Enological functions of parietal yeast mannoproteins

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Abstract

Parietal yeast mannoproteins play a very important role in the overall vinification process. Their production and release, both during winemaking and aging on lees, depends on the specific yeast strain and the nutritional conditions. The following enological functions of parietal yeast mannoproteins have been described: (a) adsorption of ochratoxin A; (b) combination with phenolic compounds; (c) increased growth of malolactic bacteria; (d) inhibition of tartrate salt crystallization; (e) interaction with flor wines; (f) prevention of haze; (g) reinforcement of aromatic components; (h) wine enrichment during aging on fine lees; (i) yeast flocculation and autolysis in sparkling wines. Further discoveries related to their enological functions are foreseeable. Yeast-derived mannoproteins may well induce chemical, sensorial and health benefits, thus greatly improving wine quality.

Outlines

Mannoproteins, which make up 35 –40% of the cell wall of Saccharomyces cerevisiae, are glycoproteins, often highly glycosylated, located in the outermost layer of the yeast cell wall, where they are connected to a matrix of amorphous *β*-1,3 glucan by covalent bonds (Klis et al. 2002). The degree of mannoprotein glycosylation is variable; in some cases, mannoproteins can contain over 90% sugars, mainly mannose (Ribéreau-Gayon et al. 2000).
Mannoproteins give the yeast cell wall its active properties and play an important role in controlling the wall's porosity (Brzobohaty and Kovar 1986; De Nobel et al. 1989, 1990), thereby regulating leakage of proteins from the periplasmic space, and entrance of macromolecules from the environment.
At different pH values, the electrical charge of mannoproteins is modified. In the pH range of wine, mannoproteins carry negative charges and, as a consequence, they may establish electrostatic and ionic interactions with the other components of the wine (Vernhet et al. 1996), resulting in the formation of either soluble or insoluble complexes in a process that is strongly dependent on their net electrical charge and on the structure of their functional groups (Samant et al. 1993). In the genus Saccharomyces, the glycan portion of mannoproteins is composed not only of neutral oligosaccharides containing mannose and N-acetylglucosamine, but also of acidic oligosaccharides containing mannosylphosphate, in quantities which vary from strain to strain (Jigami and Odani 1999). This modification can change the properties and environment of the cell surface, since mannosylphosphate gives a net negative charge to cell wall mannoproteins (Friis and Ottolenghi 1970). For other yeasts, a different composition of the glycan portion of mannoproteins has been described: the oligosaccharides of Schizosaccharomyces pombe and Kluyveromyces lactis contain galactose and N-acetylglucosamine, respectively, but not mannosylphosphate, whereas the oligosaccharides of Kloeckera brevis and Candida albicans contain as much mannosylphosphate as those of S. cerevisiae (Ballou 1976). Future studies may well reveal a remarkable variability in wine yeasts, both Saccharomyces and non-Saccharomyces strains. This research could evaluate the contribution of wine yeasts to the mannoprotein content of wine, allowing the development of a new methodology to categorize yeast strains for this characteristic. It is foreseeable that mannoproteins from non-Saccharomyces yeasts will be structurally different and may have different functional effects.
Mannoprotein from yeast was reported to be an effective bio-emulsifier; spent yeast from the manufacture of wine was demonstrated to be a possible source for large-scale production (Cameron et al. 1988; Kunst et al. 1997; Barriga et al. 1999). Future studies in this field could evaluate the biosurfactant properties of different yeast mannoproteins and their potential use in the food industry.
Mannoproteins are partially water-soluble components, released by the action of *β*-1,3 glucanases during and, above all, after alcoholic fermentation (Fleet 1991). Contact time, temperature, and agitation of the yeast biomass promote their enzymatic release (Llaubères et al. 1987). *β*-1,3 glucanases exhibit activity during yeast growth (wine fermentation), as well as in the presence of resting yeast cells (aging on lees). Mannoprotein production and release depend on the specific yeast strain (Rosi et al. 1999), as well as the nutritional conditions (Ribéreau-Gayon et al. 2000). A direct relationship between the degree of grape must clarification and the amount of yeast macromolecules recovered in the wine has been described (Guilloux-Benatier et al. 1995). It has been reported (Boivin et al. 1998) that yeast cell wall porosity increases in clarified must but that macromolecule production decreases; the quantities of these macromolecules released and their sugar composition depends upon yeast strain used to conduct the fermentation.
The different enological functions of the parietal yeast mannoproteins should now be considered.

