THE PROTECTIVE ROLE OF STEROLS DURING ACTIVE DRY YEAST REHYDRATION

Anne ORTIZ-JULIEN[1] & Jean-michel SALMON[2]
1. Lallemand SA. 19, rue des Briquetiers, Blagnac Cedex, 31702 France
2. Unité Mixte de Recherches «Sciences pour l'Oenologie», INRA, 2, place Viala, F-34060 Montpellier Cedex 1, France.
jmsalmon@ensam.inra.fr

Summary
In winemaking, the active dry yeasts (ADY) used for the tailored inoculation of musts are all commercialized in a dehydrated form. Thus, a rehydration phase is required in order to reactivate these yeasts before inoculation. This rehydration phase is particularly necessary to ensure a satisfactory plasma membrane integrity, which is an essential prerequisite to maintain yeast viability during fermentations. At the stage of rehydration, the addition of specific soluble sterols allows to improve the structure of the plasma membrane allowing the yeasts to ensure the alcoholic fermentation, specifically when the medium conditions are difficult. In this sense, the sterols are important to the protection of the yeasts during the rehydration phase.

Modifications of the plasma membrane during the yeast dehydration and rehydration phases

Yeast dehydration leads to profound modifications of the internal cell structure because of the reduction of the total cell volume (1). This cellular volume reduction leads to significant wrinkles in the plasma membrane and causes ruptures in the continuity of the membrane (2, Figure 1).

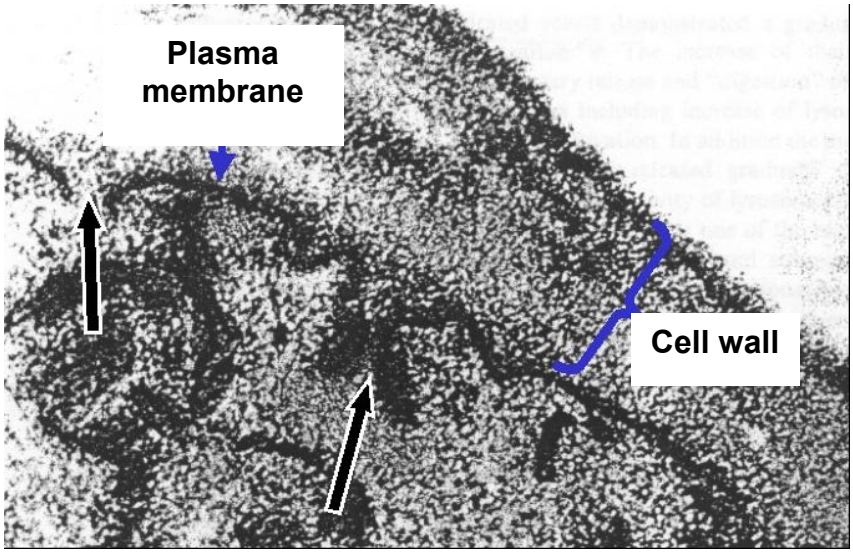

FIGURE 1: Electron microscope image of dehydrated Saccharomyces cerevisiae cells (magnification 145,000x). The plasma membrane is strongly pleated. The black arrows show the ruptures in the plasma membrane (according to reference 1).

During the rehydration phase of the active dry yeasts, the cells start by mobilizing certain lipid reserves in order to repair the damaged membranes rapidly (3). We recently demonstrated in our research unit that the yeasts could also incorporate extracellular lipids in order to repair plasma membrane defects during this rehydration stage (4). This rapid incorporation (less than 15 minutes) thus allows the cell to restore completely functional

membranes rapidly. We were specifically interested in the incorporation of sterols, a class of compounds known for their important role in cell survival during the final stages of alcoholic fermentations (5, 6). Sterol incorporation was measured with a radioactive tracer ([4-^{14}C] cholesterol) during a standard rehydration at 37°C in the presence of a sterol solution (Figure 2).

