

MODIFIED ACIDIFICATION POWER TEST APPLIED TO EVALUATE TEMPERATURE AND ETHANOL STRESS IMPACT ON YEAST FERMENTATION PERFORMANCE

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Abstract

A modified version of the acidification power test comprising two independent parameters, water acidification power (WAP) and glucose induced proton efflux (GIPE) was applied to investigate the impact of temperature and ethanol on brewery yeast fermentation; the measurements were applied on the yeast population under specific stress factors temperature and duration during storage and ethanol that may occur during fermentation process.

Keywords: *yeast, plasma membrane, viability, vitality*

Introduction

Yeast quality, in terms of its viability and vitality, depends of the integrity of the yeast plasma membrane (Van Zandycke, 2003).

The yeast cell membrane is affected by the stresses that occur during the brewing process and particularly during storage. This study is based on evaluating temperature stress impact during storage between repitching and ethanol stress impact during fermentation process, both factors that impair fermentation performance. These stresses, among others like starvation, oxygenation, agitation, duration and serial repitching cause damage to the cell membrane, which, in turn, may result in reduced yeast growth, loss of viability and a decrease in metabolic activity because of membrane fluidization or loss of transmembrane potential.

The use of membrane – excluded fluorescent dyes (excluded from the cell by the healthy membranes), such as the hemi magnesium salt 1-anilino-8-naphthalene-sulfonic acid (MgANS) or berberine, as an alternative to the brewing industry standard, methylene blue, in determining viability has already been demonstrated (McCraig, 1990).

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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 Opekarova and Sigler, 1982, and numerous improvements have been made since according to Patino, 1983. Recently the test became more accurate and reproducible resulting in the measurement of two parameters:

- WAP – water acidification power;
- GAP – glucose acidification power.

The WAP is the change in pH when cells are resuspended in water for 20 minutes and corresponds to the concentration of intracellular glycogen.

The GAP represents the change in pH when cells are resuspended in water for 10 minutes followed by 10 minutes in glucose. During this last 10 minutes, both glucose – induced and passive proton effluxes are measured.

To obtain the net *glucose induced proton efflux - GIPE*, the WAP is deducted from the GAP. The GIPE represents the ability of the cells to use extracellular reserves, without taking into account the utilization of intracellular reserves.

Experimental

A lager yeast strain, obtained from S.C. Bermas S.A. Suceava was utilized in this study. Yeast cells were harvested by centrifugation after a fermentation cycle from the conventional primary fermentation stage, 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 10^9 cells per ml sterile deionized water.

The modified version of AP test was conducted according to the method of Kara, 1988. The parameters were determined on three different yeast generations: freshly propagated yeast, fourth generation yeast and ninth generation yeast. Freshly propagated yeast was obtained after the laboratory propagation stage. For WAP parameter

determination the yeast cells was stored for 6 days at 5°C/1°C in sterile, deionized water. Before each daily measurement the yeast population was washed three times with sterile deionized water at the same temperature corresponding to the storage proposed conditions.

The ethanol stressed population GIPE parameter was determined on yeast cells harvested by centrifugation, washed three times and resuspended to a final concentration of 10^7 cells per ml in ethanol 4, 8 and 12%, incubated at 25°C in 1000 ml conical flasks (500 ml suspension). GIPE parameter was determined after three times washed and water resuspended cells to the final mentioned concentration at 2, 4 and 6 hours.

Results and Discussions

Determination of WAP parameter used to evaluate the impact of duration and temperature storage on yeast quality:

Handling and storage conditions during serial repitching are correlated with a real induced stress on yeast population in the brewery fermentation process. Glycogen levels can be affected by handling and storage conditions during serial repitching, and are indeed significantly reduced during starvation and in the presence of various stresses.

During yeast storage at 5°C and duration of 6 days, the experimental results represented in the figure 1, for the three different generations (the initial one or the freshly propagated yeast, the fourth generation yeast and the ninth generation yeast) indicates:

The passive proton efflux (WAP) determinate on the freshly propagated yeast and yeast cells from IV and IX generation at 5°C storage temperature indicates the progressively decreased of the glycogen levels after each day of starvation/storage. The decrease is drastic after the third storage day at the fourth generation yeast cells and at the freshly propagated yeast and at ninth yeast generation the decrease is obviously since the first storage day. This indicates the intracellular reserves at yeast cells from ninth generation are depleted rapidly during storage at 5°C.

The intracellular glycogen for the three generations yeast populations is different after each storage day.

