# INFLUENCE OF TEMPERATURE UPON ETHANOL YIELD OF NO-COOKING MASH FERMENTATION

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**Abstract:** *In the case of no-cooking mash there is a dangerous process of contamination and of undesirable fermentations. The fermentations of heated mashes are studied both the yield of bio-ethanol and the composition in alcohol of the mixture. Analyze of fermentation curves shows that the highest yield in bio-ethanol and an important economy of energy are obtained at 52$^0$C. Gas-chromatograms show that the secondary products are in normal limits and the images from the microscope shows an extremely reduce lactic bacterial presence.*

**Key words:** *un-cooking slurry, fermentation curves, bioethanol, Gas-chromatograms*

## Introduction

The ethanol yield depends upon raw material, flow type, conditions of sequences of operations, stages conditions. The raw material has a considerable effect on fuel ethanol yield (Lacerenza J et.al., 2006). Seed quality characteristics of grains such as: protein, starch content, moisture, kernel size, and lipid content are one of the most important factors upon yield ethanol.

Chemical and biochemical processes in ethanol flow are influenced by: chemical composition of raw material, ethanol yield depending upon starch content, starch hydrolyzing processes, yeast fermentation conditions, maintaining optimal temperature.

The corn starch content has a great influence upon ethanol yield such as: corn quality, moisture, sizes particles, (Bothast R.J., et.al, 2006).

The main operation in the classical process is the cooking of the mash presents some advantages: a well known technology, a sterile mash, a very good starch extraction and some des-advantages such as: high necessary of thermal energy, a high initial sugar concentration, undesirable reactions, etc.

The use of heated no-cooking mashes presents some advantages as: low necesary of termic energy (aprox. 10-15%) and less equipment, a reduced initial sugar concentration so that the yeat has no asmotic stress, no undesirable reactions Maillard, an excelent growth and viability of yeat(less glicerol content), a low viscozity of mashes and a high yield in ethanol, a short time of the no-cooking process and less capital than the cooking process, less cooling water, no vapor added. A des-avantage is the possible contamination of Lactobacillus sp. in the fermentation broth of bioethanol production with the decrease oo production efficiency (Watanabe I., et.al. 2007).

## Materials and methods

The performance of the hidrolyse of the corn starch in a no-cooking process is posssible by using a special packet of enzymes that determine the formation of fermentable sugar in the conditions of a low mashes viscozity (Gutt S., et.al. 2007)]. The mashes were heated at different temperatures between 45-55 degrees, keeping constant the other parameters as: raw material, dimension of particles, water quantity, enzymes quantity related to corn flour, yeast for fermentation, pH.

**Table 1** Enzymes used in hydrolyze of heated corn mash. (Gutt S. et.al, 2007). (Rotar R., et.al. 2008)

| Nr crt | Type of enzyme | The main microorganism | optim pH | Temperature (°C) | Action time (min) | Enzimatic activity | Dose (g) |
|---|---|---|---|---|---|---|---|
| 1. | Spezyme Xtra (α-amilaze) | Bacillus licheniformis | 5,4-5,8 | 55 | 120 | 14000 AAU/g | 0,04 |
| 2. | Stargen™ | Aspergillus kawaechi şi Aspergillus niger | 4-4,5 | 32 | 60 | 456 GSHU/g | 0,014 |
| 3. | Fermgen (proteaze) | Trichoderma reesei | 3,5-5,0 | 32 | 15 | 1000 SAPU/g | 0,02 |

The experimental works of many no-cooking mashes in different conditions were carried out with biosensors from Blue Sens firm that perform the monitoring of disolved oxigen, carbon dioxide content, ethanol content from mashes fermentations.

The self-recording makes possible the determination of fermentable sugar used by yeats in their metabolism and the time of their multiplication, the ethanol produced by fermentation and the optimum durata of the process in the conditions of a maximum eficiency.

The composition of the alcohol mixtures were determined by using a gascromatograf from Shimadzu firm with the following characteristics:

- gascromatograph Shimadzu with detector of flame ionization
- cromatografic copper column, 30 m length, 0,25 mm diameter
- microsyringe 5 µl
- hydrogen generator,
- computer

*Work conditions*:

- column temperature 35°C; in the first 5 minutes and than the temperatute is elevated with 6 degrees/min. until 125°C;
- detector temperature, 280°C
- injector detector, 220°C
- hydrogen debit, 40 $cm^3$/min
- propulsive gas debit( He, N), 30 $cm^3$/min.