Adsorption of ochratoxin A

Ochratoxin A (OTA) is a dangerous fungal secondary metabolite; this mycotoxin has been reported in grapes, grape juices and wines (Zimmerli and Dick 1996). Since the first observation that, during fermentation, three different strains of S. cerevisiae were able to decrease OTA added to wort by as much as 21% (Scott et al. 1995), various decontamination procedures for removal of OTA using yeasts (Bauer 1994; Piotrowska and Zakowska 2000; Bejaoui et al. 2004; Caridi et al. 2004a), yeast cell walls (Huwig et al. 2001; Ringot et al. 2005), or yeast cell wall extracts (Howes and Newman 2000; Ringot et al. 2005) have been developed. Mannoproteins play a considerable role in OTA adsorption, due to the mycotoxin-binding capacity reported for modified mannanoligosaccharide derived from the cell wall of S. cerevisiae (Devegowda et al. 1998; Zaghini et al. 1998; Baptista et al. 2004); moreover, the spontaneous nature of the OTA adsorption on yeast cell walls has been recently demonstrated (Ringot et al. 2005). Accordingly, mannoproteins may be used like a sponge, sequestering OTA in grape juices and wines. Remarkable differences in the in vitro binding activity of wine yeasts towards OTA have been reported (Caridi et al. 2004a), which may be explained by the different mannosylphosphate content in the mannoproteins of each wine yeast. In addition, it has been demonstrated that it is possible to greatly reduce the OTA content of grape must during winemaking by using expressly selected wine yeasts (Caridi et al. 2005). This has become more interesting since the decision of the European Community that wines produced from the 2005 harvest onwards must respect the maximum limit of 2.0 ppb (Anon 2005).

Combination with phenolic compounds

Phenolic compounds are of great interest in enology since they influence wine colour, taste, and stability. Yeast mannoproteins can combine with anthocyanins and tannins in wine; this combination seems to increase colour stability (Escot et al. 2001) and decrease astringency, giving softer tannins and strongly inhibiting their self-aggregation (Riou et al. 2002). The final result will be a wine with more body, better mouthfeel and with an increased resistance against oxidation. During barrel aging on lees, tannins given off by the wood are fixed both on the yeast cell wall and on the mannoproteins released by the lees; this gives a lower overall tannin concentration, a much lower proportion of free tannins and limits the ellagic tannin concentration (Chatonnet et al. 1992). It has been demonstrated (Escot et al. 2001) that the influence of mannoproteins is dependent upon the strain of yeast used, and that mannoproteins released during the fermentation itself are more reactive than those released during yeast autolysis. Mannoproteins are capable of combining with phenolic compounds, thus diminishing the total polyphenol index; this mechanism may well be exclusively physical, involving the establishment of weak and reversible interactions mainly between anthocyanins and yeast walls by adsorption (Vasserot et al. 1997). A recent paper has shown remarkable correlations between the yeast strain used for winemaking and the phenolic composition of wine, highlighting the fact that strain behaviour can somewhat modify the chromatic properties, the phenolic profile and the antioxidant power of wine (Caridi et al. 2004b).

Increased growth of malolactic bacteria

Malolactic fermentation consists of the conversion of L-malate to L-lactate and carbon dioxide, and plays an important role in winemaking because, besides lowering total acidity, it is usually believed to improve the biological stability and the sensory properties of the wines where it occurs. Parietal yeast mannoproteins have been associated with stimulation of malolactic bacteria growth in wine (Guilloux-Benatier et al. 1995). This could be due to the adsorption of the medium chain fatty acids synthesized by Saccharomyces (Guilloux-Benatier and Feuillat 1991). These compounds have been shown to inhibit bacterial growth and, therefore, their removal improves malolactic fermentation. Moreover, malolactic bacteria are able to hydrolyze mannoproteins, thus enhancing the nutritional content of the medium and also stimulating their activity (Guilloux-Benatier and Chassagne 2003).

Inhibition of tartrate salt crystallization

Precipitation of tartaric acid salt in the course of winemaking greatly lowers the acidity. Using mannoproteins, it is possible to prevent the tartrate salt insolubilization (Lubbers et al. 1993) thus getting a better control of wine stability. It has been shown that mannoproteins can effectively inhibit the crystallization of tartrate salt by lowering the crystallization temperature (Gerbaud 1996; Moine-Ledoux and Dubourdieu 2002). The crystal seeding process is slowed down by highly glycosylated mannoproteins with molecular weights between 30 and 50-kDa, which improve tartaric stability.

Interaction with flor wines

The flor technique is the process in which, at the end of winemaking, specific film-forming yeasts (flor yeasts) spontaneously develop on the surface of the wine forming a thick mat of cells (velum). During wine aging, there is an inverse relation between yeast viability and concentration of macromolecules in solution, and a direct relation between biomass and macromolecules in solution (Dos Santos et al. 2000). A 49-kDa hydrophobic cell wall mannoprotein present in a velum yeast has been identified and correlated with velum formation and surface hydrophobicity (Alexandre et al. 2000).

