

THE INFLUENCE OF LINOLEIC ACID SUPPLEMENTING ON THE WAP AND GIPE PARAMETERS UNDER DIFFERENT FERMENTATION CONDITIONS AND IN SUCCESSIVE FERMENTATION CYCLES

Rodica Rotar¹, Antoaneta Gabriela Stoicescu²

¹Ștefan cel Mare University Suceava – Food Engineering Faculty

²Dunărea de Jos University Galați – Food Science and Engineering Faculty

Abstract

We have resorted to acidification power test, in modified version and the WAP and GIPE parameters were determined to study yeast cell population supply with linoleic acid before pitching upon the inoculum quality under stress factors: successive fermentations and sudden reduction of fermentation temperature by transferring the samples from a temperature of 15°C to 4°C.

Keywords: *linoleic acid, WAP parameters, GIPE parameters*

Introduction

Yeast quality, in terms of its viability and vitality, depends of the integrity of the yeast plasma membrane (Moonjai, 2002). Yeast plasma membrane has not only been proved useful in determining viability but also vitality. Plasma membrane proton efflux can be measured by using the acidification power test and this has been demonstrated to correlate to fermentation performance (Mathieu, 1991).

The test was first designed by Murray (1984) and Jones (1987), and numerous improvements have been made since according to Van Zandycke (2003). Recently the test became more accurate and reproducible resulting in the measurement of two parameters:

Water acidification power, WAP, shows the passive efflux of protons in the absence of exogenous carbon sources and, consequently, reflects the use of carbohydrate reserves needed by the cell to maintain its metabolic activity.

Glucose-induced proton efflux, GIPE, reflects the cell capacity of using extra-cell nutrients, enzyme activity H⁺ATP-ase and integrity of

plasma membrane and can be considered an indicator of membrane functionality, tolerance to stress factors.

The proton efflux directed by adenosid triphosphatase enzyme activity from the level of yeast cell plasma membrane, by determining the two components GIPE and WAP, can be considered a useful biomarker to put into evidence the influence of stress factors and appreciate the yeast inoculum quality before pitching.

Experimental

The study is carried out on the industrial isolated strain from the production culture at S.C. Bermas S.A - Suceava - *Saccharomyces cerevisiae* (*carlsbergensis*), kept on malt wort with agar at 4°C. Hopped malt wort is used for experiments in order to create the production conditions 10.7°P (1.041g/cm³). The medium has been sterilized by auto cleavage for 15 minutes at 121°C.

Fermentations have been monitorized under three different conditions, according to table 1.

Table 1. The fermentation conditions achieved in experiments

Experiment	Medium	Inoculum	Contact time with linoleic acid
I	aerated	non-supplemented	-
II	non-aerated supplemented	non-supplemented	-
III	non-aerated	supplemented	24 hours

Yeast cells were harvested by centrifugation after each fermentation cycle, washed three times with cooled sterile water. The yeast suspension used for the measurements applied and determination of WAP and GIPE parameters was obtained by resuspending cells to a final concentration of 10⁹ cells per ml sterile deionized water.

The wort and inoculum supplementation were made according to techniques presented in the study “Experiments on conditioning modalities of yeast inoculum by linoleic acid supplementing”.

For each fermentation cycle experiment carried out two series of samples:

- first series – experiment I, II, III carried at 15°C in successive fermentation cycles II, III and IV for 120 hours used for measurements of WAP and GIPE parameters after 72 and 120 hours;
- second series – experiment I, II, III sudden reduction of fermentation temperature by transferring the samples from a temperature of 15°C to 4°C after 72 hours and a total duration of 120 hours for measurements of WAP and GIPE parameters.

Results and Discussions

WAP parameter determination:

The values of WAP parameter determined at cells cropped from 15°C media show, figure 1, during the fermentation cycles III, IV, lower values of the yeast cropped in aerated medium and of that cropped in supplemented medium, but constant values for the supplied and inoculated yeast in non-aerated medium.

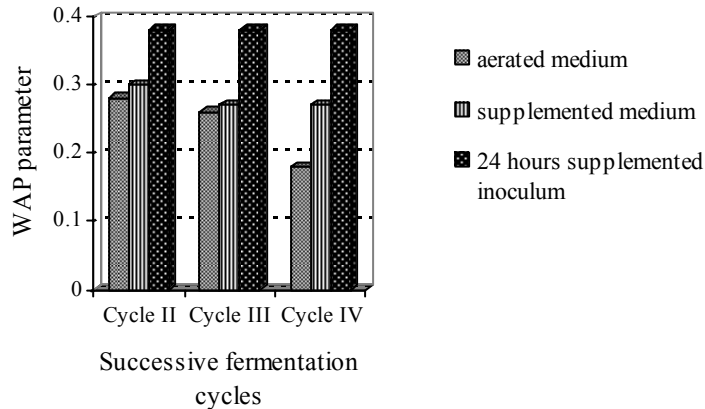


Fig.1. WAP parameter evolution determined after 72 hours fermentation at 15°C

After having fermented for 72 hours at 15°C, the cell capacity to acidify water, to utilize inter-cell storage respectively to maintain their metabolic activity decreases in the case of adopting the medium

The Influence of Linoleic Acid Supplementing on the Wap and Gipe Parameters under Different Fermentation Conditions and in Successive Fermentation Cycles

aeration or linoleic acid supplementing method and remains constant in the case of supplemented cells at linoleic acid inoculum stage.

