermentation to eliminate lactic acid bacteria as rapidly as possible by sulphuring followed by filtration. During storage, the presence of lactic acid bacteria can be tested for by analyzing the level of D(−) lactic acid in the wine. But the best 'natural' protection of wine against lactic alterations resides in its acid pH and in the relatively low temperature of the storage cellars.

4. Spoilage by acetic acid bacteria

Occurrence of acetic acid bacteria during the different stages of vinification

In the field of enology, acetic acid bacteria have received little attention[7, 17, 26, 27, 55]. This lack of interest may be explained by the fact that these bacteria are strictly aerobic and consequently they were usually considered to be unable to grow in wines except those wine surfaces in permanent contact with air.

In recent years, wineries have become concerned about the small increases in the levels of acetic acid sometimes encountered during storage of wine in wooden barrels. This has provided the basis of new investigations. The application of new microbiological analysis techniques has facilitated the selective isolation of acetic acid bacteria.

The density of the population of acetic acid bacteria has always been found to be linked to the degree of grape infection. Freshly extracted juice, from grapes at the beginning of the harvesting season, contained an average 10^2 cells per ml of acetic acid bacteria. Slightly higher levels were found on the early picked 'pourriture noble' grapes, but the levels had increased to 10^4–10^6 cells per ml on such grapes picked at the end of the harvesting season. *Gluconobacter oxydans* was the main representative of the acetic acid bacteria on sound, unspoiled red or white grapes[27]. However, *Acetobacter pasteurianus* became more prevalent as the grapes became spoiled. These two species accounted for 75–85% of the acetic acid bacteria on the *Botrytis* infected grapes.

In white wine, the must obtained from sound white grapes contained only a very small population ($< 10^2$ cells per ml) of *G. oxydans,* which quickly disappeared during fermentation. In contrast, the behavior of acetic acid bacteria was quite different during the production of Sauternes style white wines from grapes parasitized with *B. cinerea.* The initial must contained around 10^6 cells per ml of acetic acid bacteria, with *G. oxydans* being the predominant species. The yeast population was about the same. During the course of the natural alcoholic fermentation, the total population of acetic acid bacteria decreased to less than 10^3 cells per ml; *A. pasteurianus* and *A. aceti* became the predominant representatives in equal proportion.

In red wine, freshly pressed must contained about 10^4 cells per ml of *G. oxydans.* The population progressively decreased during alcoholic fermentation and at the end of this fermentation (10 days), only some cells per ml were detected in the wine. The proportion of *G. oxydans* in the population decreased and that of *A. pasteurianus* increased. On drainage of the wine from the fermentation tank, the population increased to around 10^4 cells per ml and this may be explained by contamination through contact with equipment and perhaps multiplication of

those cells present in the wine as a consequence of agitation and aeration. *A. pasteurianus* was the predominant species after draining.

Samples of wine taken at the commencement of the malolactic fermentation exhibited counts around 10^2–10^3 cells per ml and consisted mainly of *A. pasteurianus* and lesser amounts of *G. oxydans* and *A. aceti*. At the completion of malolactic fermentation (which took 3 months) cell counts remained around 10^2 cells per ml and now consisted mainly of *A. aceti* and a smaller proportion of *A. pasteurianus*.

At this stage 20 mg/l of SO_2 were added to the wine; then it was mixed, filtered and returned to the wooden barrels for storage at 10 °C. Samples taken during the next 11 months of storage exhibited counts around 10^2–10^3 cells/ml and consisted mostly of *A. aceti* and smaller levels of *A. pasteurianus*. The amount was about the same at the top and at the bottom of the barrel. At the end of this period, all the acetic acid bacteria were *A. aceti* sp. *aceti*. During that time, wine was added to the barrels, twice a week, in order to compensate for evaporation. In a few cases, *G. oxydans* was again identified immediately after this operation. But a week later this species had again disappeared. This suggests that its presence was due to an infection, brought either by the wine or by equipment used in filling up the barrels.

