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Acid adaptation improves viability of winemaking *Saccharomyces cerevisiae* UP3OY5 after freeze drying

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INTRODUCTION AND OBJECTIVES

Freeze drying is the most convenient and successful method of preserving microorganisms. It protects from contamination, increases viability and eases strain distribution (Abadias 2001). Substances such as polymers, sugars, albumin, milk, honey, polyols and amino acids have been tested for their protective effect during freeze drying. Components of the suspending media have two main functions in preserving viability of freeze dried cells. The first is to provide a dry residue with definite physical structure acting as a support material and as a receptor in rehydration, and the second is to protect the living cells biochemically against damage during freezing and/or drying. Glycogen and trehalose are the two major reserve carbohydrates in the yeast *Saccharomyces cerevisiae* and can represent up to 25 % of the dry cell weight, depending on the environmental conditions (Parrou 1997a). The trehalose content of yeast correlated well with viability after drying: when the yeast was grown anaerobically, its trehalose content and cryoresistance decreased (Diniz-Mendes 1999) .

It has been recognized that exposure to a mild stress can result in improved resistance to subsequent exposures, either to more extreme forms of the same stress or to other stresses. These phenomena are referred to as acquired stress resistance and cross protection (Bourdineaud 2003). Adaptation ability of yeast cells determines yeast viability. Indeed, adaptation of cells to different stresses (heat, ethanol, acid stresses) leads to synthesis of shock proteins, and changes in fatty acid membrane composition. Yeast strains which are the most resistant to thermal and ethanol stresses have membrane enriched with oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids (Swan 1999). Furthermore, yeast cells subjected to a heat shock accumulate trehalose and heat shock proteins. Trehalose is also involved in survival under different stress conditions as a membrane protectant (Hounsa 1998). The study of adaptation to different stresses was original and useful in improving yeast survival during freeze drying. Nevertheless, the effect of acid stress on yeast cell membranes has not been well documented.

The objectives of this work were to assess freeze drying as a dehydration method for preserving winemaking *Saccharomyces cerevisiae* cells. The effects of two carriers (maltodextrin, trehalose) and acid adaptation to improve the survival of *S. cerevisiae* during freeze drying were studied. Moreover, the impacts of acid adaptation on the intracellular glycogen, trehalose contents and the membrane fatty acid profile of *S. cerevisiae* were investigated.

MATERIALS AND METHODS

Microorganism: The winemaking *Saccharomyces cerevisiae* UP3OY5 was isolated at the University of Burgundy, France. Controls and acid-adapted cells were grown at pH 5.2 and at pH 3.5, respectively.

Freeze drying protocol: Harvested cells were washed with NaCl 0.9% solution. The pellets were resuspended in a solution containing matodextrin or trehalose as carriers before being freezed at -20°C for 24 h then dried with Flexi-Dry MP™ at -82°C.

Analytical methods: The contents of intracellular glycogen and trehalose of whole yeast cells were determined by the methods adapted from Parrou (Parrou 1997b) and Hounsa (Hounsa 1998).

Fatty acid extraction and gas chromatography analysis: Total lipids were extracted according to the method described by (Bligh 1959). The lipid extract was saponified, methylated and analyzed by gas chromatography using a Shimadzu GC17A gas chromatograph equipped with a fused silica capillary column BPX70 (Victoria, Australia)

RESULTS AND DISCUSSION

Influence of carriers and acid adaptation on the viability of S. cerevisiae UP3OY5 after freeze drying

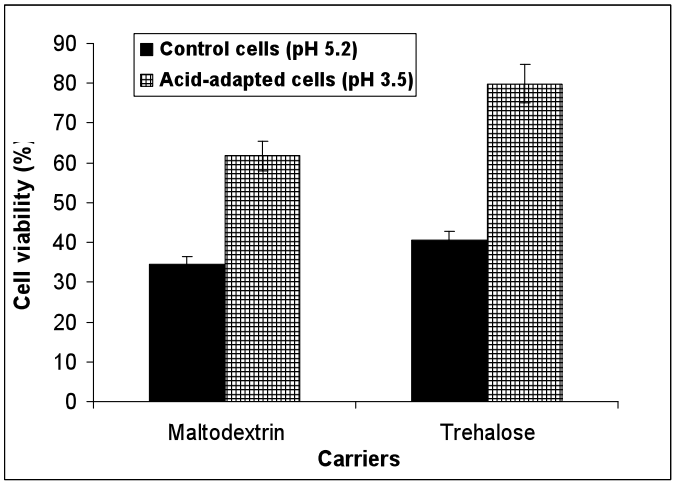

Figure 1. Cell viability of control and acid-adapted cells after freeze drying with maltodextrin and trehalose as carriers.

