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Review Article

Review: Characterization and Role of Grape Solids during Alcoholic Fermentation under Enological Conditions

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Abstract: During wine production, grape solids have a large impact on fermentation characteristics and the organoleptic qualities of the resulting wine. We review here the research carried out on grape solids. We begin by focusing on the origin, physical characteristics and composition of these solids, and the changes in these aspects occurring during fermentation. We then consider the effect of solids on fermentation, the role of sterols, the control of solids and interactions between solids and other nutrients.

Solids exert their effects on alcoholic fermentation mainly by modulating lipid supply. The balance between solid content and nitrogen is a key factor in fermentation control. The study of grape solids is recent and requires further development. Knowledge of the composition of these solids, and of sterol uptake mechanisms by yeast should facilitate improvements in fermentation control.

Key words: alcoholic fermentation, grape solids, sterols, yeast.

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Introduction

During red wine processing, alcoholic fermentation occurs in the liquid and solid phases. Maceration of solid phases allows the extraction of polyphenols from the pomace. In white wine production, alcoholic fermentation is only limited to the liquid phase. The two phases are separated, by pressing, after the alcoholic fermentation for red wine and before the fermentation for white wine. The introduction of new practices into red winemaking (e.g. thermovinification, flash release, centrifuge decanter) has made it possible to carry out fermentations in the liquid phase, at low temperature, without maceration. These new technological steps include the treatment of the harvested grapes with heat, followed by the pressing of the must before alcoholic fermentation, as for white wines. Thermovinification and flash-release lead to the extraction of polyphenols before alcoholic fermentation for the production of light, fruity wines. In both systems, pressing eliminates the pomace, but a large number of solid particles nevertheless remain in the liquid phase. These solids of various sizes, generally referred to as “sludge”, are then removed by clarification before fermentation. Several clarification methods are used (Battle et al. 1998): cold-settling, filtration, centrifugation and flotation. In some cases, clarification problems from polysaccharides produced by grapewine mold diseases such as grey rots can occur, but this will not be discussed here.

Solid particles are widely considered to contain nutrients useful for fermentation (Ribéreau-Gayon 1985), such as lipids, in particular. The principal lipid components of eukaryotic membranes are phospholipids, sterols, sphingolipids and glycerolipids. These

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molecules play a key role in maintaining cell membrane integrity. They are therefore key determinants of the growth, metabolism and viability of yeasts during alcoholic fermentation. Lipids, particularly in the forms of phytosterols and fatty acids, are a major source of nutrients for fermenting yeasts (Ribereau-Gayon et al. 1975). The lipids in grape must, due to their strong hydrophobic status, are mostly provided by the solid particles. By contrast to assimilable nitrogen, the role of which in alcoholic fermentation has been extensively studied, the effect of solid particles on fermentation kinetics and yeast metabolism has been little studied. However, enological experience suggests that the amount of these particles in grape musts affects fermentation kinetics and wine quality.

This review provides an overview of research on grape solids, focusing on the latest developments in solid particles characterization and the management of these particles during enological alcoholic fermentation. We first provide an overview of current knowledge about the characteristics of solid particles. We then discuss the role of sterols in yeast metabolism. Finally, we highlight the role of solid particles during alcoholic fermentation in white wine production, and its implications for fermentation control.

1 Grape Solids

1 1 Origin and physical characteristics. Sludge consists of suspended solids (mostly cell fragments) present in white or red grape juices after pressing. The nature and quantity of grape solids vary considerably, depending on the raw material and the process used for juice extraction. The intensity of the mechanical crushing forces applied to the berries depends on the juice extraction method, which also controls the degree of filtration/retention of solid

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69 debris from the pomace. The treatment of red grapes by flash-release or thermovinification,
70 followed by pressing and liquid phase fermentation, enhances the extraction of polyphenols
71 and polysaccharides, thereby affecting the amount of extracted suspended material and its
72 characteristics. This specific red winemaking process results in red grape juices that are
73 generally considered more turbid than white grape juices.

74 In winemaking, the grape solids present in juice are most commonly characterized by
75 determinations of turbidity and the total wet or dried suspended solids (TWSS or TDSS,
76 expressed in % (w/w)). In red musts, turbidity generally ranges from 1000 to 5000
77 nephelometric turbidity units (NTU) and TWSS ranges from 1.5 to 5 g/100 g. These parameters
78 are useful and easily accessible to winemakers, but they provide no information about the
79 nature and composition of the suspended solids or their potential impact on fermentation. In
80 addition, there is no simple and direct relationship between turbidity and TWSS (Vernhet,
81 unpublished observations, 2015). Indeed, turbidity does not only depend on the suspended
82 solid fraction, but also on particle size shape and refractive index. Thus, depending on their
83 nature and size distribution, similar quantities of suspended solid material can result in
84 different turbidities (Davies-Colley and Smith 2001).

