

EXPERIMENTS ON CONDITIONING MODALITIES OF YEAST INOCULUM BY LINOLEIC ACID SUPPLEMENTING

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Abstract

The main goal of the experiments made consisted in studying the linoleic acid supplementing effect of cropped yeast cells upon fermentation intensity, biomass generation, cell viability, fermentation degree and yeast intracellular trehalose content as an alternative of wort aeration or its unsaturated fatty acids supplementing.

Keywords: *brewery yeast, linoleic acid supplementing, wort aeration*

Introduction

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 (Moonjai, 2003).

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 (Haukeli, 1979). 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.

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Synthesis activation of phospholipids due to the oxygen presence or active lipid assimilation from wort during fermentation reduces drastically the volatile ester synthesis that plays an important part in beer flavor due to the fact that synthesis activation of phospholipids determines gene repression that codifies the alcohol acetyl transferase synthesis (Mason, 2000). In the case of yeast slurry supplementing, the linoleic acid assimilation takes place before fermentation, in the stationary growth stage, thus avoiding interferences with synthesis of acetic esters (Moonjai, 2002).

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.

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 (1.041 g/cm³). 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 $15 \cdot 10^6$ 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). Two samples of supplemented yeast inoculum were obtained: one with a 12 hour contact time and another one with 24 hours. 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 $10 \cdot 10^6$ cell/ml in 150 ml wort in siphoning tubes -equipped conic vessels.

In order to study the influence of inoculum supplementing and determine periodically the quantity of generated bio-mass, viability of cells, trehalose content and fermentation degree of fermented medium, the yeast of first fermentation cycle was inoculated in 3 l charges of hopped malt wort 10.7°P (1.041 g/cm^3) up to concentration of $10 \cdot 10^6$ cell/ml and placed on orbital agitator at 100 rpm 15°C.

Fermentations have been monitorized under four 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	non-supplemented	-
III	non-aerated supplemented	non-supplemented	-
IV	non- aerated	supplemented	12 hours
V	non-aerated	supplemented	24 hours

The malt wort has been aerated before inoculation to a concentration of 8 ppm O₂ dissolved by barbotage of sterile air for 30 minutes, at 15°C. The dissolved O₂ content: 3.6 ppm has been determined for the non-aerated malt wort, used at experiments II, III, and IV. 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.

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After each 24 hours, five days long, 100 ml of sample was drawn to determine the generated quantity of bio-mass, cell viability and fermentation degree.

Results and Discussions

Linoleic acid supplementing effect of yeast inoculum upon fermentation intensity:

One can see from the fermentation curves shown in the figure 1 which presents the CO₂ release dynamics in a 42 hour fermentation period that, at equal concentration of cells in medium, during the first 6 fermentation hours, the cells from the non-aerated and supplemented medium adapt more slowly, but in the next interval the cells from the supplemented medium, those from the 24 and 12 hour supplemented inoculum have the most progressive dynamics.

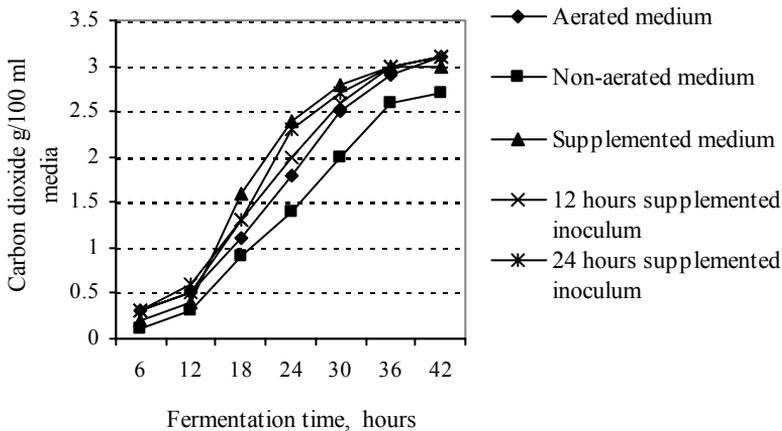


Fig.1 Linoleic acid supplementing influence upon fermentation intensity

The effect of linoleic acid –supplementing inoculum upon the biomass generation:

Cell fermentation in aerated, non-aerated or non-aerated supplemented media was compared to the fermentation of linoleic acid - supplemented cells maintained at different contact times 12, 24 hours respectively, and the results are shown in figure 2. One can see the generated bio-mass increase during the first 72 hours of fermentation

in all fermented media. The lowest bio-mass quantity present in the medium was registered in the non-aerated medium and non-supplemented one. The highest bio-mass quantity was registered in the non-aerated but linoleic acid –supplemented medium but after 96 and even 120 hours the bio mass generated is highest in linoleic acid –supplemented inoculum fermented medium.

