THE INFLUENCE OF LINOLEIC ACID SUPPLEMENTING ON TREHALOSE INTRACELLULAR YEAST CONTENT UNDER DIFFERENT FERMENTATION CONDITIONS AND IN SUCCESSIVE BEER FERMENTATION CYCLES

Rodica ROTAR
„Ştefan cel Mare" University of Suceava, Food Engineering Faculty
rodicas@usv.ro

Abstract
The main goal of the experiments made consisted in studying the linoleic-acid supplementing of yeast inoculum effect on trehalose intracellular content in different experiments and in successive beer fermentation cycles.
Keywords: brewery yeast, supplementing, linoleic acid, trehalose intracellular yeast content

Rezumat
Obiectivul studiilor experimentale constă în studiul efectului suplimentării inoculului de drojdie asupra conţinutului intracelular de trehaloză în diferite experimente şi în cicluri succesive de fermentare în industria berii.
Cuvinte cheie: drojdia de bere, suplimentare, acid linoleic, conţinut intracelular de trehaloză

Résumé
L'obiectif des études expérimentales consiste dans l'étude de l'effect de supplémenter l'inoculum de levure sur le conteau en tréhalose des cellules dans des expériments différérents et dans des cycles succesifs de fermentation.
Mots clef: levure, supplémente, acid linoleic, contenu en tréhalose des cellules

Introduction

Unsaturated fatty acids are important constituents of the yeast cell membrane [1]. Sterol and unsaturated fatty acids formed during the first hours of the fermentation, in the presence of oxygen, are mainly incorporated in the lipid fraction of the membranes(figure 1 and figure 2).

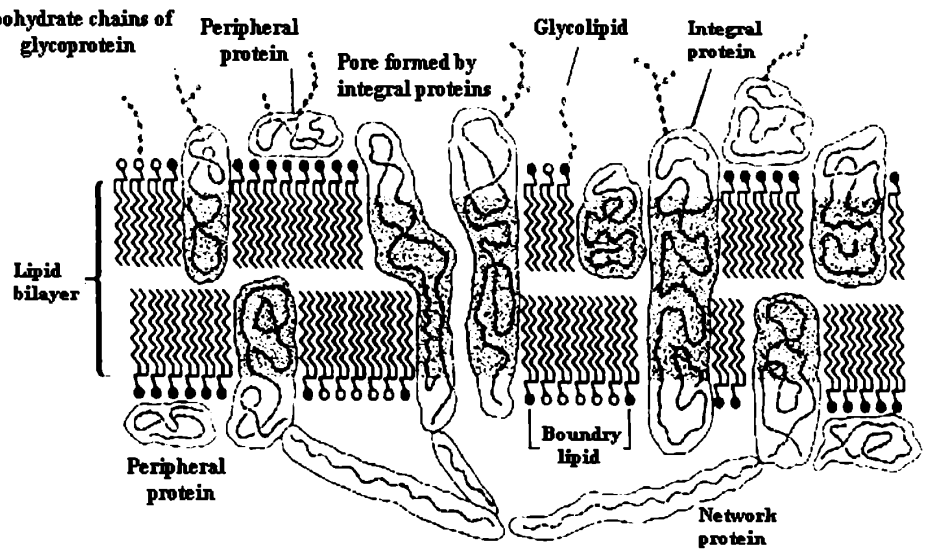

Figure 1: Ordering of phospholipids in the cell membrane [2]

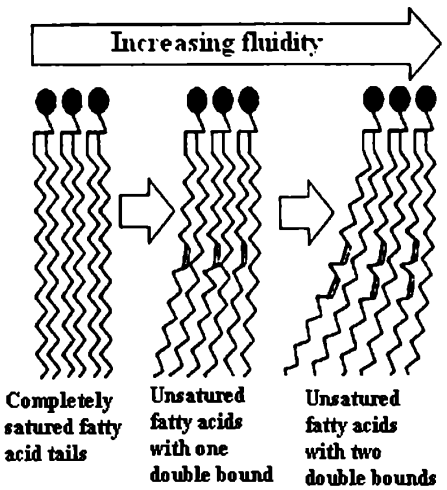

Figure 2: Unsaturated fatty acids increasing membrane fluidity [2]