85 The size distribution of suspended solids has been studied by light scattering in several
86 red musts obtained from various grape varieties by different heating (flash-release and
87 thermovinification) and extraction processes. Particles with a wide range of sizes (up to several
88 hundred micrometers) were detected, but most of the particles had micronic and submicronic
89 hydrodynamic diameters (< 2 μm). Large particles accounted for only a small fraction of the

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total suspended solids. These results are consistent with those obtained for a white must in a study based on impedance measurements with a Coulter counter in which 92% of particles were found to have a hydrodynamic diameter in the range indicated above (Davin and Sahraoui 1993).

1 2 Composition. Little is known about the precise nature and composition of grape solids. Analyses on small particles fractionated by static settling from a white must showed these particles to have the following overall composition (as a % dry weight): 72% total sugars, 8% lipids, 5.5% minerals, 5.2% pectins and about 2.6% nitrogen (Alexandre et al. 1994). On the basis of this overall composition and sugar content, it was concluded that the solid particles present in white musts consisted mostly of cell wall fragments. The lipids present contained 52.7% unsaturated fatty acids (UFA) (25% linoleic acid, 22.2% oleic acid and 5.5% palmitoleic acid) and 47.1 % saturated fatty acids (25% palmitic acid, 13.8% stearic acid, and 8.3% lauric acid) (Alexandre et al. 1994). In grapevine berries at maturity, the ratio between UFA and saturated fatty acids is different (72% and 28% respectively) (Roufet et al., 1987). Skin represents an important source of fatty acids, its content being 1.5-3 times higher than in pulp (Roufet et al., 1987). In solids from white and red musts, phytosterol concentrations range from 3 to 10 mg cholesterol equivalent/g dry weight and their composition is as follows: β-sitosterol (89%), campesterol (6%), stigmasterol (3%) and stigmastanol (3%) (Casalta, unpublished observations, 2015). This composition closely resembles that of berry skins, as reported by Le Fur et al. (1994). β-sitosterol has been shown to be the most abundant component of both the flesh and skin of grape berries (Ruggiero et al. 2013).

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Similar composition analyses, coupled with transmission electron microscopy (TEM), were performed on small suspended solids from red musts. The TEM observations provided evidence to suggest that the small particles in red musts consisted mostly of cell cytoplasm fragments, such as membranes of various origins, chloroplasts, tannosomes (Brillouet et al. 2013) and numerous amorphous and more or less spherical structures potentially corresponding to modified organelles or molecular/macromolecular aggregates. Contrary to expectations, insoluble cell wall fragments were either not present or did not account for a significant proportion of these fine particles. These particles were found to have the following composition, on average: 5.6% nitrogen, 13% lipids, 9 to 12% tannins, 0.3 to 1.2% anthocyanins, 6 to 9% neutral sugars and 3% ashes (Vernhet, unpublished observations, 2015). This composition differed from that of the small suspended solids in white musts. The principal differences were a much smaller amount of carbohydrates, a much higher amount of proteins and the presence of polyphenols, including tannins in particular. The possible presence of polyphenols in suspended solids in white musts has neither been checked. Although much lower amount are expected by comparison to red musts, this should be confirmed due to their potential impact on the overall quality. Neutral sugars were analyzed by gas chromatography after trifluoroacetic acid or Saeman hydrolysis (Harris et al. 1984, Saeman et al. 1954) and the conversion of monosaccharides into their alditol acetate derivatives. Most of the polysaccharides present in these fine particles were found to originate from water-soluble pectic polysaccharides rather than from insoluble cell wall fragments, consistent with TEM observations. Neutral sugar analyses can provide useful information about the nature of the

