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Factors influencing the fermentation process and ethanol yield

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Abstract

The factors which influence ethanol yield are: the quantity of fermenting sugars from raw material, the heating temperature of the mashes, the fermentation time, the distillation parameters. Out of the already-mentioned factors, the heating temperature of the mashes and the fermentation time have been studied. From the analyzed data it has been found that the highest percentage of alcohol can be obtained in the case of non – cooked wheat and corn mashes. In the case of cooked mashes the highest quantity of ethanol is released in the first 46 hours of the process, fact that recommends the use of a short type fermentation process for cooked mashes which ensures greater productivity. In the case of non-cooked mashes the recommended process is the longer one (72 hours) because ethanol is produced continuously, the sowed yeasts are resistant to alcohol and the osmotic stress caused by fermenting sugars is reduced compared to the process based on cooked mashes. Contamination with Lactobacillus is missing in the case of cooked mashes, and in the case of non cooked mashes it appears only at the end of the process, with a density of 1-2 bacteria per field. Compounds of the distilled mixture and their quantity have been determined by means of the gaschromatographic analysis.

Keywords: bioethanol, non cooking process, time of fermentation, gaschromatography

1. Introduction

Bioethanol, the most important alternative to the fossil fuels, obtained from corn, wheat, or sugar beets is the common biofuel produced from classic agricultural food crops.
The use of corn and wheat as a source of fermentable sugars is one of the most important possibilities of obtaining bioethanol [1]. The obtaining of mashes from cereals which will be put to fermentation with use of yeasts can be achieved through cooking in the classic way or through heating with help provided by an enzymatic mixture. The use of heated non cooking mashes presents some advantages such as: low need of thermic energy (aprox.10-15%) and less equipment, a reduced initial sugar concentration so that the yeast has no osmotic stress, no undesirable reactions Maillard, an excellent growth and viability of yeast, less glicerol content [2], a low viscosity of mashes and a high yield in ethanol, a short time of the non cooking process, less cooling water, no vapor added [3]. A disadvantage is the possible contamination of Lactobacillus sp. in the fermentation broth of bioethanol production with the decrease of production efficiency [4]. The thermodynamics of the industrial production of ethanol from one particular food crop, corn, was studied and the possibilities of improving the yield very intensive analyzed [5]. A possibility to increase the efficiency of bioethanol production is to use a short cooking process of 46 hours in case of cooked mashes and a long cooking process of 72 hours in case of non cooked mashes.

2. Materials and methods

2.1 Sample handling

The experiments were carried on two series of cereals cooked and non cooked mashes. The first determinations were performed on corn grinding and than the same analyses were done on wheat grinding. In both cases, the efficiency of using a short fermentation process of 46 hours and a long one of 72 hours has been analyzed.

The bioalcohols were obtained in bioreactors of 10 L volume and all parameters were monitored: pH, temperature, rotate speed, free carbon dioxide, free oxygen and dissolved oxygen. Also with small bioreactors from Bluesens, 1l volume, and parameters that pH, free carbon dioxide, free oxygen bioethanol were monitored. The alcohol mixture was distilled by a rotaevaporator of 500 ml volume. The analyses of alcohol mixture was performed by a gaschromatograph Shimadzu, with flame ionization detector, copper chromatographic column of 30m length and 0.25 mm internal diameter, mycrosyringe 5μl, hydrogen generator, nitrogen cylinder, helium cylinder, computer, printer.

2.2 Materials

The composition of standard solution is: 20cm^3 ethanol, 2cm^3 methanol, 2cm^3 propanol, 2cm^3 isopropanol, 2cm^3 acetaldehyde, 2cm^3 ethyl acetate, reagents from Merk company. The standard solution is obtained from the stock solution thinned until a 1:5 ratio is achieved and contains ingredients in following concentration : acetaldehyde 3,12 mg/ ml, ethyl acetate 3,28 mg/ml, methanol 3,16 mg/ml, isopropylic alcohol, 3,12 mg/ml, ethanol 31,6 mg/ml, propil alcohol, 3,2 mg/ml, amyl alcohol 3,3 mg/ml.

For determinations made on analyzed samples a standard working solution described herein before has been used. Compounds from the analyzed sample can be identified and dosed with the help of gaschromatograph through reporting to the data recorded for the standard solution. Chromatogram from figure 1 presents the compounds of standard solution utilized by gaschromatograph, and in the table no. 1 is presented the concentration of compounds from the standard solution.