The compounds of the alcoholic solution are identified and quantified with the gascromatograph and are raported to the standard alcoholic solution.

The compouns of the standard solution are: ethanol, 20$cm^3$, methanol 2$cm^3$, propyl alcohol 2$cm^3$, izopropyl alcohol 2$cm^3$, acetic aldehyde 2$cm^3$, ethyl acetate 2$cm^3$.

The standard solution is obtained from the stoc solution by a dilution 1:5 and the contration of compounds is: acetaaldehide 3.12 mg/ml, etil acetat 3.28 mg/ml, methanol 31.6 mg/ml, izopropanol 3.12 mg/ml, ethanol 31.6 mg/ml, propanol 3.2 mg/ml, amyl alcohol 3.3 mg/ml

**Results and Discussion**

The corn no-cooking mashes fermentations, heated at different temperatures are presented in figures 1-5.

The concentrations of oxigen, $CO_2$ and ethanol obtained are monitorised in every moment.

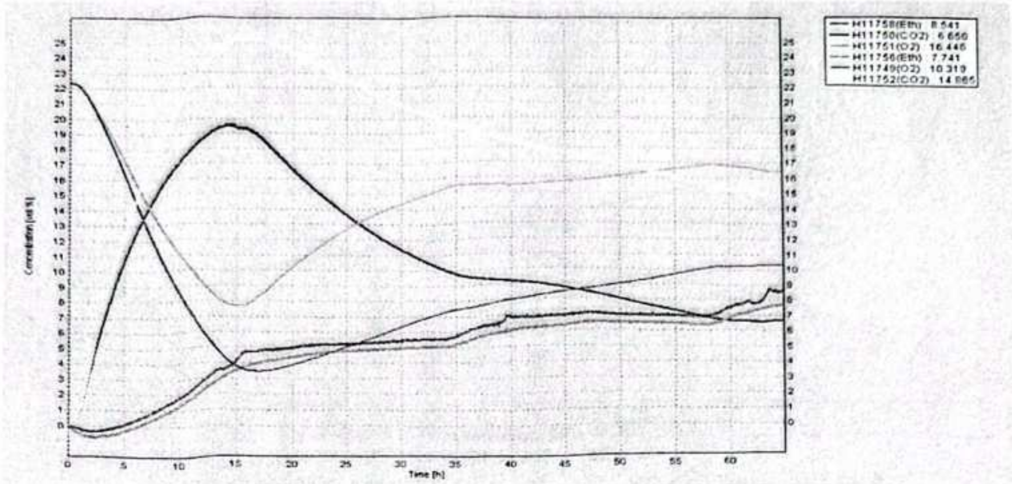

**Figure 1** No-cooking corn mash, 55$^0$C

| NO-COOKING MASH 1 | NO-COOKING MASH 2 |
| --- | --- |
| — ethanol concentration | — ethanol concentration |
| — $CO_2$ concentration | $CO_2$ concentration |
| — $O_2$ concentration | — $O_2$ concentration |