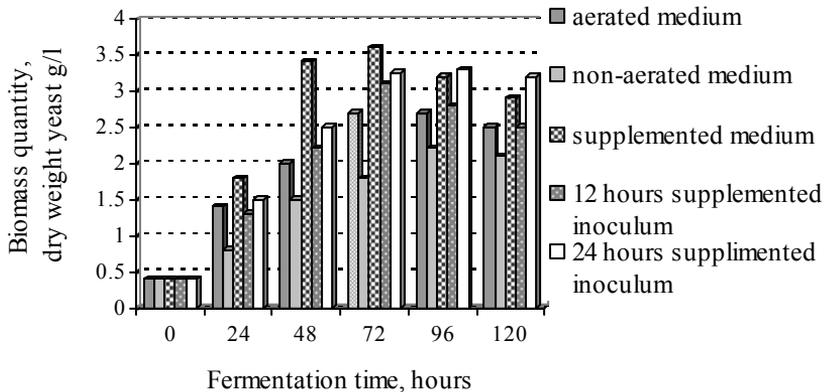


Fig. 2. The effect of linoleic acid supplementing inoculum and medium upon bio-mass generation

The comparison between the generated bio-mass from the yeast-inoculated medium and maintained at different contact times 12 and 24 hours respectively and the aerated or non-aerated but linoleic acid supplemented bio-mass leads to the conclusion that medium aeration or its supplementing with unsaturated fatty acids can be replaced by using the linoleic acid supplemented-yeast inoculum method.

Cell viability comparison from fermented media under different conditions after 24, 72, and 120 hours:

As figure 3 shows, the cell viability is lower under the non-aerated medium conditions. In the aerated medium, viability is initially low and decreases towards the fermentation end. The greatest number of viable cells was registered in the media wherein the linoleic acid was present, either under the form of a supplement for medium or yeast inoculum.

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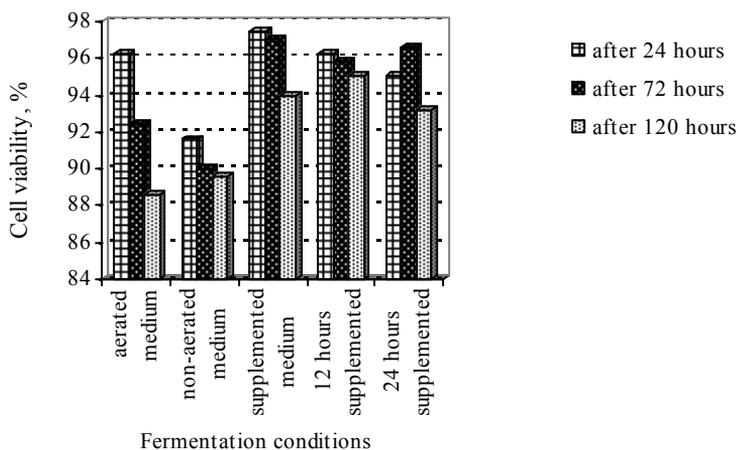


Fig. 3. Cell viability comparison from fermented media under different conditions after 24, 72, 120 hours

Linoleic acid supplementing effect upon fermentation degree:

The fermentation degree is lower under fermentation conditions of non-aerated wort and would take more than 120 hours to reach maximum fermentation degree. In the case of linoleic acid supplementing medium, its extract decreased with the highest speed, the maximum fermentation degree being reached after 72 hours. Fermentation degrees at aerated worts or pitched with 12 or 24 hour-supplemented inoculum were lower, but at not very different values (figure 4).