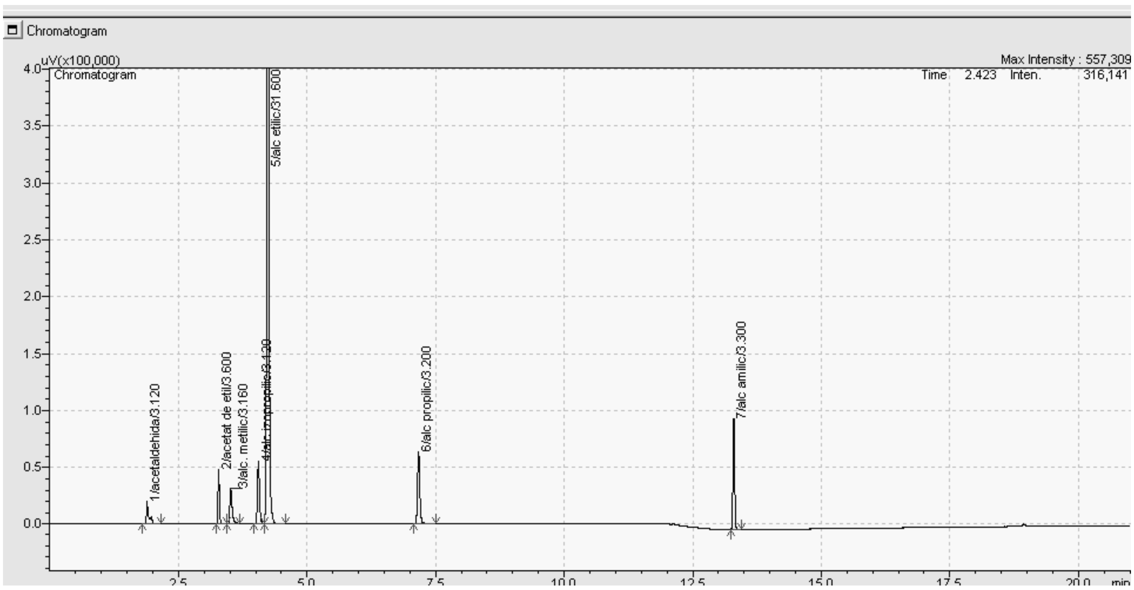

Figure 1. Chromatogram with compounds in the standard alcoholic solution

Table no. 1 Compounds and their concentration in the alcoholic solution**.**

Peak#	Compound Name	Ret.Time	Area	Conc.	Units
1	acetaldehyde	1.886	61266.5	3.12000	mg/l
2	ethyl acetate	3.280	92445.4	3.60000	mg/l
3	methanol	3.513	97107.0	3.16000	mg/l
4	izopropilic alcohol	4.053	152599.7	3.12000	mg/l
5	ethanol	4.243	1632780.0	31.60000	mg/l
6	propil alcohol	7.168	195055.2	3.20000	mg/l
7	amyl alcohol	13.299	233405.3	3.30000	mg/l

The hydrolysis of starchy raw materials can be done by acids or enzymes, but due to several advantages of enzymatic hydrolysis, it is the preferred choice for industrial applications [6]. The enzymes involved in hydrolysis include: α -alfa amylase, glucoamylase, and pullulanase. For the hydrolysis of starch a combination of specific enzymes has been used for both processes with cooked and non cooked mashes as it can be seen in tables no. 2 and 3.
The enzymatic mixtures used in corn and wheat non-cooked mashes has the following composition [7]:

Table no.2 Enzymatic mixtures used in practical determinations of non cooked mashes**.**

Nr.crt	Enzyme	Micro – organism that creates it	Optimum pH	Temp (°C)	Action time (min)	Enzymatic activity	Used doze (g)
1.	**Spezyme Xtra (α-amilază)**	Bacillus licheniformis	5,4-5,8	55	120	14000 AAU/g	0,04
2.	**Stargen™**	Aspergillus kawaechi and Aspergillus niger	4-4,5	32	60	456 GSHU/g	0,014
3.	**Fermgen (protease)**	Trichoderma reesei	3,5-5,0	32	15	1000 SAPU/g	0,02