Membranes with low levels of sterol and unsaturated fatty acids are altered. Many preteins are embedded in the membrane phospholipid bilayer and function in the transport of molecules in and out of the cells are involved in biochemical processes. Taking into consideration that, in brewery technology, the cropped yeast cells have deficits in membrane sterols and unsaturated fatty acids, necessary to good fermentation performance in the next fermentation cycle, these ones have to resynthesize these compounds [4].

One requirement to remake the content of membrane sterols and unsaturated fatty acids is the oxygen presence in the medium wherein the yeast is being inoculated [1]. Using endogenous carbon resources, the yeast cells will synthesize essential lipids being able for a new fermentation cycle. But two inconvenient may occur: insufficient aeration, insufficient revitalization respectively, growth defects and low degrees of fermentation or excessive aeration resulted in surplus bio-mass to the detriment of ethyl alcohol generation.

According to Moonjai, 2003, yeast revitalization alternatives by aerations of malt wort may be:

- aeration of water or malt wort slurried yeast cells;
- malt wort supplementing with unsaturated fatty acids;
- yeast slurry supplementing with unsaturated fatty acids.

Results indicated that the supplementation of copped yeast with unsaturated fatty acids could be an interesting alternative to wort oxygenation to restore the optimal membrane fluidity of the yeast.

In both conventional and modern techniques, the decrease of partially fermented wort temperature is a current procedure for ending the primary fermentation stage by which the beginning of final fermentation medium attenuation [3].

The goal of this study is to assess to what extent the linoleic acid yeast inoculum supplementation reduces the negative effect that the stress factor heat – shock and the undergone succesive fermentation cycles manifest.

Trehalose is one of the main reserve carbohydrates that provides balance to the plasma membrane and that is consumed by yeast in starvation stages. The significance of trehalose as an endogenous protectant is referred to in numerous literature sources, in particular comprehensive reviews [5,6].

The trehalose accumulation is iduced by stress factors and increases with the generation number. Consequently, the assessment of the cellular trehalose content may be considered biomarker of cellular viability and their ability to respond to stress factors.

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/cm^3). The medium has been sterilized by auto cleavage for 15 minutes at 121°C.

A single colony was taken from the stock culture which was pitched on malt wort with agar in inclined test tube, incubated for 48 hours at 27°C, then stored at 4°C. 5ml of medium were added to the test tube with inclined medium to obtain the laboratory inoculum slurry and the cells were transferred by slight stirring of the slurry. The slurry was inoculated in 150 ml medium from a 250 ml Erlenmeyer flask, plugged with dense cotton, incubated at 20°C for 48 hours on an orbital agitator at 150 rpm.

The cells were cropped by centrifuging and inoculated into medium up to a concentration of 15 x10^6 cells/ml.

The first fermentation cycle was made in 500 ml medium in 1000 ml Erlenmeyer flask, plugged with dense cotton and placed on the orbital agitator at 100 rpm 20°C for 72 hours.

The sample was doubled to provide the bio-mass outfit in order to obtain the linoleic acid-supplemented inoculum.

The yeast was not separated from the fermented medium in order to study the supplementing effect of yeast inoculum, at one sample, and the linoleic acid was dosed in 0,5 ml ethyl alcohol up to a final concentration of 60 mg linoleic acid/l yeast slurry (fermented medium). One sample of supplemented yeast inoculum was obtained with a 24 hours contact time. The supplemented cells and non-supplemented ones were collected by centrifuging, washed twice with sterile water at 4°C and then used for the second fermentation cycle. The cropped yeast was inoculated in hopped malt wort 10,7°P up to a concentration of 10x10^6 cell/ml in 150 ml wort in siphoning tubes-equipped conic vessels .