Metabolism of acetic acid in grape must. Relationship between the growth of acetic acid bacteria and the formation of acetic acid in wine

The development of *G. oxydans* and *A. aceti*, the main species of acetic acid bacteria developing on grapes, modifies the constitution of the grape. Glucose preferentially, but also fructose, malic and citric acids are degraded with formation of gluconic, lactic, succinic acids, acetaldehyde and ketonic substances. Nevertheless, the capacity to fix sulphur dioxide remains low. Acetic acid derives from oxidation by *G. oxydans* of the small amount of ethanol already present in the grape must. *A. aceti* forms acetic acid but it is subsequently wholly degraded in the decline phase.

An antagonistic effect between bacteria and yeasts has been discovered[21]. A strain of *Acetobacter mesoxydans* with a population ratio of 3:1 inhibits the growth of *Saccharomyces cerevisiae*. But previous development in the must of acetic bacteria, such as *G. oxydans* also inhibits its growth and metabolism of *S. cerevisiae*. In contrast, malolactic degradation by *L. oenos* is generally stimulated.

In laboratory trials, the effects of wine pH, storage temperature and momentary aeration on the growth of acetic acid bacteria and acetic acid production were examined. In this case *A. aceti* was the only representative of the acetic acid bacteria in the wine. The wine contained 12% ethanol and 20 mg per liter of free SO_2. The growth of lactic acid bacteria, which were also present in the wine, was prevented by the addition of penicillin.

Wine samples were adjusted to either pH 3.4 or 3.8 and stored at either 10 °C or 18 °C. The samples were stored in completely filled 100-ml flasks to yield an anaerobic environment. After 8 days of storage the contents of each flask were exposed to air for 3 min by pouring them into a beaker and then returning them to the flasks. Analysis were performed 8 days after this aeration.

In the samples stored under completely anaerobic conditions, at both temperatures the acetic acid bacteria decreased more rapidly at pH 3.4 than at pH 3.8; the concentrations of acetic acid stayed constant.

However, one week after the momentary aeration, results (table 12) showed little growth at 10 °C, but the cell numbers increased by 30–40-fold on storage at 18 °C. This was accompanied by a significant increase in the level of acetic acid. Slightly higher counts of bacteria were observed at the higher pH of 3.8. The counts were still higher when no SO_2 was added.

These results suggest that *Acetobacter aceti* may survive well throughout the storage of the wine in barrels. It disappears when conditions become strictly anaerobic, for instance in bottles; this decrease is faster for lower pH values. These low residual levels (10^2 cells per ml) of *Acetobacter aceti* in wines during storage may have a dramatic effect on wine quality. A short exposure of wine to air (2–3 min), as is common during normal wine-making, may lead to a rapid multiplication of this organism with consequent generation of acetic acid. This may be particularly important at high temperature and high pH. Many observations made in different cellars show definitively that acetic acid bacteria are responsible for the small increase of acetic acid sometimes observed during wine storage in barrels. This fact is a new concept for enology; it has always been thought that anaerobic acetic acid bacteria can only develop when wine is stored in barrels, because of the apparent lack of oxygen[55].

But, during normal wine-making procedures a short aeration can occur, for instance during the racking processes. Then the temperature and, to a lesser extent, the pH values are the most significant factors which affect the bacterial growth and the production of acetic acid, which appears even though oxygen may subsequently be absent; free SO_2, in the concentrations used as a preservative of red wines, does not sufficiently protect them against the metabolism of acetic acid bacteria.

The practical consequences which arise from these results are: 1) the necessity for a low temperature (10–15 °C) during the storage of the wine in barrels; 2) the necessity of avoiding any aeration at higher temperature, for instance during the summer time; however, in red wine making, this aeration is indispensable for the normal evolution of the phenolic compounds involved in wine color[55].

Table 12. Effect of wine pH and storage temperature on the growth of acetic acid bacteria and concentration of acetic acid in wine after aeration[a]

Storage time	Storage temperature	Wine pH	Acetic acid bacteria (cells/ml) ($\times 10^2$)	Acetic acid (mg/l)
0			5	370
15 days	10 °C	3.4	15	380
15 days	10 °C	3.8	15	420
15 days	18 °C	3.4	140	500
15 days	18 °C	3.8	200	520

[a] The wine from Cabernet Sauvignon grapes contained 12% ethanol and 20 mg of free SO_2 per liter; it contained *A. aceti* only. After 8 days of storage, the contents of the flakes were exposed to air for 3 min by pouring them into beakers and then returning them to the flasks. Analyses were performed 8 days after this aeration.