Table no. 3 Enzymatic mixtures used in practical determinations of cooked mashes

Nr.crt.	Enzyme	Micro – organism that creates it	Optimum pH	Temp. (°C)	Action time (min)	Enzymatic activity	Used doze (g)
1	**AMYLEX**	Bacillus licheniformis	5,4-5,8	105-110	5-7	940 GSAU /g	1,44
2	**Optimash (endo-glucanase)**	Geosmithia emersoni	3-6,5	75	60	5625 u/g	1,292
3	**DIAZYME X4 (amiloglucozidase)**	Aspergillus niger	4,2-4,4	60	120	400 GAU/ml	1
4	**Fermgen (protease)**	Trichoderma reesei	3,5-5,0	60	60	1000 SAPU/g	1,8

2. Experimental

2.1. Fermentation of cooked wheat mashes.

The behaviour of cooked wheat mashes during fermentation has been analyzed. In the analysis sample fated for cooking SPEZYME EXTRA enzyme has been added for liquefaction and the mash has been cooked for an hour at 100 ^{0} C. The obtained content has been let to cold until temperature reached 60^{0}C (optimum enzyme temperature) and enzyme GC 536 has been added for intensifying the enzymatic hydrolysis of proteins from the mash. The mash is let to saccharify on the water bath for an hour at constant temperature of 60^{0}C. The end of saccharification is verified through the reaction of a solution with iodine in KI. After saccharification the mash pH is verified and its value must be between 3 and 4.5, the optimum values for the FERMGEN enzyme. The pH correction is done with lactic acid. When the temperature of the mash reaches 34^{0}C FERMGEN enzymes will be added for a quicker hydrolysis of proteins and to assure many nutrients for the yeast added in the fermentation stage. After a repose of 15 minutes the yeast will be added to the mash which will be put in a fermentation cell with sensors which assure the monitoring of the fermentation parameters on the entire process (72 hours) [8], figure 2. For analysis two samples of cooked mashes has been achieved (72 hours) [8], figure 2.

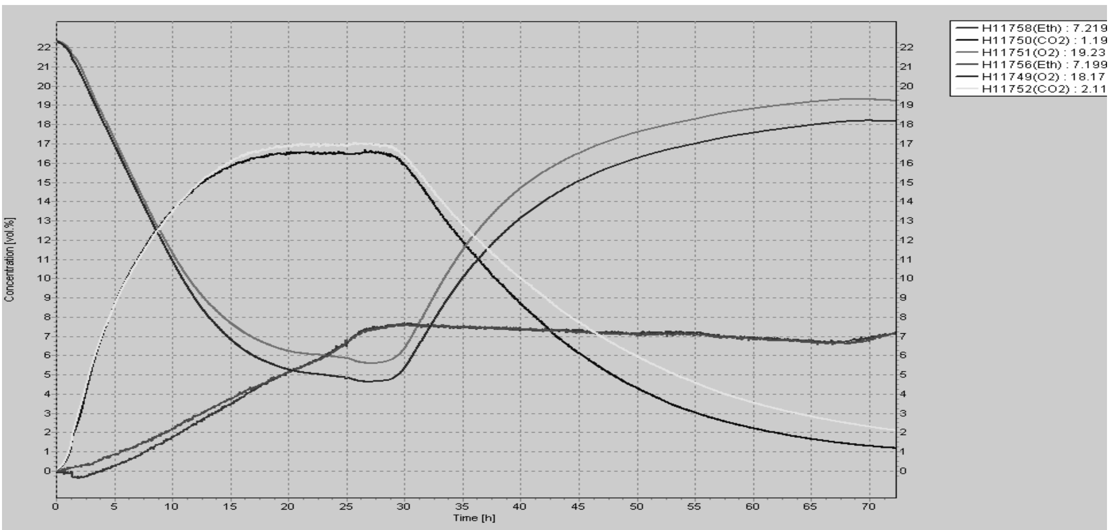

Figure 2.Variation of compounds concentrations in gas form during fermentation phase of cooked wheat mashes

COOKED MASH 1

Ethanol concentration

CO_2 concentration

O_2 concentration

COOKED MASH 2

Ethanol concentration

CO_2 concentration

O_2 concentration

The content of fermentation cells is put to distillation in a rotaevaporator and with help provided by a gaschromatograph the compounds of distilled sample and their concentrations are identified.