Linoleic acid supplementing influence upon yeast intracellular trehalose content under different fermentation conditions:

The trehalose content at the cells cropped from 12 hour supplemented inoculum fermented medium, which is from the start very high, decreases drastically during the first 24 hours, afterwards it increases and reaches a maximum at 48 hours, in the case of cells cropped from 12 hour supplemented inoculum fermented medium, and at 72 hours in the case of cells cropped from supplemented medium. The maximum values reached are significantly different: 23 mg trehalose/g su yeast after 48 hours at cells from supplemented

inoculum fermented medium and 49 mg trehalose/g su yeast after 72 hours at cells from supplemented medium. Considerable increases of trehalose content at yeasts cropped from aerated or non-aerated media were not registered (figure 5).

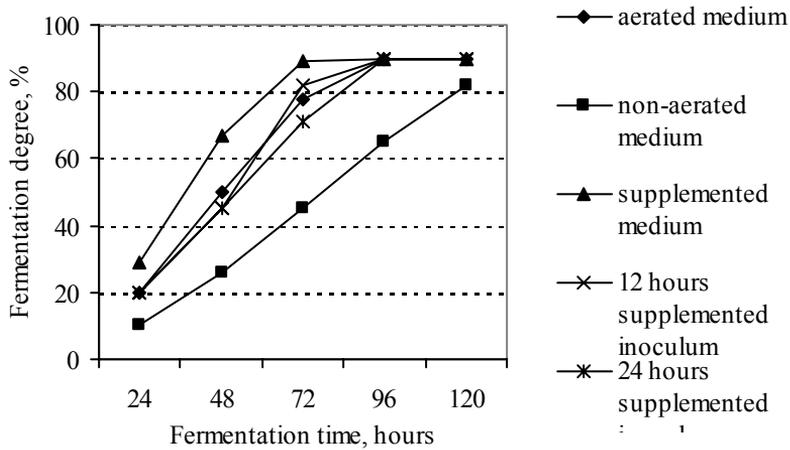


Fig. 4. Fermentation degree evolution under different conditions

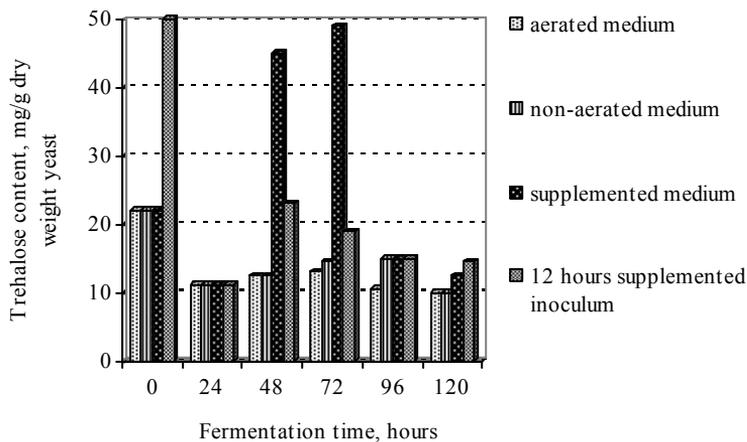


Fig. 5. Trehalose content of yeast cells under different fermentation conditions

Conclusions

The data related to fermentation intensity, biomass generation, cell viability, fermentation degree and yeast intracellular trehalose content indicate that the cells from the supplemented medium, those from the 24 and 12 hour supplemented inoculum have the most progressive dynamics of fermentation intensity. Also, the bio-mass generated is highest in linoleic acid-supplemented inoculum fermented medium. The greatest number of viable cells was registered in the media wherein the linoleic acid was present, either under the form of a supplement for medium or yeast inoculum. Fermentation degrees at aerated worts or pitched with 12 or 24 hour-supplemented inoculum were lower, but at not very different values. The trehalose content at the cells cropped from 12 hour supplemented inoculum fermented medium, with initial value incomparably higher, decreases drastically during the first 24 hours, which indicates the increasing cells capacity to use trehalose in the lag phase related to other parameters strongly ameliorated. The supplementation of cropped yeast with unsaturated fatty acid – linoleic acid can be considered as an interesting alternative to wort oxygenation to restore optimal membrane function.

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