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132 polysaccharides associated with small particles, but such analyses do not take into account the
133 acidic sugars accounting for 30 to 40% of soluble cell wall polysaccharides in grape. The total
134 sugar content is therefore underestimated. It has been suggested that these soluble
135 polysaccharides were present in suspended solids due to their involvement in aggregation with
136 tannins and/or proteins or their adsorption onto other suspended particles. The presence of
137 proteins and tannins in suspended solids is consistent with that of cell fragments and
138 organelles, such as membrane fragments and tannosomes. In addition, proteins and tannins are
139 probably present in the form of amorphous aggregates or adsorbed on cell fragments. Beside
140 their propensity to interact with proteins, the tendency of tannins to adsorb at interfaces is well
141 known and has been studied in enology in different contexts, including that of their interactions
142 with grape cell walls (Cartalade and Vernhet, 2006; Hanlin et al., 2010, Bindon et al, 2010).
143 Aggregation phenomena are likely to be more frequent in red grape juices than in white grape
144 juices, due to the simultaneous presence of proteins and tannins. This could explain the higher
145 amount of proteins in suspended solids of red musts. The lipids present in small particles were
146 analyzed by thin layer chromatography. They were found to be apolar lipids (sterols,
147 diacylglycerides and triacylglycerides) and polar phospholipids and glycolipids (steryl glycosides
148 and esterified steryl glycosides).

149 **1. 3 Changes in solids during fermentation.** Few studies have investigated the changes
150 in solids occurring during enological alcoholic fermentations. Casalta et al. (2009) showed that
151 the physical behavior of the sludge particles depended on the CO_2 production of the yeast
152 during white winemaking. This study showed that solid particle dynamics could be divided into

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three phases clearly linked to the fermentation activity of the yeast. The first phase corresponded to the lag phase and the earliest part of the growth phase. Solids rapidly settled at the bottom of the tank during the first hour, resulting in a large decrease in turbidity. The second phase corresponded to the main part of the growth phase. Medium-sized and large solid particles (diameter exceeding about 1 μm) broke up into smaller particles (0.1-0.2 μm), probably due to strong agitation mediated by the bubbling of CO_2. Indeed, the medium-sized and large particles were found to consist of several small aggregated particles. At this point in the progression of the fermentation, the solids were dispersed in the medium and any deposits rapidly disappeared. The third phase was completed during stationary phase. Some of the small particles aggregated together again, leading to the appearance of new medium-sized and large particles, which then deposited with the yeast lees at the bottom of the tank. At the end of this last phase, the solids became more compacted, decreasing the height of the layer of the sediment. Changes in must turbidity during fermentation were thus much more complex than expected: the solids were completely dispersed during fermentation, but mean turbidity never reached initial levels. Initial turbidity cannot, therefore, be considered representative of solid particle dynamics.

2 Effect of solids on fermentation

2.1 Role of sterols and UFA in yeasts. Solids are an important source of nutrients for yeasts during fermentation, principally due to their non-soluble grape phytosterol content (Luparia et al. 2004). They compensate, at least partially, for oxygen (Andreasen and Stier, 1953) and long-chain fatty-acid deficiencies (Cabanis and Flanzy 1998). At the start of the

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alcoholic fermentation, they are required for growth, with 2 to 4 mg phytosterols/L required for maximal growth (Deytieux et al. 2005). These molecules subsequently enable the yeasts to tolerate high ethanol concentrations, and they decrease the risks of sluggish and stuck fermentations. They play a key role in yeast metabolism, which has been described in detail.

In the presence of oxygen, cells can *de novo* synthesize their own major sterol, ergosterol, in the membrane of the endoplasmic reticulum, and transport it to the plasma membrane. In the absence of oxygen, cells take up sterols from the environment and transport the lipid back into the membrane of the endoplasmic reticulum, where the free sterol becomes esterified and is stored in lipid droplets. Steryl ester formation is thus a reliable readout for assessing the retrograde transport of endogenous sterols from the plasma membrane to the endoplasmic reticulum (Jacquier and Schneiter 2012). However, stigmasterol is directly incorporated into the plasma membrane in its free form (Luparia et al. 2004). Steryl esters are the main components of yeast lipid particles (Leber et al. 1994).

