# THE INFLUENCE OF LINOLEIC ACID SUPPLEMENTING OF YEAST INOCULUM ON FLOCULATION OF YEAST CELLS IN DIFFERENT EXPERIMENTS AND IN SUCCESSIVE FERMENTATION CYCLES

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**Abstract**
The main goal of the experiments made consisted in studying the linoleic acid supplementing of yeast inoculum effect of cropped yeast cells flocculation in different experiments and in successive fermentation cycles.
Keywords: brewery yeast, supplementing, linoleic acid, yeast cells floculation capacity

**Rezumat**
Obiectivul studiilor experimentale constă în studiul efectului suplimentării inoculului de drojdie asupra capacităţii de floculare a celulelor în diferite experimente şi în cicluri succesive de fermentare.
Cuvinte cheie: drojdia de bere, suplimentare, acid linoleic, capacitate de floculare

**Résumé**
L'obiectif des études experimentales consiste dans l'étude de l'effect de supplémenter l'inoculum de levure sur la capacité de floculation des cellules dans des experimentes differérentes et dans des cycles succesifs de fermentation.
Mots clés : levure, supplémente, acid linoleic, capacité de floculation

## Introduction

For the production brewer, predicting fermentation performance may also involve the determination of flocculation capacity of yeast slurry. Predicting the capacity of yeast to consistently flocculate following serial repitching is of great importance to the brewing industry [SMA, 96].

The genetics and biochemistry of flocculation are not completely understood but the term includes two clearly different physical aspects of yeast behaviour during fermentations:

- The aggregation of yeast cells into flocks which may be due to either non-separation of cells after budding or coalescence of single cells into clumps late in fermentation;
- The rate of sedimentation of cells from the fermented beers.

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 [MOO,03].

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

The main goal of the experiments made consisted in studying the linoleic acid supplementing of yeast inoculum effect of cropped yeast cells flocculation in different experiments and in successive fermentation cycles.

## Experimental

The study is carried out on the industrial isolated strain from the production culture at S.C. Bermas S.A - Suceava - Saccharomyces 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 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. After each fermentation cycle the supplemented yeast cells were harvested and suffered the same treatment. 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 fermenting tubes-equipped conic vessels.

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, 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.

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 flocculation measurement is made using the Helm's test ethanol – improved variant.

Preparation procedure of yeast sample consisted in: suspend a 5 to 6 g sample of yeast in 30 ml calcium sulphate solutions, centrifuge and decant the supernatant, wash once with 30 ml calcium sulphate solution and centrifuge.

Determination of sediment volume consisted in: measure 10 ml calcium sulphate containing 4% ethanol into a 15 ml centrifuge tube, add 1g of the washed yeast and suspend, place in a water bath at 20°C for 20 min., shake to resuspend, after a further 10 min. measure the volume of yeast suspension.

Flocculation is expressed as the mean percentage.

## Results and discussions

The results shown in figures 1 – 4, refer to yeast flocculation capacity, in four successive fermentation cycles, started for each cycle from initial moment and for 24 hour intervals until the 5th fermentation day offers the possibilities for the following comparisons:

**Comparison of yeast cells flocculation capacity values at the initial moment for the non - supplemented yeast inoculated in aerated or supplemented medium**

It is observed that, from one fermentation cycle to another, before the medium inoculation, there are no evident differences between yeast flocculation capacity values from aerated or supplemented medium. Yeast flocculation capacity at the initial moment is 1,4% maximum increased from one fermentation cycle to another.