Conclusion

Concerning alcoholic fermentation, the concept of survival factors and the fact of the influence of certain fatty acids formed by yeast on the fermentability of media rich in sugars have an important theoretical and practical significance. New results modify the way in which problems of grape must fermentation are interpreted, and the solutions proposed. Yeast ghosts may adsorb toxic substances produced during the fermentation. But the applications of this process can extend with efficacy beyond that field, in conditions which induce difficulties in fermentation. These properties make the material the most effective activator of wine fermentation known. Yeast ghosts could be used to overcome a premature stoppage of alcoholic fermentation or to induce a second fermentation in stuck wine. Their addition has no effect on wine taste or aroma. But the mechanism of action of fatty acids toxic to yeast will be examined.

Concerning the malolactic fermentation a technical solution consists in the implantation in wine of selected lactic acid bacteria after reactivation. Immobilization of lactic acid bacteria on an inert supporting material functioning as a chemical reactor still requires a considerable amount of research to be done[16]. One might also consider the transfer of genes coding for the malolactic enzyme into bacteria or yeasts which are more resistant to wine. Immobilization of the malolactic enzyme itself, of which the properties are known[33], is also a potential solution.

On the other hand, acetic acid and lactic acid bacteria are present at all stages of wine-making. A lower pH and higher ethanol concentration limit the risks of alteration. During the conservation, a low temperature, good use of SO_2 and frequent checking ensures control of bacterial growth and metabolism.

1 Amachi, T., Chemical structure of a growth factor (TJF) and its physiological significance for malolactic bacteria, in: Lactic Acid Bacteria in Beverages and Food, pp. 103–118. Eds J. G. Carr, C. V. Cutting and G. C. Whiting. Academic Press, London 1975.

2 Amerine, M. A., and Joslyn, M. A., Table Wines, 2nd edn. University of California Press, Berkeley 1970.

3 Andreasen, A. A., and Stier, J. T. B., Anaerobic nutrition of *Saccharomyces cerevisiae*. J. cell. comp. Physiol. *41* (1953) 23–36.

4 Barre, P., Recherches sur les bactéries lactiques des vins. I. Isolement et classification. Annls Technol. agric. *15* (1966) 173–180.

5 Barre, P., Killer factor activity under vinification conditions. VIth International Specialized Symposium on Yeasts, Montpellier 1978.

6 Bevan, E. A., and Somers, J. M., Somatic segregation of the Killer (k) and neutral (N) determinants in yeasts. Genet. Res. *14* (1969) 71–79.

7 Bidan, P., Sur quelques bactéries isolées des vins en fermentation malolactique. Annls Technol. agric. (1956) 597–617.

8 Blackwood, M., Etude des bactéries acétiques de raisins et de vins. Conn. Vigne Vin *3* (1969) 227–243.

9 Bonnamour, C., Contribution à l'étude des produits secondaires de la fermentation malolactique des vins. Thèse de 3ème cycle. Université de Bordeaux II, Bordeaux 1984.

10 Bousbouras, G. E., and Kunkee, R. E., Effect of pH on malolactic fermentation in wine. Am. J. Enol. Vitic. *22* (1971) 121–126.

11 Bréchot, P., Chauvet, J., Dupuy, P., Croson, M., and Rabatu, A., Acide oléanolique, facteur de croissance anaérobie de la levure de vin. Annls Technol. agric. *20* (1971) 103–110.

12 Carre, E., Recherche sur la croissance des bactéries lactiques en vinification. Désacidification biologique des vins. Thèse 3ème cycle. Université de Bordeaux II, Bordeaux 1982.

13 Chalfan, Y., Goldberg, I., and Mateles, R., Isolation and characterization of malolactic bacteria from Israeli red wines. J. Fd Sci. *42* (1977) 939–943.

14 Costello, P. J., Morrison, G. J., Lee, T. H., and Fleet, G. H., Numbers and species of lactic acid bacteria in wine during vinification. Fd Technol. Aust. *35* (1983) 14–18.

15 Demel, R. A., and De Kruyff, B., The function of sterols in membranes. Biochim. biophys. Acta *457* (1976) 109–132.

16 Divies, C., and Siess, M. H., Etude du catabolisme de l'acide L-malique par *Lactobacillus casei* emprisonné dans un gel de polyacrylamide. Annals Inst. Pasteur, Paris *127 B* (1976) 525–539.