In order to study the influence of inoculum supplementing and determine periodically trehalose content, the yeast of first fermentation cycle was inoculated in 3 l charges of hopped malt wort 10,7°P (1,041g/cm^3) up to concentration of 10x10^{6}cell/ml and placed on orbital agitator at 100 rpm 15°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

The malt wort has been aerated before inoculation to a concentration of 8 ppm O_2 dissolved by barbotage of sterile air for 30 minutes, at 15°C.

The dissolved O_2 content: 3,6 ppm has been determined for the non-aerated malt wort, used at experiments II and III.

The malt wort supplementing was achieved by dosing the linoleic acid in 3 ml ethyl alcohol to a final concentration in wort of 15 mg linoleic acid/l. The same quantity of ethyl alcohol was added to the wort used in all fermentation samples.

After 3 days of fermentation at 15°C, a series of three samples (I – aerated medium inoculated with non - supplemented yeast, II – linoleic acid supplemented medium inoculated with non - supplemented yeast and III – non - aerated medium, non – supplemented but inoculated with linoleic acid supplemented yeast) have undergone sudden temperature decrease, by transffering samples from

15°C to 4°C temperature in order to assess the wort or the inoculum linoleic acid supplementations upon the ability of yeast cells to respond to stress after they have been harvested from fermented mediums under different conditions.

The cells were cropped by centrifuging and three times washed with sterile water at 4°C and werw resuspended in the same composition medium to assess the impact of stress factors, heat shock and succesive fermentation cycles, cycle III and IV.

The trehalose content, useful for assessing yeast cells vitality was measured by the anthrone method. Trehalose, extracted with trychloracetic acid, reacts with anthrone under heat conditions, in the presence on sulphuric acid; an blue - green product results. The method requires the prior ethalon curve drawing – absorbtion function of glucose concentration of ethalons (25, 50, 100 μg glucose/cm^3 respectively) obtained by dilutions from a glucose stock solution in saturated benzoic acid solution. The yeast susspension is two times extracted with an equal amount of tryclhoracetic acid, each extraction being followed by centrifuging. The supernatant was object to glucide quantitive determination technique using anthrone. The absorbtion value increases with the amount of glucose, for concentration values up to 150 μg glucose/cm^3.

Results and discussion

According to resulting experimental data, presented in figure 8.20, one may observe that in the 2nd fermentation cycle, the cellular trehalose content decreases in the case of the sample maintained in the last 2 fermentation days at the same temperature at 15°C. The yeast begins to feel the lack of nutrients in all three experiment alternatives and consumes a reduced amount of cellular trehalose.

Consequent to the sample transfer at 4°C, data show the aerated and supplemented medium yeast synthetises trehalose as a response to the stress factor – heat shock.

But the effect of temperature decrease is hardly perceived by linoleic acid supplemented yeast; therefore there is an even larger ability to tolerate its impact.