Sterol uptake is a multistep process. It involves interaction between external sterols and the cell wall, the incorporation of sterol into the plasma membrane and its subsequent integration into the intracellular membranes for turnover. The sorting of sterols and their transport between membranes remain poorly understood, but both vesicles and non-vesicular pathways appear to be involved. In one such pathway in *Saccharomyces cerevisiae*, exogenous sterols are transported from the plasma membrane to the endoplasmic reticulum. Yeasts do not take up exogenous sterol under aerobic conditions, but they are auxotrophic for sterol in the absence of oxygen (Fornairon-Bonnefond et al. 2002). Uptake assays have shown that 16

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genes are required for sterol uptake/transport and esterification (Reiner et al., 2005), and it has been suggested that incorporation into the plasma membrane is an early step in sterol uptake (Reiner et al. 2006). Preliminary studies have described some of the physiological properties and effects of sterols on aerobic metabolism (Smith and Parks 1993, 1997), cell cycle completion (Dahl et al. 1987), sterol uptake (Lorenz et al. 1986) and sterol transport (Tuller and Dam 1995). Sterols are essential lipid components of yeast membranes and are responsible for ensuring the integrity of the membrane. Many studies have shown that sterols are important regulators of membrane permeability and fluidity (Daum et al. 1998) and that these molecules play a key role in plasma membrane H^+- ATPase activity. They also regulate the cellular metabolic cycle in aerobic conditions and exogenous sterol uptake (Daum et al. 1998). The sterol pathway appears to make a significant contribution to the oxygen consumption capacities of cells under anaerobic conditions (Rosenfeld et al. 2003). Yeast growth in the absence of both anaerobic growth factors (sterols and fatty acids) and oxygen leads to the accumulation of large amounts of squalene in membranes, resulting in extremely low levels of cell viability (Jollow et al. 1968, Jahnke and Klein 1983, Fornairon-Bonnefond et al. 2002).

UFA are also important in yeast metabolism. UFA/SFA ratio is important for maintaining the membrane fluidity at low temperature. In the absence of oxygen, yeast cannot synthetize these compounds and solids may be a source of them.

Yeast cells uptake fatty acids with subsequent rapid incorporation into glycerolipids. The uptake kinetics are consistent with a dual mode of transport: one is a saturable, energy-independent process suggestive of a carrier-mediated transport, the other is apparently a

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simple diffusion that predominates at high substrate concentrations. (Kohlwein and Paltauf, 1983).

During grape maturation, change in fatty acids level is low, except for linolenic acid, which decreased consistently (Roupet et al., 1987) (This loss was concerned with neutral and glycolipid fractions).

2.2 Control of solids. Given the impact of solids on both the fermentation itself and the characteristics of the wine produced, the degree of grape juice clarification required depends on the objectives of the winemaker. There are several reasons for clarifying white grape juices before fermentation: i) a large proportion of oxidative enzyme activities (plant cell polyphenol oxidase or mold laccase, which could deplete the must of oxygen), and of elemental sulfur and other vineyard residues (source of the H_2S produced by yeasts during fermentation) are associated with pulp and skin fragments, ii) there is evidence to suggest that grape tissues contain an esterase that limits the accumulation of esters produced by the yeast during fermentation (Boulton et al. 1996).

However, several studies (Ribéreau-Gayon 1985, Houtman and Duplessis 1986, Feuillat et al. 1989) have reported negative effects of excessive must clarification. In addition to its effect on yeast nutrition, sludge may have a physical effect on fermentation, by favoring CO_2 nucleation (Groat and Ough 1978, Axcell et al. 1988). Dissolved CO_2 can be adsorbed onto fine particles at specific nucleation sites, at which the CO_2 bubbles increase in size before being released from the particle as free CO_2 bubbles (Kühbeck et al. 2007). This mechanism decreases the concentration of dissolved CO_2 in the liquid phase, thereby decreasing its toxicity to the

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yeast (Jones and Greenfield, 1982). Nevertheless, Casalta et al. (2012) have shown that the effects of sludge on alcoholic fermentation result principally from the provision of lipids. These authors compared alcoholic fermentation in a highly clarified must without solids with that of the same must supplemented with solids depleted of lipids. Yeast population and fermentation kinetics were found to be very similar in both sets of conditions.

Turbidity is the principal criterion used by enologists to evaluate the level of clarification. In white wine production, it is usually recommended to maintain turbidity at a sufficiently high level (50-150 NTU) to ensure that the lipid requirements of the yeast are satisfied (Charrier et al. 2013).

It should also be stressed that the use of new clarifying technologies, such as centrifuge decanters, rather than classical pressing systems, leads to the production of smaller solid particles during red and white/rosé winemaking, presumably providing the yeast with better access to nutrients during fermentation (Eudier et al. 2011, Duquene et al. 2014).