17 Dupuy, P., and Maugenet, J., Oxydation de l'éthanol par *Acetobacter rancens*. Annls Technol. agric. *11* (1962) 219–225.

18 Feuillat, M., Bidan, P., and Rosier, Y., Croissance des bactéries lactiques à partir des principaux constituants azotés du vin. Annls Technol. agric. *26* (1977) 435–447.

19 Gallander, J. F., Deacidification of eastern table wines with *Saccharomyces pombe*. Am. J. Enol. Vitic. *28* (1977) 65–68.

20 Geneix, C., Lafon-Lafourcade, S., and Ribéreau-Gayon, P., Effet des acides gras sur la viabilité des populations de *Saccharomyces cerevisiae*. C.r. Acad. Sci. Paris *296* (1983) 943–945.

21 Gilliland, R. B., and Lacey, J. P., Lethal action by an *Acetobacter* on yeasts. Nature *202* (1964) 727–728.

22 Gnaegi, F., La désacidification des vins. Revue suisse Vitic. Arboric. Hortic. *14* (1982) 147–154.

23 Gnaegi, F., Aerny, J., Bolay, A., and Crettenand, J., Influence des traitements viticoles antifongiques sur la vinification et la qualité du vin. Revue suisse Vitic. Arboric. Hortic. *15* (1983) 243–250.

24 Grncarevic, M., and Radler, F., A review of the surface lipids of grapes and their importance in the drying process. Am. J. Enol. Vitic. *22* (1971) 80–86.

25 Hadjinicolaou, D., Incidence des opérations préfermentaires sur la fermentescibilité des moûts et les caractères organoleptiques des vins blancs secs. Thèse 3ème cycle. Université de Bordeaux II, Bordeaux 1981.

26 Joyeux, A., Lafon-Lafourcade, S., and Ribéreau-Gayon, P., Métabolisme des bactéries acétiques dans le moût de raisin. Conséquences à l'égard de la fermentation alcoolique et malolactique. Sci. Aliments *4* (1984) 247–255.

27 Joyeux, A., Lafon-Lafourcade, S., and Ribéreau-Gayon, P., Evolution of acetic acid bacteria during fermentation and storage of wine. Appl. envir. Microbiol. *48* (1984) 153–156.

28 Kabara, J. J., Toxicological bacteriocidal and fungicidal properties of fatty acids and some derivatives. J. Am. Oil Chem. Soc. *56* (1979) 750–767.

29 Kirsop, B. H., Oxygen in brewery fermentation. J. Inst. Brew. *80* (1974) 252–259.

30 Kunkee, R. E., Malolactic fermentation. Adv. appl. Microbiol. *9* (1967) 263–267.

31 Kunkee, R. E., Ough, C. S., and Amerine, M. A., Introduction of malolactic fermentation by inoculation of must and wine with bacteria. J. Enol. Vitic. *15* (1964) 178–183.

32 Lafon-Lafourcade, S., Etude de la dégradation de l'acide L-malique par les bactéries lactiques non proliférantes isolées des vins. Annls Technol. agric. *19* (1970) 141–154.

33 Lafon-Lafourcade, S., 'Wine and Brandy' in Biotechnology, pp. 81–163. Eds H. J. Rehm and G. Reed. Verlag Chemie, Weinheim 1983.

34 Lafon-Lafourcade, S., Carre, E., and Ribéreau-Gayon, P., Occurrence of lactic acid bacteria during the different stages of vinification and conservation of wines. Appl. envir. Microbiol. *46* (1983) 874–880.

35 Lafon-Lafourcade, S., Dubourdieu, D., Hadjinicolaou, D., and Ribéreau-Gayon, P., Incidence des conditions de travail des vendanges blanches sur la clarification et la fermentation des moûts. Conn. Vigne Vin *14* (1980) 97–109.

36 Lafon-Lafourcade, S., Geneix, C., and Ribéreau-Gayon, P., Inhibition of alcoholic fermentation of grape must by fatty acids produced by yeasts and their elimination by yeast-ghosts. Appl. envir. Microbiol. *47* (1984) 1246–1249.