Prevention of haze

Haze is a common problem in white wines, caused by the slow denaturation and flocculation of grape proteins. A polysaccharide active in promoting the stability of wine has been isolated and characterized from its total colloidal fraction (Waters et al. 1994): it is a high mass mannoprotein with a molecular weight of 420-kDa, present in a very low concentration in wine – 0.007% of total polysaccharides – which derives from fermenting yeasts. The presence in wines of this glycoprotein, termed haze-protective factor, reduces the visible haziness by decreasing the particle size of the haze (Waters et al. 1993). This observation explains the lees-induced protein stabilization of white wines. The improvement by the lees of the wine's thermal stability is due neither to removal of the unstable protein fractions nor to the proteolytic activities present in yeasts, but rather to the addition of yeast mannoproteins (Ledoux et al. 1992). Improvement in the protein stability of white wines during barrel-aging on the lees is a well-known phenomenon. However, the grape proteins responsible for the instability of white wines are not digested or adsorbed by the lees during aging: they become heat-stable in the presence of a 32-kDa, N-glycosylated, heat-stable mannoprotein.

Reinforcement of aromatic components

The flavour of wine is a sensory perception that varies with the individual, the context of the consumer experience and the chemical composition of the product; the final response is the outcome of complex chemosensory interactions that are difficult to predict because of the influences of many variables (Fleet 2003). It has been shown (Feuillat et al. 1987) that wine clarification and stabilization processes exert a negative influence upon sensory properties when the rate of eliminated macromolecules reaches 30%. When the macromolecule content of the wine is reduced by filtration, losses of colour intensity, aroma, and flavour are observed; intensity of aroma and persistence of flavour are lessened. Aroma stabilization is dependent upon the hydrophobicity of the aroma compounds, and the protein component of the mannoproteins is important for overall aroma stabilization (Lubbers et al. 1994). Interactions between mannoproteins and aromatic compounds can lead to modifications of volatility and aromatic intensity of wines; in this case, mannoproteins are free to interact and to fortify the existing aroma components.

Wine enrichment during aging on fine lees

The aging of wine on yeast lees at the end of the alcoholic fermentation can greatly affect the concentration of nutrients, including amino acids, peptides and protein, through passive release or yeast autolysis. This winemaking practice improves structure, richness and roundness of wine; it is used to either protect the wine from oxidation or to add complexity of aroma and flavour to the wine. It has been demonstrated (Feuillat 1998; Charpentier and Feuillat 1993) that periodic stirring of the wine while on lees increases the mannoprotein level and the amount of yeast-derived amino acids and that wines aged on their lees in barrel exhibit an increase in colloidal macromolecules. The liberation of amino acids and glucose during barrel aging on lees has also been studied (Guilloux-Benatier et al. 2001), with and without the addition of exogenous β-1,3 glucanase preparations. Little or no increase in amino acids in wine stored on lees versus wine stored on lees with the addition of β-1,3 glucanase was found.

Yeast flocculation and autolysis in sparkling wines

Mannoproteins contribute, together with all the other cell wall constituents, to the flocculation of yeast strains (Suzzi et al. 1984; Klis et al. 2002) specifically used in the manufacture of several sparkling wines. Using traditional methods, the wine is subjected to an aging process in contact with the yeast that has produced the fermentation; during aging, the yeast undergoes autolysis, which significantly changes the sensorial characteristics of the wine (Martìnez-Rodriguez et al. 2001). Yeast autolysis is a very slow process which involves hydrolytic enzymes, may require many months and usually increases the complexity of the wine. Thus, the following methods of increasing mannoprotein levels may be explored: (a) the selection of yeasts which produce high levels of mannoproteins during alcoholic fermentation, or which autolyse rapidly upon its completion, and (b) the addition of exogenous mannoproteins or of β-1,3 glucanase to wines stored on lees. Attempts have been made to reduce the time required for aging by adding various yeast autolysates. However, the product thus obtained is slightly oxidized and acidic whereas the natural product is judged to be more mature (Feuillat 2003). The rate of autolytic release of mannoproteins from the yeast cell wall (Sanz et al. 1985) may, however, be greatly accelerated using autolysogenic strains of S. cerevisiae (Zambonelli et al. 1991).

Conclusion

It appears that parietal mannoproteins can play a very important role in the overall vinification process. Further discoveries related to their enological functions are foreseeable. Yeast-derived mannoproteins may well induce chemical, sensorial and health benefits, thus greatly improving wine quality.

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