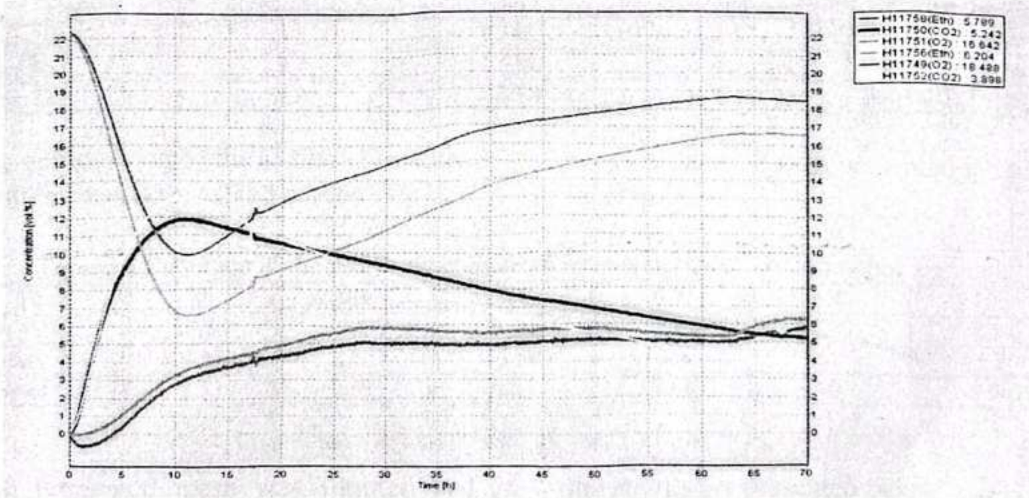

**Figure 2** No-cooking corn mash, 45 $^0$C

| NO-COOKING MASH 1 | NO-COOKING MASH 2 |
| --- | --- |
| — ethanol concentration | — ethanol concentration |
| — $CO_2$ concentration | $CO_2$ concentration |
| — $O_2$ concentration | — $O_2$ concentration |