2.3 Interaction between solids and other nutrients. In their role as the lipid suppliers, solids interact with other nutrients, including oxygen and assimilable nitrogen (amino acids and ammonium) in particular (nitrogen considered as a non-gaseous nutrient). Oxygen is involved in the ergosterol metabolic pathway. Its addition is therefore one of the best ways of compensating for lipid deficiencies in yeasts. Sablayrolles and Barre (1986) estimated oxygen requirements at about 10 mg/L in the absence of lipids in the must. The timing of oxygen addition is at least as important as the amount added. Oxygen is most effective when added at the end of the growth phase (Sablayrolles and Barre 1986, Vivas and Cros 1991), when the

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yeasts have used up their stock of lipids. Another advantage of adding oxygen at this stage is that it limits the risk of oxygen use by polyphenol oxidases. Indeed, oxygen consumption by oxidases is probably much less effective than oxygen assimilation by yeasts at this time point, but not at the inoculation stage.

Assimilable nitrogen is generally considered to be the principal limiting nutrient in winemaking fermentations (Bely et al. 1990). However, Casalta et al. (2012) showed that, in white winemaking, grape solids or optimized oxygen additions were required for complete consumption of the assimilable nitrogen present in the must. In the absence of solids, the lipid stock of the inoculated yeast is diluted to an excessive extent, preventing the yeast from incorporating nitrogen. Such conditions are associated with higher cell mortality and a greater risk of stuck fermentation. At higher assimilable nitrogen content, larger quantities of grape solids are required. These data highlight the importance of taking the balance between assimilable nitrogen and lipids into account in the control of white wine production (Casalta et al. 2013, Tesnière et al. 2013).

Conclusion

This review highlights the key role of solid particles during alcoholic fermentations to produce wine. Solid particles provide the yeast with essential nutrients and are, thus, a determinant factor in alcoholic fermentation.

The main mechanisms underlying the impact of solids on fermentation kinetics have been deciphered. Nevertheless, further knowledge about grape solids is required to address several unresolved issues: i) Understanding solid composition and structure during

fermentation, and the changes in solid composition and structure throughout this process. ii) The bioavailability of nutrients should be investigated, with the definition of more appropriate criteria for characterizing the nature and properties of sludge and determining its efficiency. iii) Understanding of the mechanisms by which yeasts take up sterols from solids for the maintenance of membrane structure and metabolism. iv) Improvements to the description of the impact of solids on aroma synthesis during fermentation.

From a practical point of view, the main challenge will be optimizing control strategies by taking solid management into account, together with other key control components, such as the addition of oxygen and nitrogen.

Literature Cited

Alexandre, H., T. Nguyen Van Long, M. Feuillat and C. Charpentier. 1994. Contribution à l'étude des bourbes : influence sur la fermentescibilité des moûts. Rev. Fr . Œnol. 146: 11-20.

Andreasen, A.A. and T.J.B. Stier. 1953. Anaerobic nutrition of *Saccharomyces cerevisiae.* I. Ergosterol requirement for growth in a defined medium. J. Cell. Comp. Physiol. 41:23-36.

Axcell, B., L. Kruger, and G. Allan. 1988. Proceedings of the 20th Convention of the Institute of Brewing. Australia and New Zealand 201. 2. Berry, D. R.

Battle, G.L., J.C. Boulet, A. Brugirard, M. Dubernet, J.L. Escudier, M. Moutounet, B. Saint-Pierre and A. Vernhet. 1998. Ch. 19. Clarification, stabilisation des vins *In* Œnologie fondements scientifiques et technologiques Tec & Doc (ed.) pp. 920-1001. Lavoisier Publishers, Londres, Paris, New York

Bely, M., J.M. Sablayrolles and P. Barre. 1990. Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in enological conditions. J. Ferm. Bioengin. 70:246-252.

Bindon, K.A., P.A. Smith, and J.A. Kennedy. 2010. Interaction between grape-derived proanthocyanidins and cell wall material. 1. Effect on proanthocyanidin composition and molecular mass. J. Agric. Food Chem. 58:2520-2528.

Boulton, R.B., V.L. Singleton, L.F. Bisson and R.E. Kunkee. 1996. Preparation of musts and juice. *In* Principles and practices of winemaking, Chapmann et Hall (ed), pp. 65-101. International Thomson Publishing, New York.

306 Brillouet, J.M., C. Romieu, K. Solymosi, V. Cheynier, H. Fulcrand, J.L. Verdeil and G. Conéjéro. 2013. The
307 tannosome is an organelle forming condensed tannins in the chlorophyllous organs of Tracheophyta,
308 Annals of Botany 112:1003-1014.