Control and acid-adapted cells harvested at the beginning of stationary phase were freeze dried with 40% maltodextrin or trehalose solutions as carriers. When *S. cerevisiae*

UP3OY5 strain was adapted to acid condition by growing at pH 3.5 for 28-30 h, the viability of acid-adapted cells (79.9%) was significantly higher than that of non-adapted cells (40.5%) after freeze-drying with trehalose as a carrier (Figure 1). Among the two carriers used in this study, trehalose was proven to be more efficient in preserving both non-adapted and acid-adapted yeast cells., the viability of non-adapted cells with trehalose (40.5%) was higher compared to that with maltodextrin (34.4%). These viabilities were higher than those obtained in previous work carried out by (Diniz-Mendes 1999), who only achieved 25% cell survival after freeze drying.

Impact of acid adaptation on intracellular glycogen and trehalose in S. cerevisiae UP3OY5

As shown in Table 1, the intracellular glycogen content of acid-adapted cells (7.2 mg g^{-1} dry cells) was found higher than that of non-adapted cells (5.6 mg g^{-1} dry cells). Glycogen is a major reserve carbohydrate in the yeast *Saccharomyces cerevisiae*, especially when cells enter starvation phase (Parrou 1997a), which could explain the increase in viability of acid-adapted cells. In the case of intracellular content of trehalose, the contrary results were observed, i.e. acid-adapted cells contained significantly lower intracellular trehalose content compared to non-adapted cells.

Table 1: Intracellular contents of glycogen and trehalose of control cells and acid-adapted cells

Intracellular compounds (mg g^{-1} dry cells)	**Control cells**	**Acid-adapted cells**
Glycogen	5.6 ± 0.9	7.2 ± 0.3
Trehalose	62.0 ± 3.7	11.2 ± 1.39

Our results were not in accordance with those obtained in a number of previously published work (Arguelles 1997; Diniz-Mendes 1999; Hubalek 2003). In those studies, trehalose content represented up to 25 % of the dry cell mass, depending on the environmental conditions (Parrou 1997a). This discrepancy could be the reflection of the variability among *S. cerevisiae* strains and the variation in resistance mechanisms to freeze drying stresses.

Impact of acid adaptation on the membrane fatty acid profile in S. cerevisiae UP3OY5

In *S. cerevisiae* UP3OY5 cells grown under control, eight main fatty acids were identified: decanoic acid (C10:0), dodecanoic acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic (C18:2) (Figure 2). Palmitic (C16:0) was the major saturated fatty acid identified in this strain. Palmitoleic acid (C16:1) was the major unsaturated fatty acid followed by oleic acid (C18:1). In cells grown under acid condition (pH 3.5), the membrane fatty acid profile was altered compared to that of control cells. It was worthy noting that the general propensity of the fatty acid profile for acid-adapted cells was that of an increase in the molar percentage of saturated acids, and a decrease in that of monounsaturated palmitoleic (C16:1) and oleic (C18:1) acid.

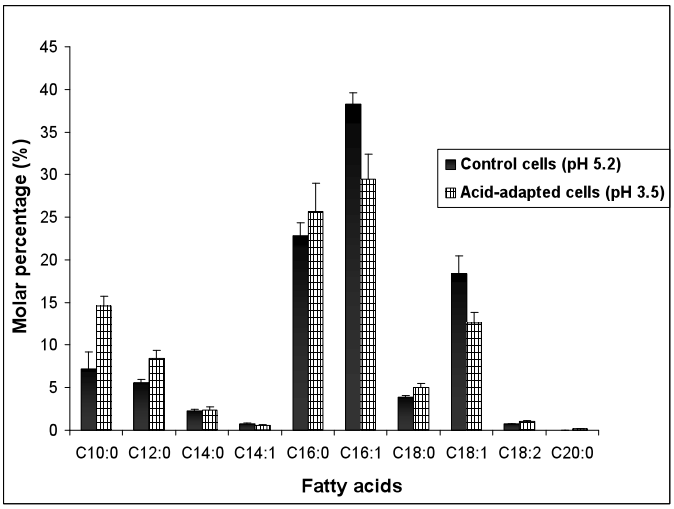

Figure 2. Fatty acid profiles of control and acid-adapted yeast cells.

CONCLUSIONS

Trehalose was shown to be a useful protecting agent for *S. cerevisiae* cells after freeze drying. Improvement in yeast cell viability after freeze drying was obtained by pre-adapting yeast to acid condition. This study also suggested the key role of acid adaptation in acquiring cross protection mechanism that could permit yeast to better survive to freeze drying. Acid adaptation induced an increase in the intracellular glycogen content and the proportion of saturated fatty acids in the cell membrane while the content of trehalose decreased. These biochemical changes could account for the increase in viability of acid-adapted cells after freeze drying.

ACKNOWLEDGMENTS

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