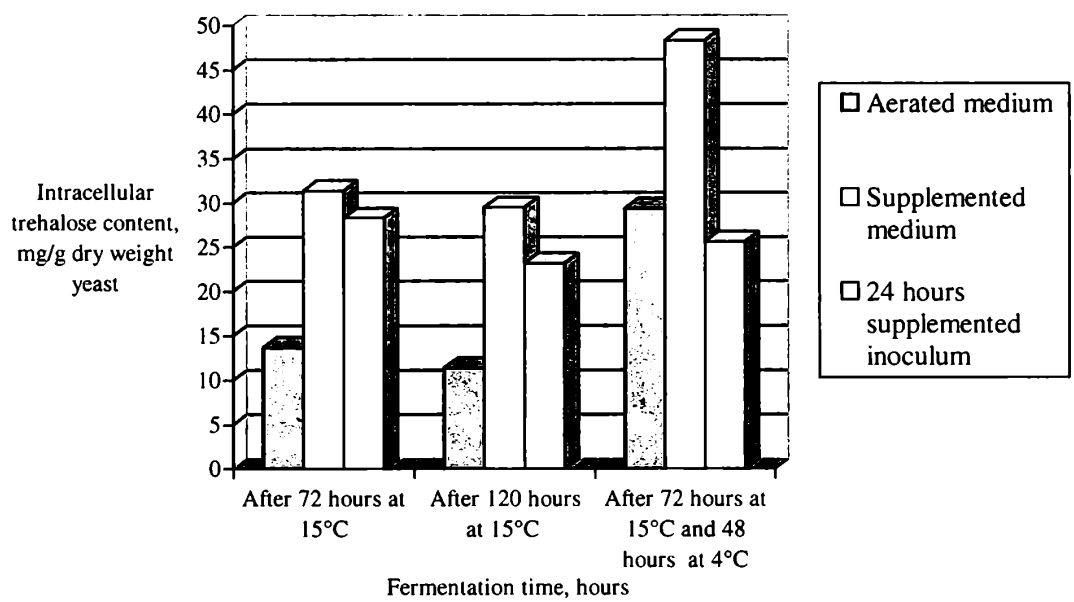

Figure 3: Intracellular yeast trehalose content in different conditions, at 2nd fermentation cycle

Within the 2nd fermentation cycle, the effect of stress factor – heat shock while fermenting in aerated or supplemented medium ciclul II de fermentare, determines yeast to react and activate the

trehaloso – syntetasic complex, which is indicated by the increasing content of trehalose in the case of the harvested yeast cell samples.

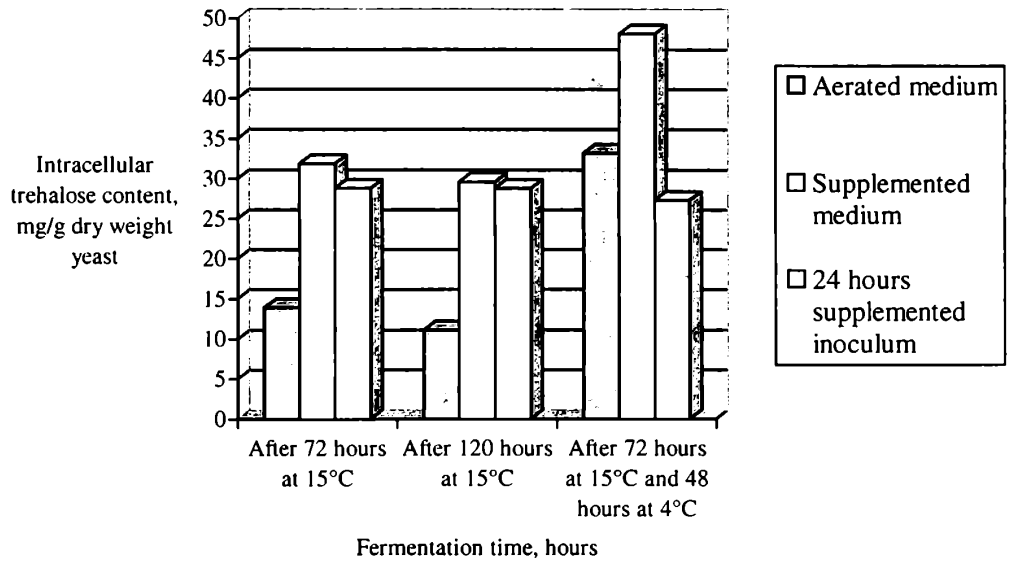

Figure 4: Intracellular yeast trehalose content in different conditions, at 3rd fermentation cycle