WAP parameter values, determined at yeast cropped from media after 120 hours of fermentation at 15°C, figure 2, show decreasing values during fermentation cycles in the case of cells cropped in aerated medium or supplemented with linoleic acid, but, constant values for the cells supplemented with linoleic acid at inoculum stage.

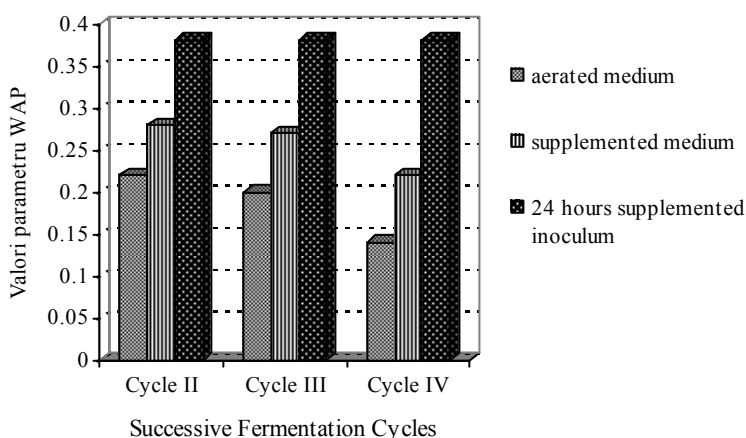


Fig. 2. WAP parameter evolution determined after 120 hours fermentation at 15°C

The sample transfer to 4°C and cell maintenance in media for other 48 hours at this fermentation temperature do not determine major changes of the WAP values at the cell level as compared to the transfer moment according to represented data in figure 3.

A slight decrease of WAP values may be noticed in the case of fermentation cycle III and cycle IV on the yeast in aerated medium and that cropped from acid linoleic-supplemented medium. The yeast supplemented with linoleic acid at the inoculum stage has the capacity to maintain its metabolic activity both under fermentation conditions at 15°C and under temperature decrease conditions, all along the successive fermentation cycles, as compared to the non-supplemented cells.

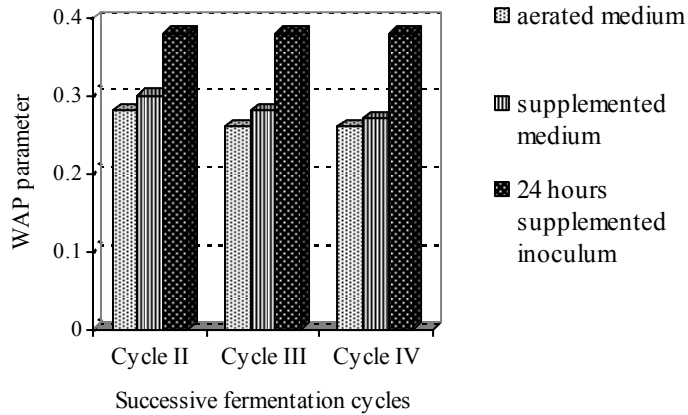


Fig. 3. WAP parameter evolution determined after 72 hours fermentation at 15°C and 48 hours at 4°C

GIPE Parameter Determination:

After 72 hour-fermentation at 15°C, GIPE parameter, represented in figure 4, decreases during the succession of fermentation cycles in the case of non-supplemented cells, but it keeps constant at those supplemented with linoleic acid at inoculum stage.

After 120 hour-fermentation at 15°C, figure 5, the cell membrane integrity loss during successive fermentation cycles is evident at cells cropped from aerated or supplemented medium.

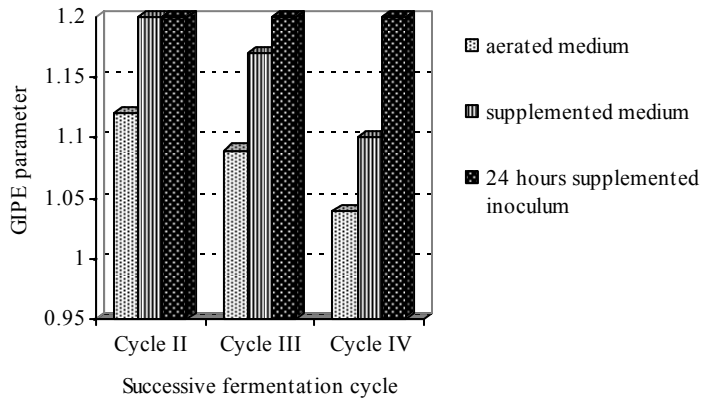


Fig. 4. GIPE parameter evolution determined after 72 hours fermentation at 15°C

The Influence of Linoleic Acid Supplementing on the Wap and Gipe Parameters under Different Fermentation Conditions and in Successive Fermentation Cycles

The obtained GIPE values mark a slight decrease in the case of cells supplemented with linoleic acid at inoculum stage.