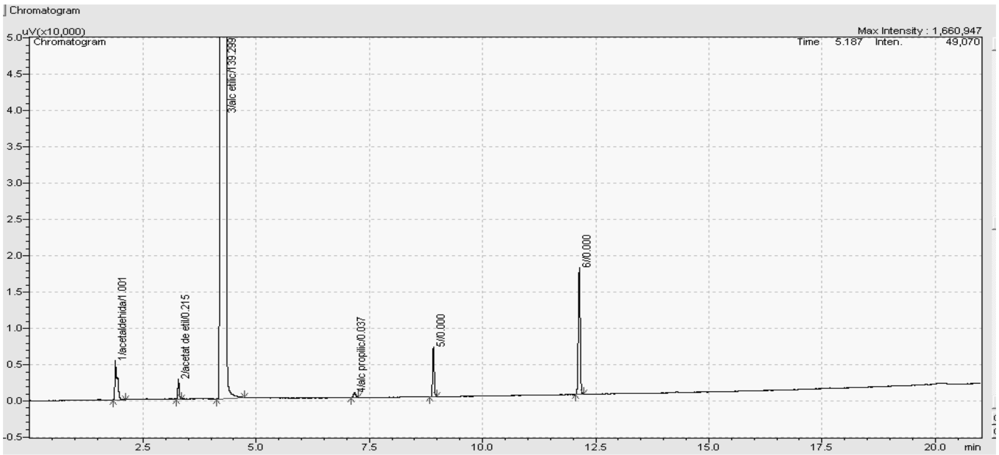

Figure 3. Chromatogram of compounds obtained from the fermentation of cooked wheat mash.

Table no.4. Compounds and their concentrations obtained from the fermentation of wheat cooked mash

Results - Peak Table

Peak Table | Compound | Group | Calibration Curve

Peak#	Compound Name	Ret.Time	Area	Conc.	Units
1	**acetaldehyde**	1.888	19660.8	1.00123	mg/ml
2	**ethyl acetate**	3.281	5532.6	0.21545	mg/ml
3	**ethanol**	4.305	7197599.0	139.29870	mg/ml
4	**propil alcohol**	7.170	2229.6	0.03658	mg/ml
5		8.915	16708.2	0.00000	
6		12.139	50222.2	0.00000	

2.2 Fermentation of non cooked wheat mashes

In the case of non cooked mashes the warming at 55^{0}C for 2 hours and the enzyme SPEZYME EXTRA for liquefaction has been used. After cooling by 32 ^{0} C, STARGEN 001 is added for intensifying the enzymatic hydrolysis of starch. After saccharification phase the mash should have a value of pH between 5,3 – 5,7, and it is rectified with lactic acid before fermentation phase. When the temperature of the mash reaches 34^{0}C, FERMGEN enzyme is added for a quicker hydrolysis of proteins and to assure more nutrients for the yeast added in the fermentation phase.

The mash is let to rest for about 15 minutes, and after that melted yeast in water is added to it and the whole mixture is put in a fermentation cell with sensors which assure the monitoring of fermentation parameters for 72 hours.

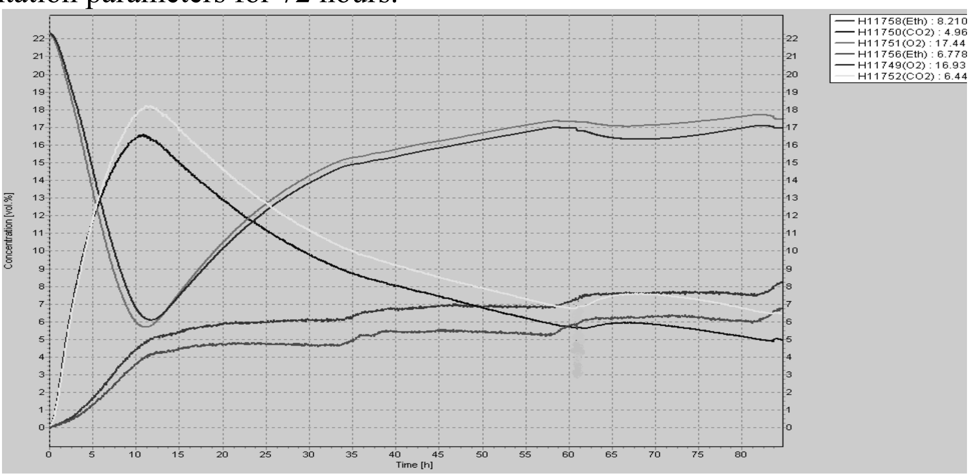

Figure 4. Variation of compounds concentrations in gas form during fermentation phase of non cooked mashes.