309 Cabanis, J.C. and C. Flanzy. 1998. Lipides. *In* Œnolologie – Fondements Scientifiques et Technologiques.
310 Flanzy C. Tec & Doc (ed), pp. 3-34. Lavoisier, Paris.

311 Cartalade, D. and A. Vernhet. 2006. Polar interactions in flavan-3-ol adsorption on solid surfaces. J. Agric.
312 Food Chem. 54, 3086-3094.

313 Casalta, E., E. Aguera, Ph. Liénard and J.M. Salmon. 2009. Physical dynamics of sludges during wine
314 fermentation in liquid phase. J. Int. Sci. Vigne Vin 43:225-230.

315 Casalta, E., M.F. Cervi, J.M Sablayrolles and J.M. Salmon. 2012. Effet combiné des niveaux d'azote
316 assimilable et de bourbes : nouveau parameter à prendre en compte pour la maîtrise de la fermentation
317 alcoolique. Rev. Fr. Œnol. 255:9-15.

318 Casalta, E., M.F. Cervi, J.M. Salmon and J.M. Sablayrolles. 2013. Effect of combined levels of assimilable
319 nitrogen and sludges on alcoholic fermentation under enological conditions. Aust. J. Grape and Wine
320 Res. 19:47-52.

321 Charrier F., L. Guérin, A. Roland and A. Samson 2013. Opérations préfermentaires , In "Les vins blancs
322 de la démarche marketing à la vinification: les clés d'un pilotage réussi", Ed. France Agricole, Paris, pp
323 146-163.

324 Dahl, C., H.P. Biemann and J. Dahl. 1987. A protein kinase antigenically related to pp60 src possibly
325 involved in yeast cell cycle control: positive in vivo regulation of sterol. Proc. Natl Acad. Sci. USA,
326 84:4012-4016.

327 Daum, G., N.D. Lees, M. Bard and R. Dickson. 1998. Biochemistry, Cell Biology and Molecular Biology of
328 Lipids of *Saccharomyces cerevisiae*. Yeast 14:1471-1510.

329 Davies-Colley, R.J. and D.G. Smith. 2001. Turbidity, suspended sediments and water clarity : a review. J.
330 Am Water Ressources Association 35: 1085-1101.

331 Davin, A. and A. Sahraoui. 1993. Débourbage rapide des moûts de raisin par flottation à l'aide de bulles
332 générées au sein du liquide par dépressurisation. Rev. Française Oenol. 140 : 53-64

333 Deytieux, C., L. Mussard, M.J. Biron and J.M. Salmon. 2005. Fine measurement of ergosterol
334 requirements for growth of *Saccharomyces cerevisiae* during enological fermentations. Appl. Microbiol.
335 Biotechnol. 68:268-271.

336 Duquene, P., A. Samson, C. Rapilly, B. Bernet and J.M. Salmon. 2014. Positionnement du décanteur
337 centrifuge en vinification en blanc et rosé. Rev. Fr. Œnol. 266:8-16.

338 Eudier, L., A. Samson, S. Caille, E. Aguera, M. Bes, G. Dangleville, L. De Vlieger and J.M. Salmon. 2011. Le
339 décanteur centrifuge Alfa-Laval : apport d'un nouvel outil pour le prétraitement de la vendange thermo
340 traitée avant fermentation. Rev. Œnol. 138:18-20.

341 Ferreira, R.B., M.A. Piçarra-Pereira, S. Monteiro, V.B. Loureiro and A.R. Teixeira. 2002. The wine
342 proteins. Trends in Food Science and Technology 12, 230-239.

343 Feuillat, M., 1995. La clarification des moûts en vinification en blanc ou débourbage, Rev. Fr. Œnol.
344 153:69-74.

345 Feuillat, M., M. Freyssinet, and C. Charpentier. 1989. L'élevage sur lies des vins de Bourgogne. II
346 Evolution des macromolécules: polysaccharides et protéines. Vitis 28:161-176.

347 Fornairon-Bonnefond, C., V. Desmaretz, E. Rosenfeld. and J.M. Salmon. 2002. Oxygen addition and sterol
348 synthesis in *Saccharomyces cerevisiae* during enological fermentation. J. Biosci. Bioengin. 93:176-182.

349 Fornairon-Bonnefond, C., E. Aguera, C. Deytieux, J.M. Sablayrolles and J.M. Salmon. 2003. Impact of
350 oxygen addition during enological fermentation on sterol contents in yeast lees and their reactivity
351 towards oxygen. J. Biosci. Bioengin. 95:496-503.