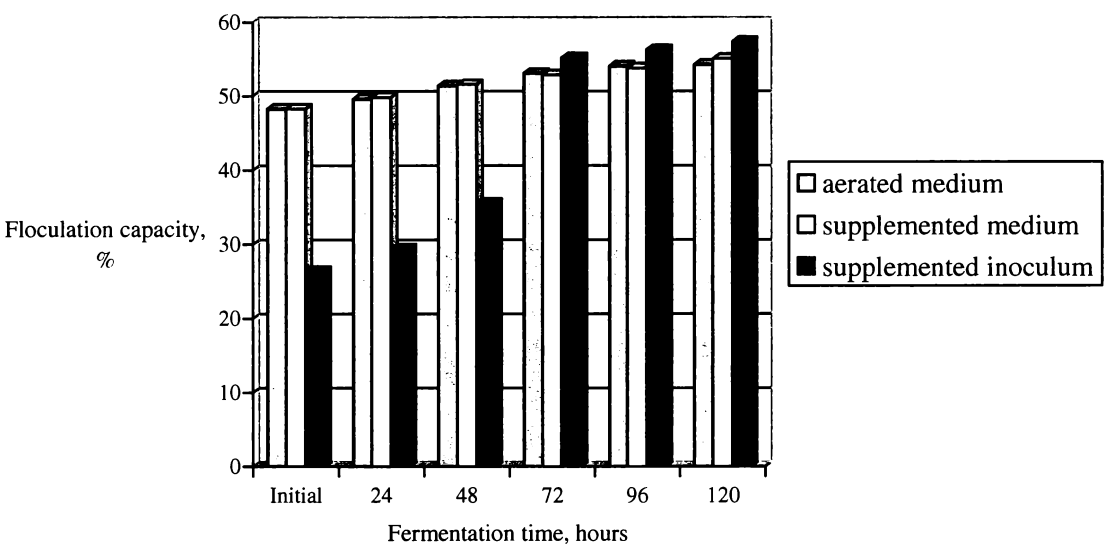


**Fig. 1: Yeast cells floculation capacity from the first fermentation cycle**

**Comparison of yeast cells flocculation capacity values at the initial moment for the supplemented yeast inoculated in non - aerated medium**

Supplemented yeast flocculation capacity is, at the initial moment, at low value.

At inoculum stage, before the 1st, 2nd, 3rd and 4th fermentation cycle supplemented flocculation capacity is: with 1,6 % increased in inoculum stage before cycle II and cycle I comparatively; with 3 % increased in inoculum stage before cycle III and cycle II comparatively; with 4% increased in inoculum stage before cycle IV and cycle III comparatively.

**Comparison of yeast cells flocculation capacity value in the same fermentation cycle for the non – supplemented yeast: (registered value after 120 hour) – (registered value at the initial moment)**

Non – supplemented yeast flocculation capacity increased in the same fermentation cycle, without significant differences of registered value.

- Non – supplemented yeast inoculated in aerated medium, flocculation capacity – first cycle:6; second cycle: 8,1; third cycle: 9; fourth cycle: 11,3;
- Non – supplemented yeast inoculated in supplemented medium, flocculation capacity – first cycle:6,8; second cycle: 9; third cycle: 10,1; fourth cycle: 11,5;

The differences between flocculation capacity value are increased towards the fourth fermentation cycle.

**Comparison of yeast cells flocculation capacity value in the same fermentation cycle for the supplemented yeast: (registered value after 120 hour) – (registered value at the initial moment)**

Linoleic acid supplemented cells have, at the initial moment a decreased flocculation capacity which significantly raise until the last fermentation day.

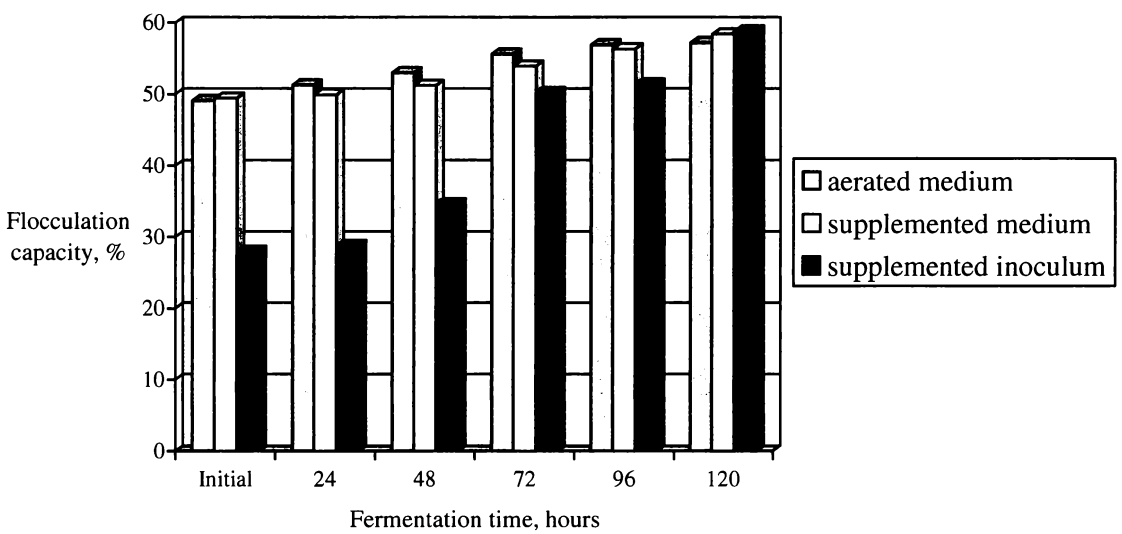


**Fig. 2: Yeast cells flocculation capacity values from the second fermentation cycle**

- After the first fermentation cycle the registered difference is 31;
- After the second fermentation cycle the registered difference is 30,8;
- After the third fermentation cycle the registered difference is 29,8;
- After the fourth fermentation cycle the registered difference is 29.

The differences between flocculation capacity value decrease towards the fourth fermentation cycle.

It is observed that the 24 hour linoleic acid contact determine the deflocculation of yeast cells which is more intense at the first fermentation cycle. Deflocculation capacity of supplementing operation decreases towards the fourth fermentation cycle.