NON COOKED MASH 1	NON COOKED MASH 2
Ethanol concentration	Ethanol concentration
CO_2 concentration	CO_2 concentration
O_2 concentration	O_2 concentration

The mixture from the fermentation cells is distilled by a rotaevaporator and the results of the chromatographic analysis of the distillate are presented in figure no.5 and table no.5

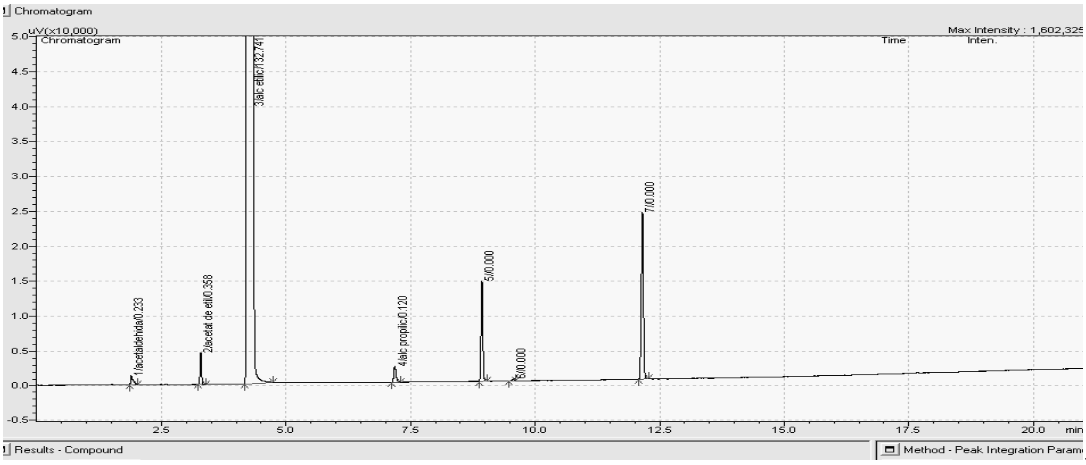

Figure 5. Chromatogram of compounds obtained from the fermentation of non cooked wheat mash.

Table no.5 Identification of the compounds obtained from the fermentation of non cooked mashes and their concentrations

Peak Table | Compound | Group | Calibration Curve

ID#	Name	Ret. Time	Conc.	Units	Peak#	Area	Height
1	acetaldehyde	1.892	0.23290	mg/ml	1	4573	1306
2	ethyl acetate	3.288	0.35822	mg/ml	2	9199	4552
5	ethanol	4.314	132.74081	mg/ml	3	6858751	1597031
6	propil alcohol	7.184	0.11971	mg/ml	4	7297	2323

2.3 Short and long fermentations of wheat mashes

In the case of wheat mashes the influence of fermentation time on ethanol content from the non cooked mashes was analyzed and the results from short fermentation process of 46 hours were compared to those from the long fermentation process of 72 hours. From the three samples which fermented simultaneous, one was stopped at 46 hours, and the two other continued fermentation processes for 72 hours. The three fermentation curves are displayed in figure no 6.