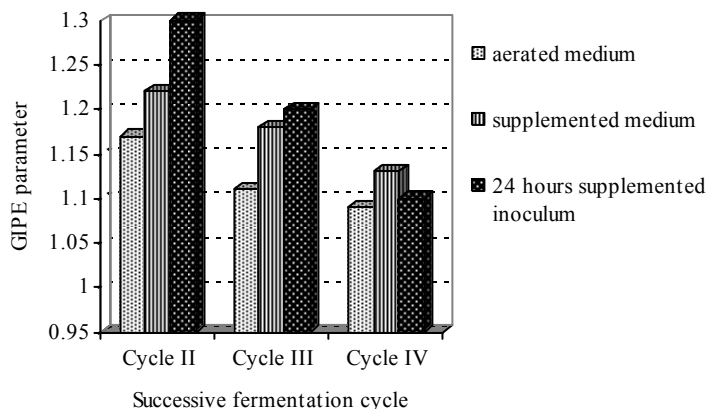


Fig. 5. GIPE parameter values determined after 120 hours fermentation at 15°C

The sample transfer at 4°C determines a significant decrease of GIPE parameter, figure 6, in the case of cells cropped in aerated or supplemented medium, fact that shows the feeble tolerance of these cells to sudden temperature decreases, partial loss of cellular integrity.

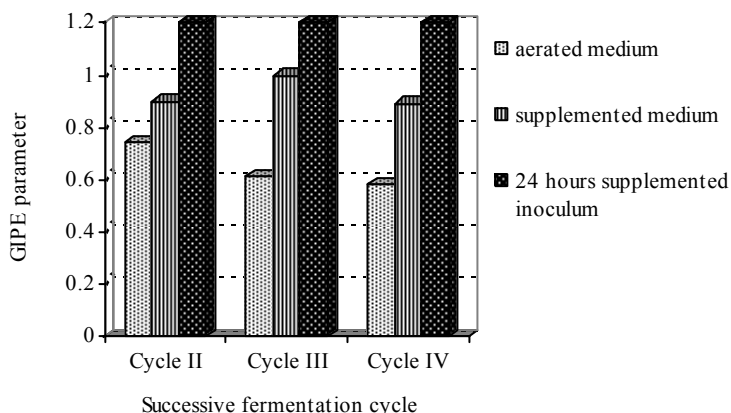


Fig. 6. GIPE parameter evolution determined after 72 hours fermentation at 15°C and 48 hours at 4°C

Conclusions

Cell supplementing method at inoculum stage proves to be efficient regarding the increase of cell capacity of maintaining their metabolic activity in successive fermentation at a temperature of 15°C. The heat shock determined by temperature decrease from 15°C to 4°C determine, in the case of cells that have fermented either in aerated medium, or linoleic acid-supplemented medium, the activating of trehalose - synthetasic complex. The WAP parameter which evaluates the cells' capacity of using the inter-cell supply of carbohydrates in order to maintain the metabolic activity when the nutrient shortage becomes critical, decreases sensitively during the successive fermentation cycles.

In the case of linoleic acid-supplemented inoculum, the temperature decrease is tolerated by cells due to the fluidity increase of cell membrane even at decreased temperatures, fact showed by the consumption of a reduced trehalose quantity, and the WAP parameter is constant both after the fermentation has been completed and in the case of sample transfer at 4°C.

One can see that the supplemented cells are not influenced by the temperature decrease of fermenting medium. One can notice, from the data obtained for the GIPE parameter, after 120 hour-fermentation at 15°C, that the cells supplemented with linoleic acid at inoculum stage are less tolerant to prolonged fermentation at this temperature and at the end of cycle III and IV running through, the cells have lower GIPE values, so lower tolerance to the stress factor to which the cells are subjected to during successive fermentations at temperature of 15°C.

References

- Jones, R.P., Greenfield, P.F. (1987). Ethanol and the fluidity of the yeast plasma membrane. *Yeast*, 3, 223-232.
- Mathieu, C., van den Berg, L., Iserentant, D. (1991). Prediction of yeast fermentation performance using the acidification power test. *Proc. Conv. Lisbon*, 23, 273-280.
- Moonjai, N., Verstrepren, K.J., Delvaux, F.R., Derdelinckx, G., Verachttert, H. (2002). The effects of linoleic acid supplementation of cropped yeast on its subsequent fermentation performance and acetate ester synthesis, *J. Inst. Brew.*, 108(2), 227 – 235;

The Influence of Linoleic Acid Supplementing on the Wap and Gipe Parameters under Different Fermentation Conditions and in Successive Fermentation Cycles

- Murray, C.R., Barich, T., Taylor, D. (1984). The effect of yeast storage conditions on subsequent fermentations. *Tech. Q. Master Brew. Assoc. Am.* 21, 189-194.
- Van Zandycke, S.M., Siddique, R., Smart, K.A. (2003). The role of membrane in Predicting Yeast Quality. *MBAA TQ*, 40(3), 169-173.