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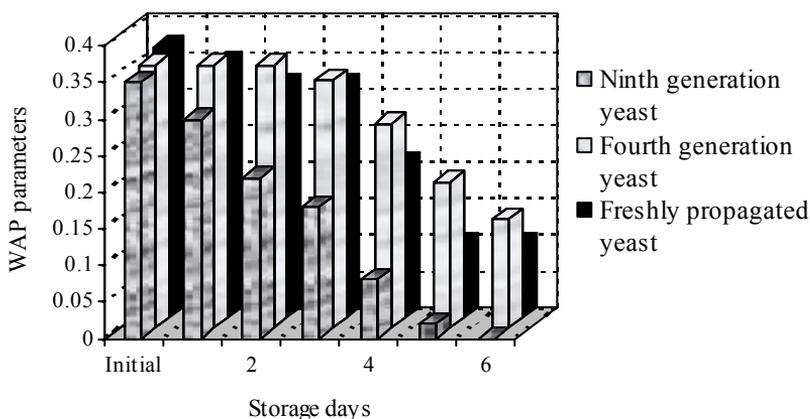


Fig. 1. The influence of storage duration at 5C on WAP parameter

The level of the intracellular glycogen for the freshly propagated yeast is lower than the fourth generation yeast after the same storage duration, but at the initial moment, the level of the intracellular glycogen indicates that the fourth generation yeast cells has already been adapted and thus the potential to replicate in the fermenter is increased.

The WAP parameters for the ninth generation yeast cells indicates a lower level of intracellular glycogen than the fourth generation yeast cells and the freshly propagated yeast cells which suggests the decreasing of cells vitality amount the population meaning yeast cells ageing overall serial repitching.

The intracellular glycogen is consumed more slowly at the 1°C temperature storage yeast than the 5°C temperature storage yeast fact that confirms the recommendation regarding the yeast storage temperature between repitching.

Though the initial WAP value of the fourth generation yeast cells was lower than the freshly propagated yeast cells, since the second storage day the WAP value became obviously higher than both other yeast cells studied. That indicates a superior intracellular glycogen level, a decrease of lag phase, a better tolerance to stress factors and fermentation performance.

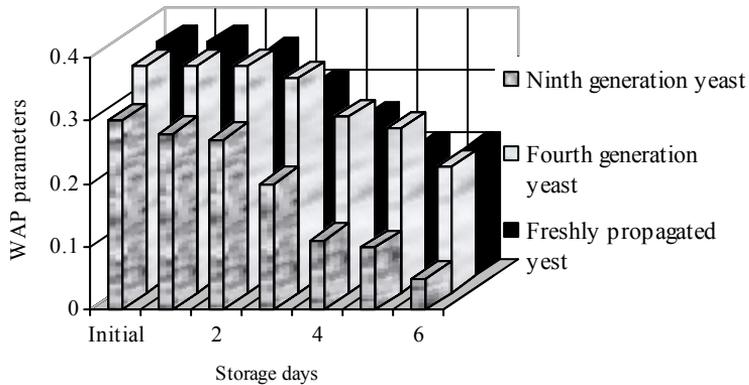


Fig. 2. The influence of storage duration at 1C on WAP parameter

The WAP parameter values depend on the stress factor – storage temperature. In fact, the intracellular reserve of glycogen is as rapidly consumed as the storage temperature is higher.

Lower levels of glycogen in pitching yeast extend the lag phase in the fermentor and affect overall fermentation performance (Martens, 1986), suggesting that this intracellular carbohydrate is a good indicator of stored yeast quality (Murray, 1984). However, direct measurement of glycogen levels requires complex enzyme assays involving lengthy methods and, sometimes, specialized equipment (Pierce, 1970).

Even the hypothesis that the 1°C storage temperature maintains yeast repitching quality does not need further studies for confirmation, since the WAP indirectly measures glycogen levels and is very operator friendly and rapid, this study indicates that this assay may be utilized to determine the potential of yeast slurry to perform.

The influence of ethanol concentration in fermentation medium on yeast quality – GIPE parameter evaluation:

GIPE directly reflects the activity of the ATPase and the plasma membrane integrity and represents an indicator of membrane functionality and viability. The ATPase promotes the efflux of protons as a result of glycolysis. The protons are extruded to enable the cell to

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counteract increases in intracellular pH (Goffeau, 1981), thereby maintaining the pH buffering capacity of the cytoplasm.

Damage to the membrane or the ATPase enzyme will, therefore, cause changes in proton efflux. The GIPE was observed to be inversely correlated with intracellular levels of trehalose (Van Zandycke, 2003) and, since accumulation of this reserve carbohydrate occurs in the presence of yeast handling stresses, the GIPE represents an indirect determinant of the brewing yeast cells stress resistance.