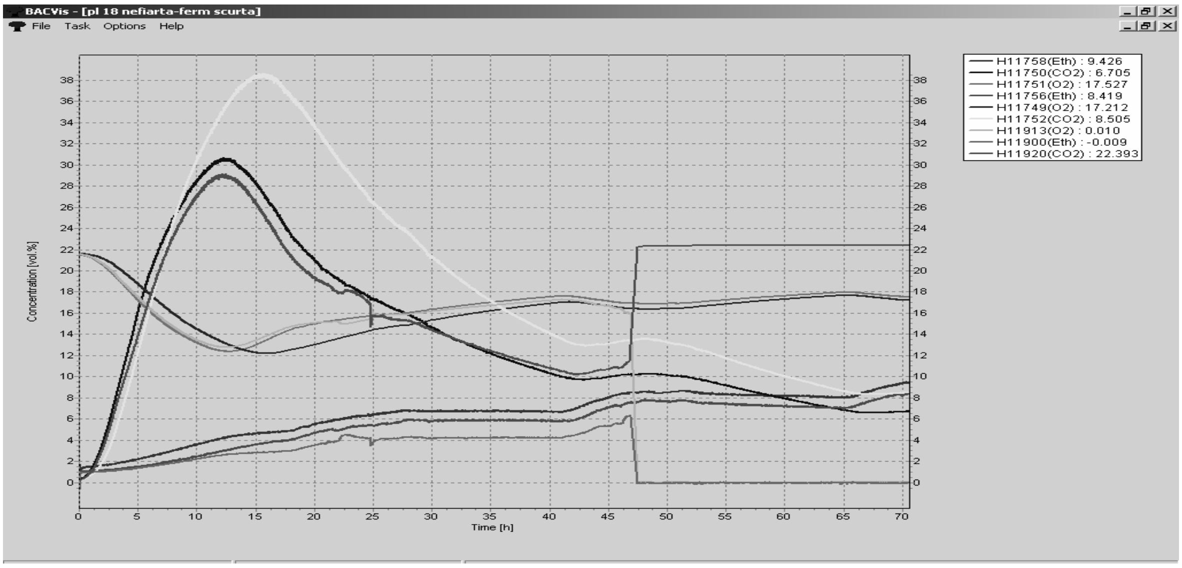

Figure 6. Comparison between non cooked wheat mashes in both short 46 hours and long 72 hours fermentation processes.

From the analysis of fermentation curves we can see that after 46 hours of fermentation the content of alcohol for all 3 samples was between 6,2 and 8 volume %. After 72 hours the bioethanol content was between 8 and 9,4 volume %. As a conclusion, in the case of non cooked mashes the recommended fermentation is the long one of 72 hours.

2.4. Fermentation of cooked corn mashes

By using corn as a starch source, the preparation of samples for fermentation is approached in the same way, and the enzymatic mixtures are the same. The results of fermentation of both cooked mashes are given in figure no.7

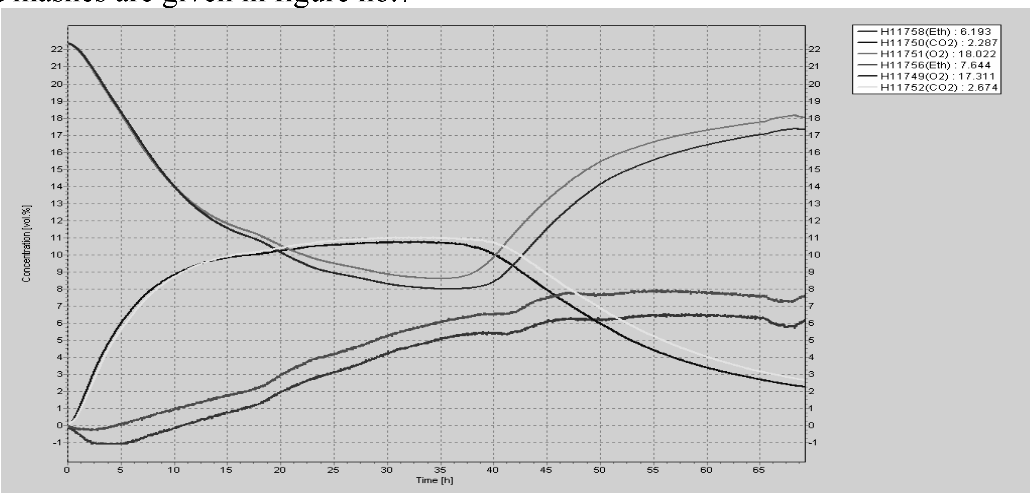

Figure 7. Variation of compounds concentrations in gas form during fermentation phase of cooked corn mashes

COOKED MASH 1	COOKED MASH 2
Ethanol concentration	Ethanol concentration
CO_2 concentration	CO_2 concentration
O_2 concentration	O_2 concentration

The results of chromatographic analysis with identification of distilled compounds and their concentrations are presented in the figure 8 and in the table 6.