37 Lafon-Lafourcade, S., Larue, F., Bréchot, P., and Ribéreau-Gayon, P., Les stéroïdes, facteurs de survie des levures au cours de la fermentation alcoolique du moût de raisin. C.r. Acad. Sci. *284* (1977) 1939–1942.

38 Lafon-Lafourcade, S., Larue, F., and Ribéreau-Gayon, P., Evidence for the existence of 'survival factors' as an explanation for some peculiarities of yeast growth, especially in grape must of high sugar concentration. Appl. envir. Microbiol. *38* (1979) 1069–1073.

39 Lafon-Lafourcade, S., and Ribéreau-Gayon, P., Premières observations sur l'utilisation des levures sèches en vinification en blanc. Conn. Vigne Vin *10* (1976) 277–292.

40 Larue, F., Les facteurs de survie de la levure, leur rôle sur la fermentation alcoolique du moût de raisin. Thèse 3ème cycle. Université de Bordeaux II, Bordeaux 1978.
41 Larue, F., La notion de facteur de survie et les implications en vinification, in: Actualités oenologiques et viticoles, pp. 231-235. Eds P. Ribéreau-Gayon and P. Sudraud. Dunod, Paris.
42 Larue, F., Evolution des activités hexokinase et alcool-deshydrogénase, au cours de la fermentation de *Saccharomyces cerevisiae*. IVème Symposium International sur les levures. Montpellier 1984.
43 Larue, F., Geneix, C., Lafon-Lafourcade, S., and Ribéreau-Gayon, P., Premières observations sur le mode d'action des écorces de levure. Conn. Vigne Vin *18* (1984) 155–163.
44 Larue, F., Lafon-Lafourcade, S., and Ribéreau-Gayon, P., Relationship between the sterol content of yeast cells and their fermentation activity in grape must. Appl. envir. Microbiol. *39* (1980) 808–811.
45 Larue, F., Lafon-Lafourcade, S., and Ribéreau-Gayon, P., Inhibition de *Saccharomyces cerevisiae* dans le moût de raisin. C.r. Acad. Sci. *294* (1982) 587–590.
46 Middelbeck, E.J., Hermans, J.M.H., and Stumm, C., Production, purification and properties of a *Pichia Kluyveri* Killer toxin. J. Microbiol. *45* (1979) 437–450.
47 Millar, O.G., Griffiths-Smith, K., Algar, E., and Scopes, R.K., Activity and stabilty of glycolytic enzymes in the presence of ethanol. Biotech. Lett. *4* (1982) 601–606.
48 Nagodawithana, T.W., With, H., and Cutaia, A.Y., Study of the feed back of ethanol on selected enzymes of the glycolytic pathway. J. Am. Soc. Brew. *35* (1977) 179–193.
49 Navarro, J.M., and Durand, G., Fermentation alcoolique. Influence de la température sur l'accumulation d'alcool dans des cellules de levure. Annls Inst. Pasteur, Paris *129* (1978) 215–221.
50 Navarro, J.M., and Finck, J.O., Evolution de l'activité 'kexokinase' de *Saccharomyces uvarum* fermentant le saccharose. Cell. molec. Biol. *28* (1982) 85–89.
51 Nordström, K., Formation of esters from acids by brewer's yeast. II. Formation from lower fatty acids. J. Inst. Brew. *70* (1963) 42–55.
52 Nordström, K., Formation of esters from acids by brewer's yeast. IV. Effect of higher fatty acids and toxicity of lower fatty acids. J. Inst. Brew. *70* (1964) 233–242.
53 Palfree, R., and Bussey, H., Yeast killer toxin purification and characterization of the protein from *S. cerevisiae*. Eur. J. Biochem. *93* (1979) 147–151.
54 Radler, F., The metabolism of organic acids by lactic acid bacteria in: Lactic Acid Bacteria in Beverages and Food, pp. 17–27. Eds J.G. Carr, C.V. Cutting and G.C. Whiting. Academic Press, London 1975.
55 Ribéreau-Gayon, J., Peynaud, E., Ribéreau-Gayon, P., and Sudraud, P., Sciences et Techniques du Vin, vol. 2. Dunod, Paris 1975.
56 Ribéreau-Gayon, J., Peynaud, Ribéreau-Gayon, P., and Sudraud, P., Sciences et Techniques du Vin, vol. 4. Dunod, Paris 1977.
57 Ribéreau-Gayon, P., Lafon-Lafourcade, S., and Bertrand, A., Le débourbage des moûts de vendange blanche. Conn. Vigne Vin *9* (1975) 117–139.