352 Gallander, J.F. and A.C. Peng. 1980. Lipid and fatty acid composition of different grape types . Amer. J .
353 Enol . Vitic . 31:24-27.

354

355 Groat, M. and C.S. Ough. 1978. Effects of Insoluble Solids Added to Clarified Musts on Fermentation
356 Rate, Wine Composition, and Wine Quality. Am. J. Enol. Vitic. 29:112-119.

357 Hanlin R.L, M. Hrmova, J.F. Harbertson and M.O. DOWNEY, 2010. Review: Condensed tannin and grape
358 cell wall interactions and their impact on tannin extractability into wine. Aus. J. Grape and Wine Res.
359 16:173-188.

360 Harris, P.J., R.J. Henry, A.B. Blakeney and B.A. Stone. 1984. An improved procedure for the methylation
361 analysis of oligosaccharides and polysaccharides. Carbohydr. Res. 127:59-73.

362 Høj, P.B., D.B. Tattersall, K.S. Adams, K.F. Pocock, Y. Hayasaka, R. van Heeswijck. and E.J. Waters. 2001.
363 The 'haze proteins' of wine – a summary of properties, factors affecting their accumulation in grapes,
364 and the amount of bentonite required for their removal from wine. Rantz, J.M. , ed. Proceedings of the
365 ASEV 50th Anniversary Meeting; 19-23 June 2000; Seattle, WA, USA (American Society of Enology and
366 Viticulture: Davis, CA, USA) pp. 149-154.

367 Houtman, A.C. and C.S. du Plessis. 1986. Nutritional deficencies of clarified white grape juices and their
368 correction in relation to fermentation. S. Afr. J. Enol. Vitic. 1:39-46.

369 Jacquier, N. and R. Schneiter. 2012. Mechanisms of sterol uptake and transport in yeast J. Steroid
370 Biochem. Mol. Biol. 129:70-78.

371 Jahnke, L., and H.P. Klein. 1983. Oxygen requirements for formation and activity of the squalene
372 epoxydase in *Saccharomyces cerevisiae*. J. Bacteriol. 155:488-492.

373 Jollow, D., G.M. Kellerman and A.W. Linnane. 1968. The biogenesis of mitochondria. III. The lipid
374 composition of aerobically and anaerobically grown *Saccharomyces cerevisiae*. As related to the
375 membrane systems of the cells. J. Cell Biol. 37:221-230.

376 Jones, R. P. and P.F. Greenfield. 1982. Effect of carbon dioxide on yeast growth and fermentation.
377 Enzyme Microb. Technol. 4:210-223.

378 Kennedy J.A., C Saucier and Y. Glories. 2006. Grape and Wine Phenolics: History and Perspective. Am. J.
379 Enol. Vitic. 57:239-248.

380 Kohlwein S.D. and F. Paltauf. 1983. Uptake of fatty acids by the yeasts *Saccharomyces uvarum* and
381 *lipolytica.* Biochimica et Blophysica Acta. 792:31O-317.

382 Kühbeck, F., M. Müller, W. Back, T. Kurz, and M. Krottenthaler. 2007. Effect of hot trub and particle
383 addition on fermentation performance of *Saccharomyces cerevisiae* Enzyme Microb. Technol. 41:711-
384 720.

385 Leber, R., E. Zinser, G. Zellnig, F. Paltauf, and G. Daum. 1994. Characterization of lipid particles of the
386 yeast, *Saccharomyces cerevisiae*. Yeast 10:1421–1428.

387 Le Fur, Y., C. Hory, M.H. Bard and A. Olsson. 1994. Evolution of phytosterols in Chardonnay grape berry
388 skins during last stages of ripening. Vitis 33:127-131.

389 Lorenz, R. T., R.J. Rodriguez, T.A. Lewis, and L.W. Parks. 1986. Characteristics of sterol up-take in
390 *Saccharomyces cerevisiae*. J. Bacteriol. 167:981-985.

391 Luparia, V., V. Soubeyrand, T. Berges, A. Julien and J.M. Salmon. 2004. Assimilation of grape phytosterols
392 by *Saccharomyces cerevisiae* and their impact on enological fermentations. Appl. Microbiol. Biotechnol.
393 65:25-32.

394 Mauricio, J.C., J.J. Moreno, E.M. Valero, L. Zea, M. Medina and J.M. Ortega. 1993 Ester formation and
395 specific activities of in vitro alcohol acetyltransferase and esterase by *Saccharomyces cerevisiae* during
396 grape must fermentation. J. Agric. Food Chem. 41:2086-2091.