The flocculation capacity of the linoleic acid supplemented cells, during the same fermentation cycle rapidly increase on the last interval of fermentation period. After 72 hour of fermentation the flocculation capacity value are obviously increased in all fermentation cycles, comparativly the previous day. After the data check at all fermentation cycles obtained while measuring the fermentation value achieved in succesive fermentations, one may notice that the maximum fermentation degree value is obtained in the interval 72 – 96 hour.

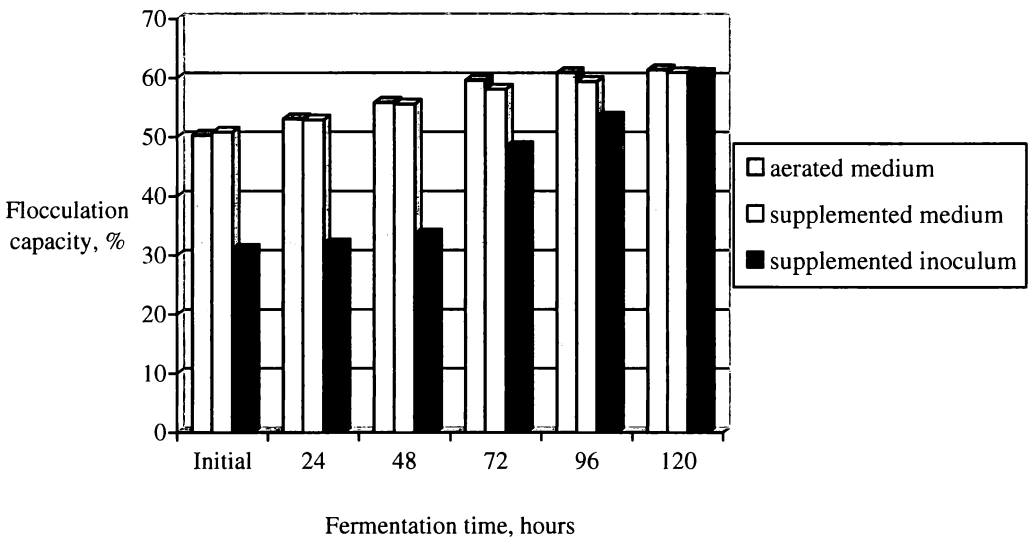


**Fig. 3: Yeast flocculation capacity values for the third fermentation cycle**

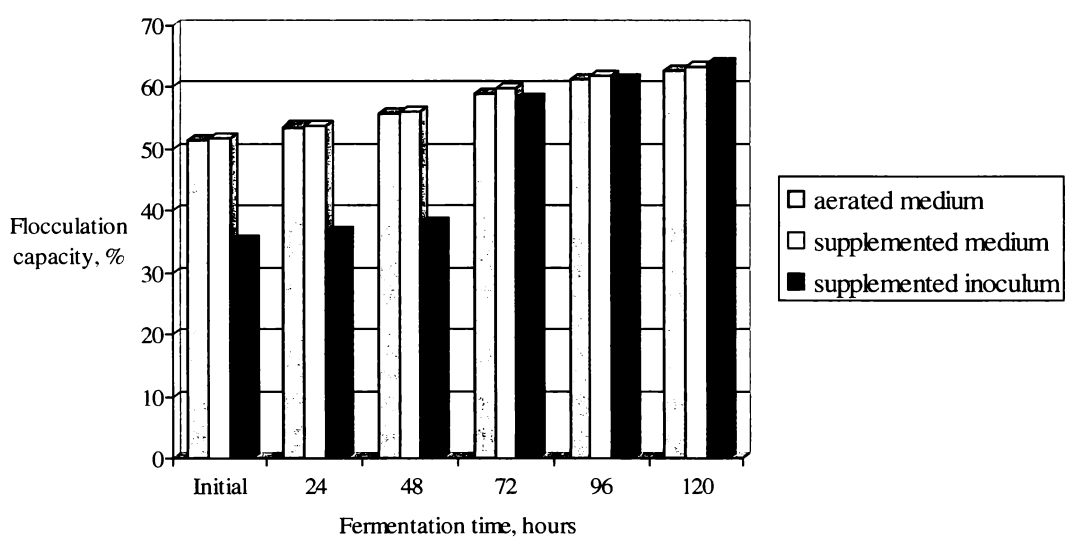


**Fig. 4: Yeast flocculation capacity values from the fourth cycle**

The sudden increase of flocculation capacity of linoleic acid – supplemented cells indicates reaching of the maximum fermentation degree value.

**Conclusions:**

- 24 hour contact maintaining with linoleic acid determines cell deflocculation;
- The flocculation capacity increases suddenly when the maximum fermentation degree value, in the case of linoleic acid supplemented inoculum, is reached;
- The flocculation capacity value at non – supplemented yeast increase at constant speed, from higher and higher start value towards the fourth fermentation cycle, therefore the maximum fermentation degree reached value is lower and lower towards the fourth fermentation.

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