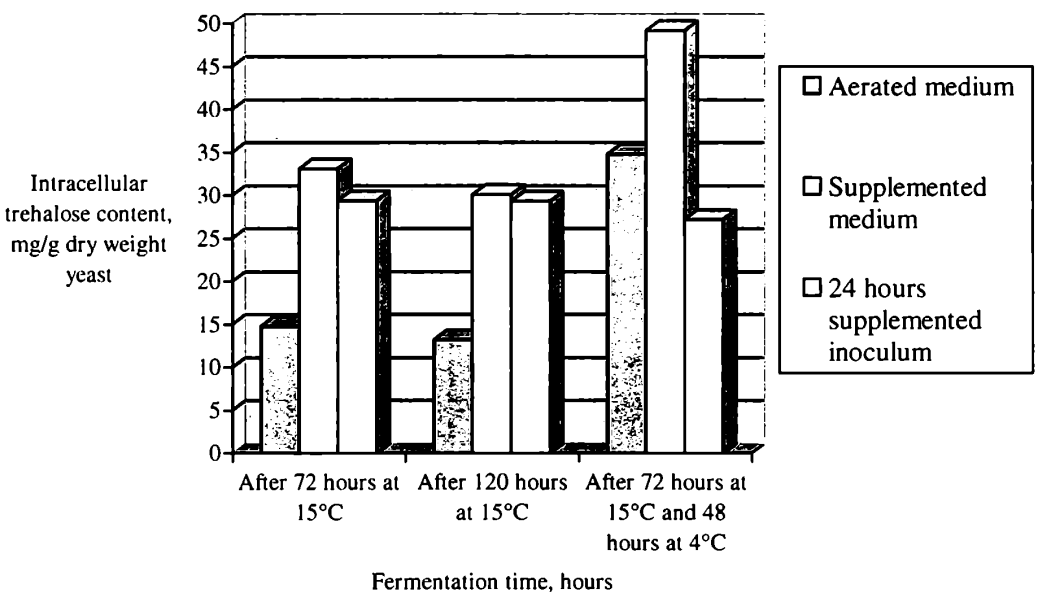

Figure 5: Intracellular yeast trehalose content in different conditions, at 4th fermentation cycle

The supplemented yeast tolerates to a certain extent the heat – shock, within the second cycle of fermentation, considering that is already protected by the damages that the sudden temperature decrease would induce, because of the high content of linoleic acid acyl radicals from the cellular wall structure and that consumes trehalose.

For the 3rd and 4th fermentation cycles, data show, in figure 4 and 5, that after 72 hours at 15°C, the initial level of cellular trehalose content is slightly higher, according to Smart`s hypothesis, who considers that the repeated fermentation cycles are a stress factor that yeast is object to and that responds to by means of reserve carbohydrates synthesis, glycogen and trehalose; the more generations, the higher the synthetised amounts.

The low cellular trehalose content after two more fermentation days at 15°C indicates an active metabolism which continues to be able to assimilate nutrients. Those been unavailable, it uses the trehalose reserve. One may noticed that in the case of the supplemented yeast, the trehalose assimilated is quite reduced.

On the other hand, the sudden change of temperature (to 4°C) induces trehalose synthesis in the case of the cells fermenting in aerated or supplemented medium. In the case of the linoleic acid supplemented cells, the trehaloso – syntetasic complex is no longer activated, so these cells tolerate the sudden temperature decrease from 15°C to 4°C, but consume part of the reserve trehalose.

Conclusions

Linoleic acid assimilation during the cells maintainig 24 hours contact time determins an increase in their capacity of tolerating the sudden temperature decrease that, in both modern and conventional technique may often induce damages upon the yeast cells function of strain genetic resistance.

To conclude, the tested yeast strain capacity to tolerate the effects of stress factors increases when in the phosfolipides in the membrane structure there is a high amount of acyl radicals of the unsaturated fatty acids. This phenomenon may be explained by the increase of cellular membrane fluidity, slightly affected by the decrease of temperature, because the 24 hours maintainig in contact and the linoleic acid assimilation have determined the decrease of the value saturated fatty acids/unsaturated fatty acids.

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