397 Reiner, S., D. Micolod and R. Schneiter. 2005. *Saccharomyces cerevisiae*, a model to study sterol uptake
398 and transport in eukaryotes. Biochem. Soc. Trans. 33:1186-1188.

399 Reiner, S., D. Micolod, G. Zellnig and R. Schneiter. 2006. A genome wide screen reveals a role of
400 mitochondria in anaerobic uptake of sterols in yeast. Mol. Biol. Cell 17:90-103.

401 Ribereau–Gayon, P., S. Lafon-Lafoucarde and A. Bertrand. 1975. Le débourbage des moûts de vendange
402 blanche. Connaissance de la Vigne et du Vin 9:117-138.

403 Ribereau-Gayon, P. 1985. New development in wine microbiology. Am. J. Enol. Vitic. 36:1-10.

404 Rosenfeld, E., B. Beauvoit, B. Blondin and J.M. Salmon. 2003. Oxygen consumption by anaerobic
405 *Saccharomyces cerevisiae* under enological conditions: Effect on fermentation kinetics. Appl. Environ.
406 Microbiol. 69:113-121.

407 Rossignol, T., L. Dulau., A. Julien and B. Blondin. 2003. Genome-wide monitoring of wine yeast gene
408 expression during alcoholic fermentation. Yeast 20:1369-1385.

409 Roufet, M., C.L. Bayonove and R.E. Cordonnier. 1987. Etude de la composition lipidique du raisin *Vitis*
410 *vinifera L.*: évolution au cours de la maturation et localisation dans la baie. Vitis 26:85-97.

411 Ruggiero, A., N. Vitalini., S. Bernasconi. and M. Iriti. 2013. Phytosterols in grapes and wine and effects of
412 agrochemicals on their levels. Food Chem. 14:3473-3479.

413 Sablayrolles J.M. and P. Barre. 1986. Evaluation of oxygen requirement of alcoholic fermentation in
414 simulated enological conditions. Sci. aliments 6:373-383.

415 Sablayrolles J.M., C. Dubois, C. Manginot, J.L. Roustan and P. Barre. 1996. Effectiveness of combined
416 ammoniacal nitrogen and oxygen additions for completion of sluggish and stuck wine fermentations. J.
417 Ferm. Bioengin. 82:377-381.

418 Saeman, J.F., W.E. Moore, R.L. Mitchell, and M.A. Millet. 1954. Techniques for the determination of pulp
419 constituents by quantitative paper chromatography. Tappi 37:336-343.

420 Salmon, J.M., 2006. Interactions between yeast, oxygen and polyphenols during alcoholic fermentations:
421 practical implications. LWT-Food Sci. Technol. 39:959-965.

422 Salmon, J.M. 2011. Particules solides : impact sur le déroulement de la fermentation. Rev. Fr. Œnol.
423 247:12.

424 Sauvage, F.-X., B. Bach, M. Moutounet and A. Vernhet, 2010. Proteins in white wines: thermo-sensitivity
425 and differential adsorbtion by bentonite. Food Chemistry 118:26-43.

426 Smith S. J and L.W. Parks. 1993. The ERG3 gene in *Saccharomyces cerevisiae* is required for the
427 utilization of respiratory substrates and in heme-deficient cells. Yeast 9:1177-1187.

428 Smith S. J. and L.W. Parks. 1997. Requirement of heme to replace the sparking sterol function in the
429 yeast *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1345:71-76.

430 Tesnière, C., P. Delobel, M. Pradal and B. Blondin. 2013. Impact of nutrient imbalance on wine alcoholic
431 fermentations: nitrogen excess enhances yeast cell death in lipid-limited must. Plos One 8:e61645.

432 Tuller, G. and G. Daum. 1995. Import of sterols into mitochondria of the yeast *Saccharomyces cerevisiae*.
433 FEBS Letters 372:29-32.

434 Vivas, N. and M. Cros. 1991. Influence du contrôle de l'anaérobiose et de la température sur le cycle de
435 croissance et le métabolisme des levures. Application aux cuves à remontage séquence. Progrès Agricole
436 et Viticole 108:163-165.

437 Waters, E.J., G. Alexander, R. Muhlack, K.F Pocock, C. Colby, B.K. O'Neill, P.B Høj, and P. Jones. 2005.
438 Preventing protein haze in bottled white wine. Aust. J. Grape and Wine Res. 11:215-225.