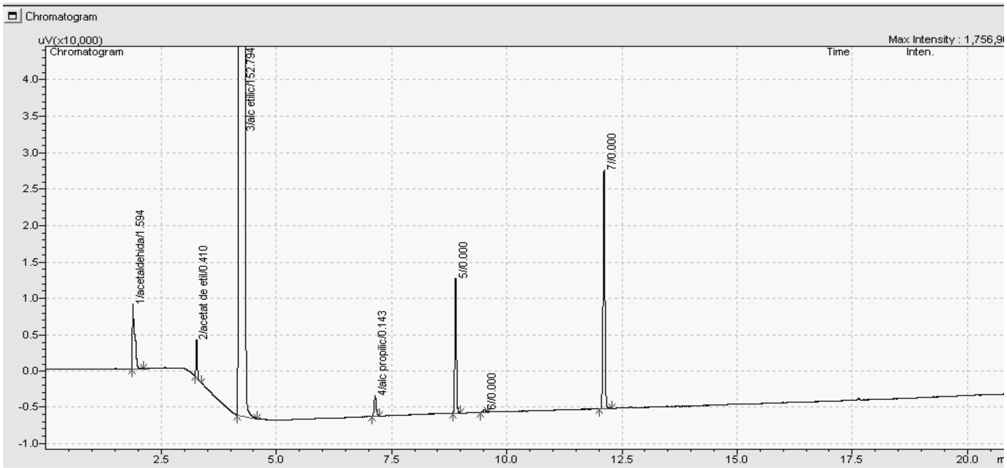

Figure 8. Chromatogram of compounds obtained from the fermentation of cooked corn mash.

Table no.6 Identification of the compounds obtained from the cooked corn mash and their concentrations**.**

Results - Peak Table

Peak Table | Compound | Group | Calibration Curve

Peak#	Compound Name	Ret.Time	Area	Conc.	Units
1	acetaldehyde	1.884	31294.6	1.59368	mg/ml
2	ethyl acetate	3.267	10531.9	0.41013	mg/ml
3	ethanol	4.290	7894901.8	152.79394	mg/ml
4	propil alcohol	7.142	8701.5	0.14275	mg/ml
5		8.889	45256.2	0.00000	
6		9.518	1251.5	0.00000	
7		12.112	90191.9	0.00000	

2.5. Fermentation of non cooked corn mashes.

Non cooked corn mashes were heated at 55 ^{0}C, in the same way as in the case of non cooked wheat mashes. Fermentation curves of both samples are presented in figure no.9.

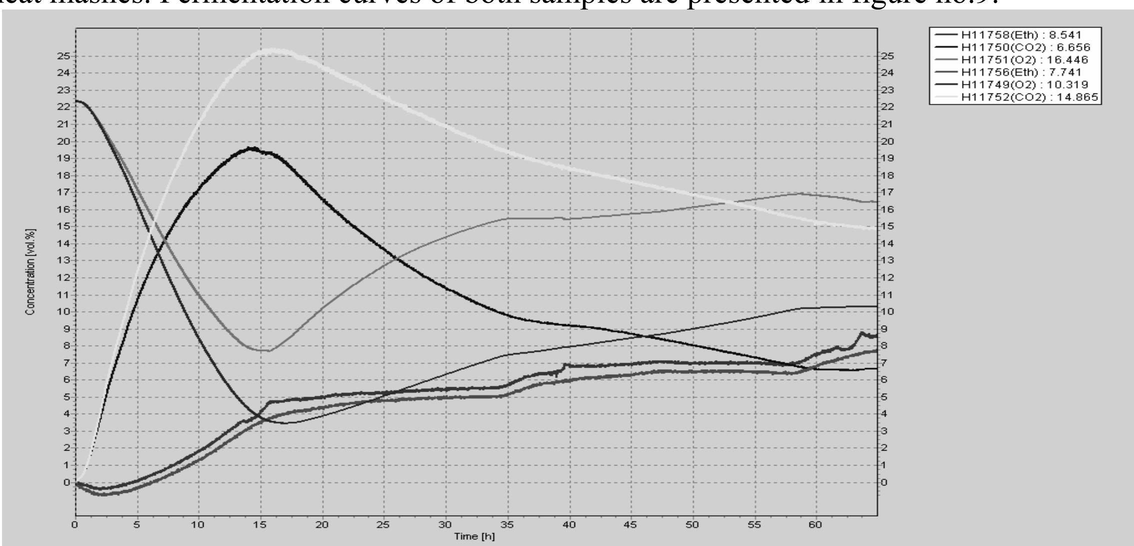

Figure 9. Variation of compounds concentrations in gas form during the fermentation phase of non cooked corn mashes at 55^{0}C

NON COOKED MASH 1	NON COOKED MASH 2
Ethanol concentration	Ethanol concentration
CO_2 concentration	CO_2 concentration
O_2 concentration	O_2 concentration

The content of fermentation cell is distillated by a rotaevaporator. From the distilled sample a gaschromatographic analysis has been done. Compounds of the distilled sample and their concentrations are identified, as showed in figure no.10 and table no. 8.