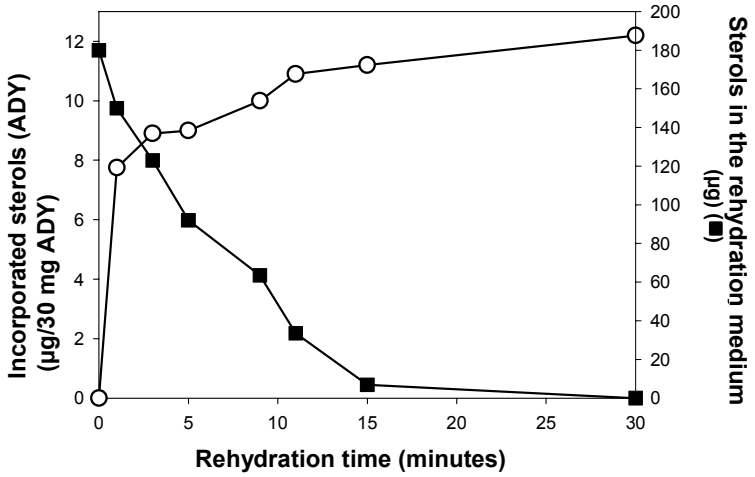

FIGURE 2: Course of sterol incorporation measured with [4-^{14}C] cholesterol during the rehydration of the reference strain (1 g) in 10 ml of a glucose solution (0.5 g) in the presence of a sterol solution (25 mg) at 37°C.

Thus, it is clear that during rehydration, active dry yeasts can incorporate extracellular sterols efficiently and rapidly in order to repair the cellular membranes damaged during dehydration (4).

Qualitative effect of different sterols on yeast growth and viability

During alcoholic fermentation, yeasts imperatively have to incorporate exogenous sterols in order to grow. In grape musts, sterols are present as phytosterols whose chemical nature is different from the sterols, which are normally synthesized by the yeasts under aerobic conditions (5). These phytosterols are mainly found in the grape skin and are generally extracted during maceration (7). A recent study carried out in our laboratory has demonstrated that a concentration of 5 mg l^{-1} of these phytosterols and above was sufficient to enable the growth of yeasts under winemaking conditions (Figure 3), thus allowing a satisfactory onset of fermentation (5).
However, because of the different chemical structure of the phytosterols and the sterols usually found in the yeast membranes, yeast viability decreased prematurely when phytosterols were the only sterol source available to the yeasts (Figure 4).

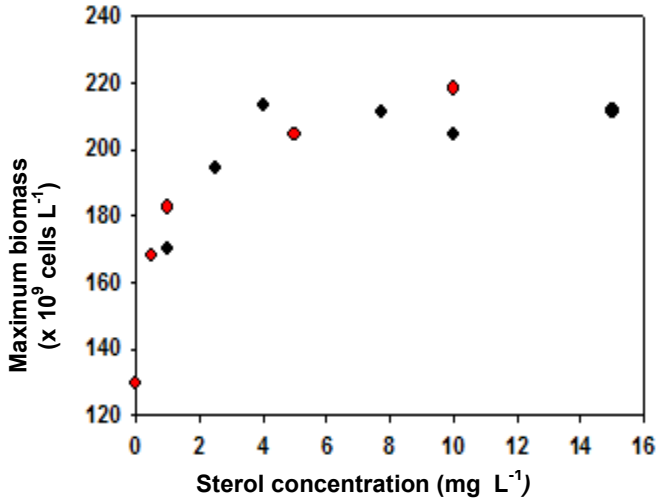

FIGURE 3: Effect of the concentration of diverse grape phytosterols (red dots) or yeast sterols (black dots) on the maximum biomass yield of the reference yeast during fermentations.

Accordingly, the corresponding fermentation rates in stationary phase were significantly affected and could lead to stuck fermentations under unfavourable conditions (5).

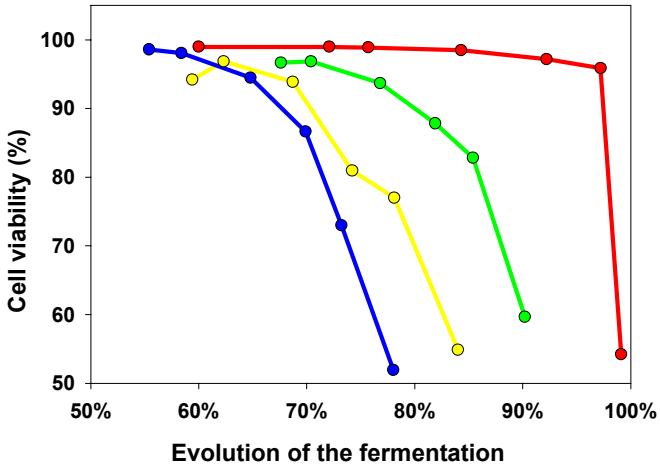

FIGURE 4: Evolution of the cell viability during the fermentation of the reference yeast strain in the presence of grape phytosterols (1 mg l^{-1}, blue curve; 5 mg l^{-1}, yellow curve; 10 mg l^{-1}, green curve) or of yeast sterols (10 mg l^{-1}, red curve).