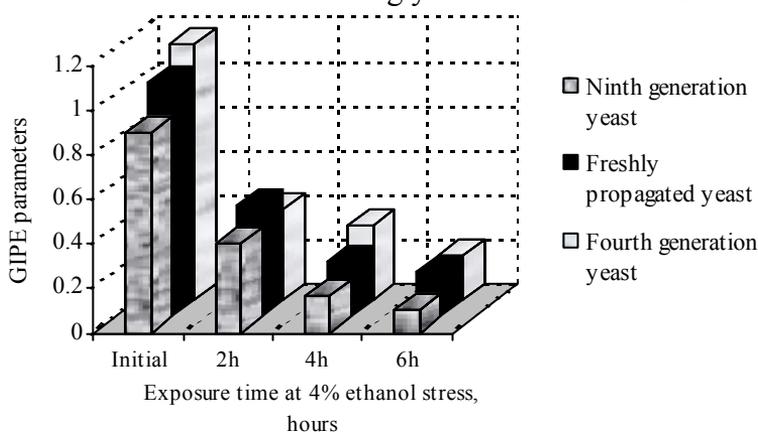


Fig. 3. The glucose induced proton efflux at 4% ethanol stress

The impact of 4, 8 and 12% ethanol on the GIPE of yeast cells populations was determined initial and at 2, 4 and 6 hours.

The GIPE decreased significantly after 2 hours of exposure to all ethanol concentrations and continued to decline on further exposure up to 6 hours (figure 5), confirming previous observations that sublethal concentrations of ethanol influence plasma membrane functionality.

The GIPE parameter values for the freshly yeast cells population are higher than the values determined for the fourth and the ninth generation yeast cells after different exposure periods which indicates superior membrane functionality for the cells.

The initial fourth generation yeast GIPE parameter value is higher than the values corresponding the other populations indicating an heterogeneity of the fourth generation yeast cells population and a decreasing of ninth generation yeast cells membrane functionality.

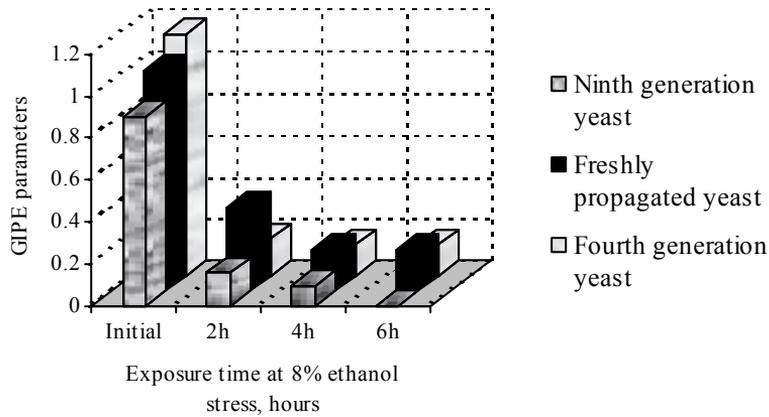


Fig. 4. The glucose induced proton efflux at 8% ethanol stress

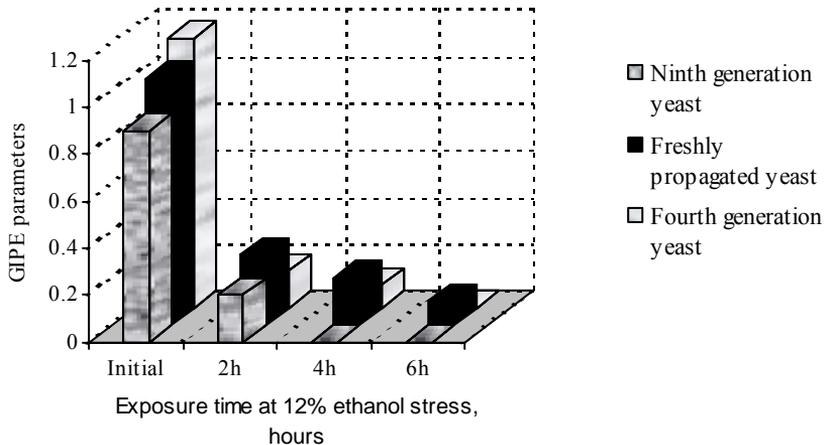


Fig. 5. The glucose induced proton efflux at 12% ethanol stress

Conclusions

Ethanol represents a chemical stress, especially during high – gravity brewing when concentrations can range from 7–14% (vol.) (Casey, 1983). Ethanol causes an increase in proton influx because of enhanced membrane permeability and the accumulation of unsaturated fatty acids. The increase in the ratio of unsaturated to saturated fatty acids (Jones, 1987) results in the fluidization of the plasma membrane that has been linked to ion and enzymes release (Mochaba, 1997).

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Furthermore, the inhibition of the activity of the ATPase proton pump is normally associated with ethanol toxicity (Cartwright, 1986).

The modified AP test is a viability and yeast activity indicator that reflects production slurry's potential to perform during the initial stages of fermentation. The proton efflux depending on the adenosine trifosfatase enzyme activity of the yeast cell plasma membrane, evaluated by measuring the two parameters WAP and GIPE, is a useful biomarker for the determination of stress impact factors and evaluation of yeast quality during serial repitching.

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