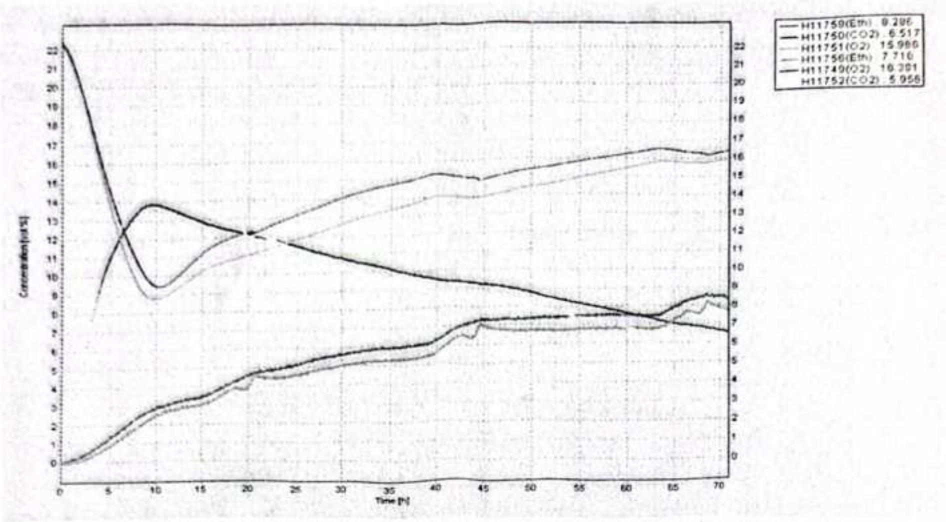

**Figure 3** No-cooking corn mash, 49 $^{0}$C

NO-COOKING MASH 1

- ethanol concentration
- $CO_2$ concentration
- $O_2$ concentration

NO-COOKING MASH 2

- ethanol concentration
- $CO_2$ concentration
- $O_2$ concentration

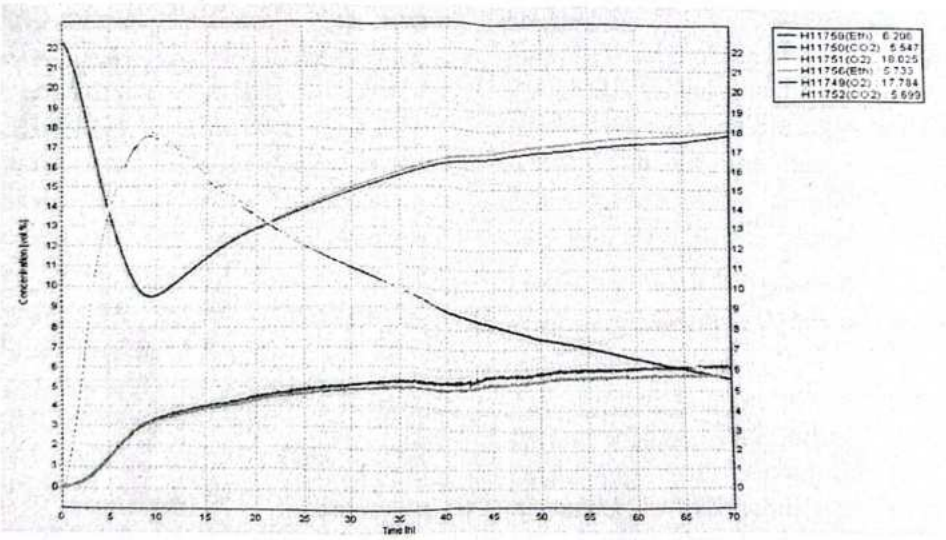

**Figure 4** No-cooking corn mash, 51 $^{0}$C

NO-COOKING MASH 1

- ethanol concentration
- $CO_2$ concentration
- $O_2$ concentration

NO-COOKING MASH 2

- ethanol concentration
- $CO_2$ concentration
- $O_2$ concentration

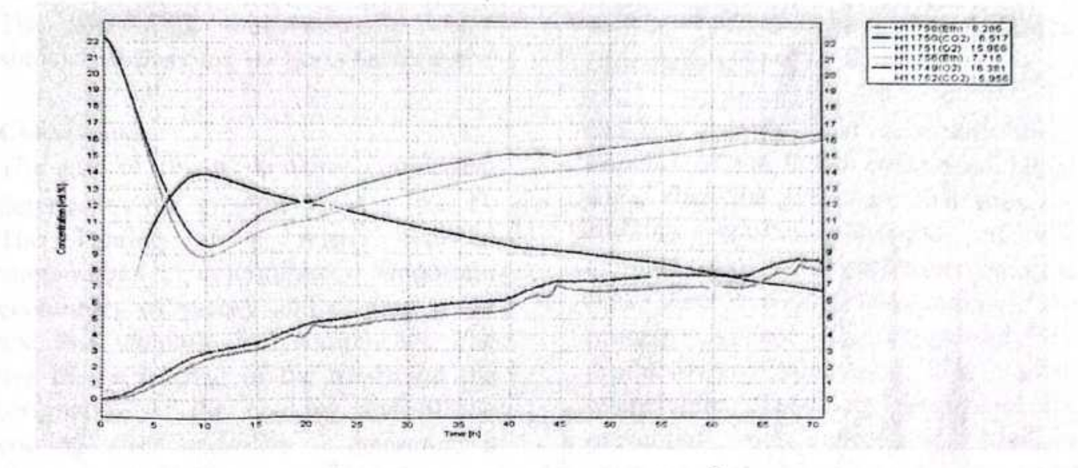

**Figure 5** No-cooking corn mash, 52$^0$C

| NO-COOKING MASH 1 | NO-COOKING MASH 2 |
|---|---|
| ethanol concentration | ethanol concentration |
| $CO_2$ concentration | $CO_2$ concentration |
| $O_2$ concentration | $O_2$ concentration |

The evolution of main parameters are presented in the table 2.

**Table 2** The evolution in time of main parameters

| Time | S.u.% | $n_D$ | pH | microbiological |
|---|---|---|---|---|
| inițial (0h) | 6.6 | 1.3423 | 4.37 | No lactic bacteria |
| After 36 h | 3.7 | 1.3383 | 4.03 | No lactic bacteria |
| After 72h | 5.8 | 1.3520 | 4.24 | No lactic bacteria |

The composition of the alcoholic mixture from fermentation of the no-cooking corn mash, at 52$^0$C is presented in the tabel no.3.

**Tabele 3** Evolution in time of parameters from fermentation of corn no-cooking mash

| Time | 8h | 10h | 36h | 42h | 65h | 70h |
|---|---|---|---|---|---|---|
| $CO_2$ %v | 13.63 | 13.85 | 9.92 | 9.28 | 6.99 | 6.6 |
| $O_2$ %v | 9.27 | 8.77 | 13.59 | 13.91 | 15.79 | 15.88 |
| Ethanol %v | 2.53 | 2.91 | 6.03 | 7.08 | 8.01 | 8.68 |

The fermented mash was filtrated and analysed with the gascromatograf and the results are presented below:

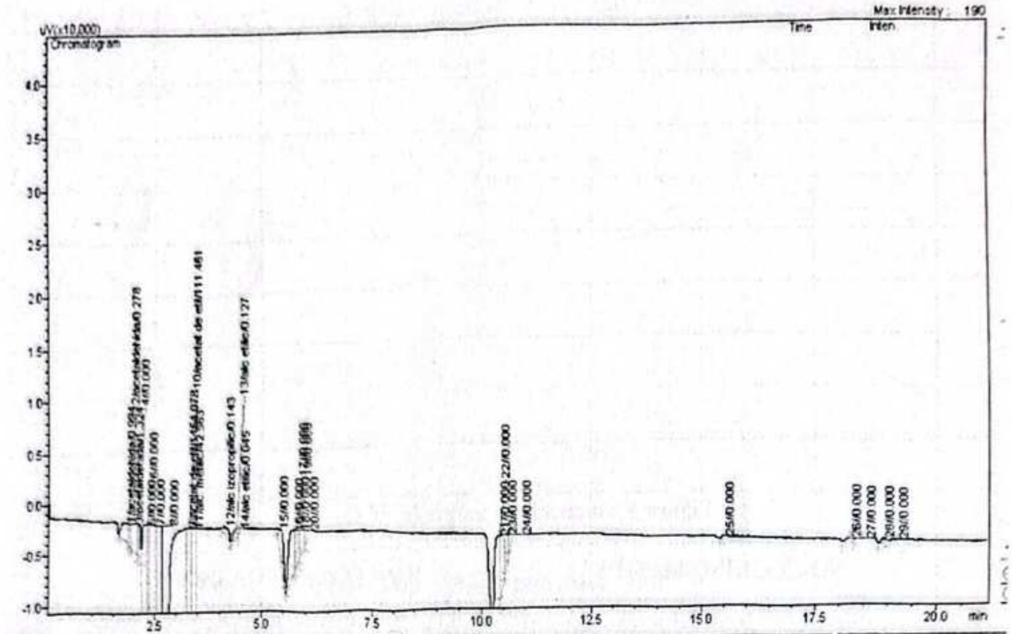


**Figure 6** The gascromatrograme at 52 $^0$C

Results - Peak Table

Peak Table | Compound | Group | Calibration Curve

| Peak# | Compound Name | Ret.Time | Area | Conc. | Units |
|---|---|---|---|---|---|
| 1 | acetaldehida | 1.888 | 19660.8 | 1.00123 | mg/ml |
| 2 | acetat de etil | 3.281 | 5532.6 | 0.21545 | mg/ml |
| 3 | alc etilic | 4.305 | 7197599.0 | 139.29870 | mg/ml |
| 4 | alc propilic | 7.170 | 2229.6 | 0.03658 | mg/ml |
| 5 | | 8.915 | 16708.2 | 0.00000 | |
| 6 | | 12.139 | 50222.2 | 0.00000 | |

**Figure 7** The identification and concentration of compounds from the heated corn mash at 52 $^0$C

The best results are obtained in the case of the corn mashes heated at 52 $^0$C. In these conditions, the greatest quantity of ethanol and the small quantity of secondary alcohols are obtained.

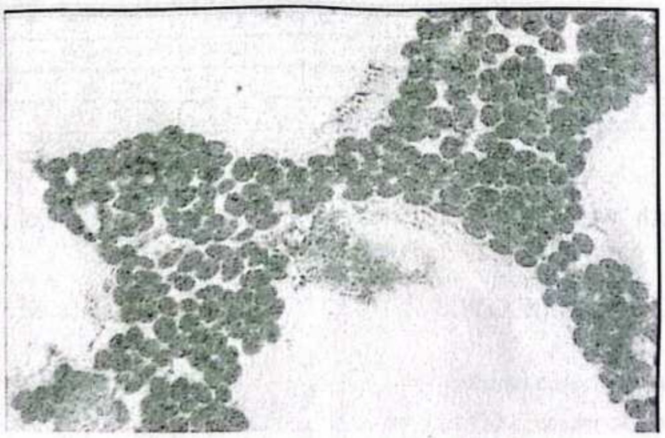

**Figure 8** Microscopic image of the mash heated at 52 $^0$C after 22 hours [5]

The analyse of the microscop image shows that there are no lactic bacteria and no danger of the contamination of heated corn mash at 52 $^0$C.

## Conclusions

The use of the no-kooking corn mash determines the greatest yield at 52 $^0$C. The heating at a relativ reduced temperature determines important economies of energy and cooling water and less technological equipments. The use of the heating of the mash and the resignation of the cooking and of the cooling after hydrolise determine a decrease of the viscozity and an increase of the ethanol contain. The yeat has an excelent viability and no osmotic stress because of the small contain of initial sugar than the procedure that uses the cooking mashes. Because of the elimination of some operations from the flow sheet (cooking and cooling), the process is not so long and the productivity increases. The small temperature of heating determines the prevention of undezirable Maillard reactions that determine an increase of ethanol yield.

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