However, numerous pre-fermentation treatments, specifically the clarification steps frequently carried out for white or rosé vinifications (such as racking, 8) lead to the flocculation of pectic aggregates to which phytosterols remain adsorbed. Thus, the musts are deficient in yeast assimilable sterols from the beginning of the fermentation. Under these conditions, starting with the rehydration of the active dry yeast and onwards, it is essential to favour an optimal cell membrane conformation by incorporating yeast specific sterols.

Effect of the incorporation of solubilized yeast sterols during rehydration on yeast fermentation performance

The effect of adding solubilized yeast sterols during the rehydration of active dry yeasts was then studied. The effect on the yeast performance was remarkable. Under difficult fermentation conditions (medium containing 8 mg l^{-1} of phytosterols), the fermentation duration could be reduced by about 20 to 25 hours (Figure 5).

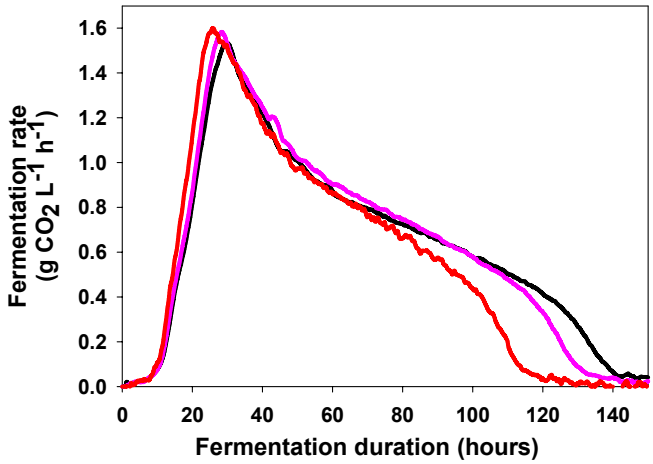

FIGURE 5: Effect of the addition of solubilized yeast sterols during rehydration of the reference strain. Rehydration without addition (black curve), in the presence of solubilized yeast sterols (12 mg l^{-1}, pink curve; 24 mg l^{-1}, red curve). The fermentation courses were followed at 24°C with a fermentation medium containing 8 mg l^{-1} of phytosterols.

The positive effect of the addition of solubilized yeast sterols during the rehydration phase on the fermentation course was due to the very significant increase of cell viability during the stationary phase of the alcoholic fermentation (Figure 6).

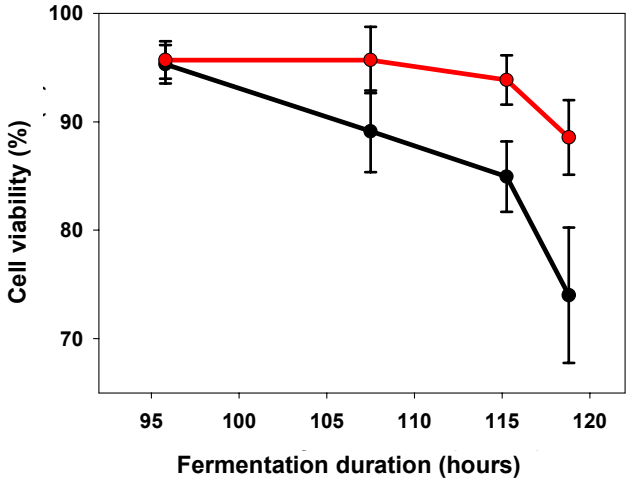

FIGURE 6: Effect of the addition of solubilized yeast sterols during the rehydration of the reference strain. Rehydration without addition (black curve), in the presence of solubilized yeast sterols (24 mg l^{-1}, red curve). Cell viabilities were followed at 24°C during the fermentation of a medium containing 8 mg l^{-1} of phytosterols.

The cell viability differences measured for the different conditions reached up to 20% by the end of the fermentation. Towards the end of the fermentations, the ethanol content becomes particularly toxic for the cell metabolism. Under these conditions, the cell populations with the highest cell viability have an advantage to complete fermentations.

Conclusions

The recent discovery of the capacity of active dry yeasts to incorporate exogenous sterols during the rehydration phase allows to envisage the specific addition of sterols during this critical technological stage. However, even though the yeasts can assimilate numerous sterols of various origins, the high efficiency of sterols of yeast origin has been proven. Under these conditions, the addition of specific and soluble yeast sterols during rehydration allows to improve the structure of the plasma membrane and accordingly enables the yeast to better ensure alcoholic fermentation, especially under difficult conditions.

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