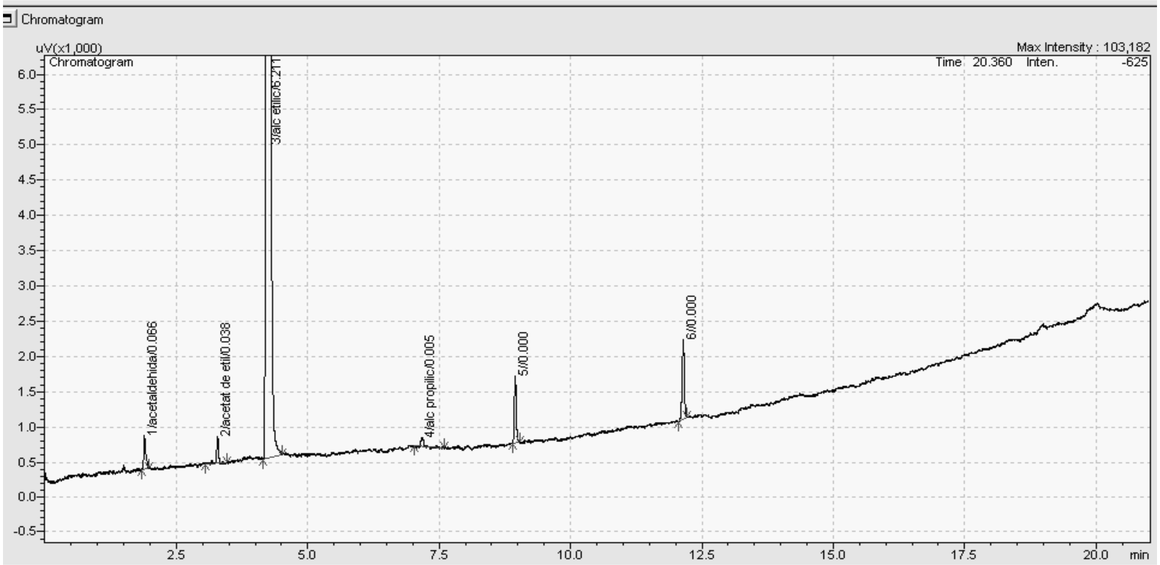

Figure 10. Chromatogram of compounds obtained from the fermentation of non cooked corn mash.

Table no. 8 Identification and concentrations of the compounds obtained from the fermentation of non cooked corn mash.

Peak#	Compound Name	Ret.Time	Area	Conc.	Units
1	acetaldehyde	1.888	7646.3	0.38939	mg/ml
2	ethyl acetate	3.261	8137.6	0.31689	mg/ml
3	ethanol	4.266	6345687.8	122.81124	mg/ml
4	propil alcohol	7.095	6475.7	0.10624	mg/ml
5		8.906	20422.6	0.00000	
6		12.155	44365.6	0.00000	

3. Conclusions

A maximum concentration of ethanol is obtained after 46 hours of cooked mashes fermentation (7,5 % ethanol alcoholic volume for wheat mashes and 8,2% for corn) with concentration decreasing after that. In the case of non cooked mashes the maximum ethanol content is obtained after 72 hours of fermentation. In the same case of non cooked mashes it can be seen that the highest percentage of ethanol from distilled mixture is obtained in the case of the procedure that utilizes the heating of the wheat mash at 55^0C using an enzymatic mixture from table no 2 which releases constantly glucose from the starch without creating osmotic stress for the yeasts. Also the small viscosity of non cooked mashes allows a proper development of yeasts and a better fermentation compared to cooked mashes. In the case of non cooked mashes due lower heating temperature then in the case of cooked mashes, unwanted Maillard reactions are not present, and in the same time an excellent growth and viability of yeast is approached which is demonstrated by the increase of CO_2 and O_2 consumption. A saving of energy is possible in the case of using non cooked mashes through heating at only 55^0C against 100^0C in the case of cooked mashes and also an important saving of cooling water.

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