58 Ribéreau-Gayon, P., Lafon-Lafourcade, S., Dubourdieu, D., Lucmaret, V., and Larue, F., Metabolisme de *Saccharomyces cerevisiae* dans le moût de raisins parasités par *Botrytis cinerea;* Inhibition de la fermentation; formation d'acide acétique et de glycérol. C.r. Acad. Sci. *289* (1979) 441–444.
59 Samson, F.E., Katz, A.M., and Harris, D.L., Effects of acetate and other short-shain fatty acids on yeast metabolism. Archs Biochem. Biophys. *54* (1955) 406–417.
60 Sapis-Domercq, S., Bertrand, A., Mur, F., and Sarre, C., Influence des produits de traitement de la vigne sur la microflore levurienne. Conn. Vigne Vin *10* (1976) 369–390.
61 Schopfer, J.F., Bourbes et débourbages des moûts dans la vinifiction en blanc. Revue suisse Vitic. Arboric. Hortic. *1* (1969) 102–109.
62 Slonimsky, P.P., Adaptation respiratoire: développement du système hémoprotéique induit par l'oxygène, pp. 242–247. Academic Press, New York 1955.
63 Sozzy, T., Gnaegi, F., d'Amico, N., and Hose, H., Difficultés de fermentation malolactique du vin dues à des bactériophages de *Leuconostoc oenos*. Revue suisse Vitic. Arboric. Hortic. *14* (1982) 17–23.
64 Strehaino, D., Moreno, M., and Goma, G., Fermentation alcoolique, observation cinétique. Conn. Vigne Vin *13* (1979) 281–291.
65 Sylver, J., and Leighton, T., Control of malolactic fermentation in wine, Isolation and characterization of a new malolactic organism. Am. J. Enol. Vitic *32* (1981) 64–72.
66 Torres-Alegre, V.M., Formation des acides gras et autres produits secondaires au cours de la vinification, Interprétation statistique des résultats. Thèse Docteur-Ingénieur, Université de Bordeaux II, Bordeaux 1982.
67 Tortora, P., Hanozet, G.M., Guerritore, A., Vincenzini, M.T., and Vanni, P., Selective denaturation of several yeast enzymes by free fatty acids. Biochim. biophys. Acta *522* (1978) 297–306.
68 Traverso-Rueda, S., A quantification of the stimulatory effect of sterols on the growth rate of *Saccharomyces cerevisiae* under vinification conditions. Ph. D. Dissertation, University of California, Davis 1980.
69 Webb, R.B., Laboratory studies of the malolactic fermentation. Am. J. Enol. Vitic. *13* (1962) 189–195.
70 Webb, R.B., and Ingraham, J.L., Induced malolactic fermentation. Am. J. Enol. Vitic. *11* (1960) 59–63.

0014-4754/86/080904-11$1.50 + 0.20/0

Wine technology: Current trends

by D. Dubourdieu

Institut d'Œnologie, Université de Bordeaux, 351 Cours de la Libération, F-33405 Talence Cedex (France)

Key words. Wine technology; wine-making; aging; clarification; stabilization.

Introduction

In the last decade, the consumption of wine in the world has been marked by a decreasing demand for those standard products of minimum quality which have few distinctive characteristics.

On the other hand, wines which have the specific qualities of the great cultivars on good viticultural soil are gaining a wider and wider following. The current high prices of wines from the prestigious growths are testimony to this fact. Moreover, throughout the wine-producing world, the more modest wines, whose originality and quality are recognized by the consumer, are becoming more and more popular. Modern enology has brought about this change.

Technological research initially took as its objective the elimination of major defects that might have an influence on the quality of a wine as regards its presentation, aroma and taste. Thus, the first technological work, particularly that of J. Ribéreau-Gayon from the 1930s onwards, concentrated on the chemical nature and manifestations of cloudiness and sediments of various origins that can occur in wines. Preventive techniques guaranteeing the physico-chemical stability of wines have been the result of