STUDY ON ENZYME HYDROLYSIS TO OBTAIN BIOETHANOL

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Abstract

The stage of starch hydrolysis is that providing yeasts with fermentescent sugars which are converted into alcohol. Grain starch is hydrolyzed by enzyme preparations which efficiency influences the alcohol quantity obtained, co-products quantity as well as energy consumption. The paper presents the efficiency of two enzyme preparations upon wheat and corn mashes, expressed by the content of fermentescent sugars which determine the quantity of alcohol obtained.
Keywords: *hydrolysis, enzyme, wheat and corn mashes.*

Introduction

Starch, by its two components, has four types of bonds in its structure: 1.4α-glycosidic inside amylose and amylopectin macro-molecules, 1.4α- marginal glycosidic, 1,3α-glycosidic and 1,6α-glycosidic (Jurcoane, 2004). Starch complete hydrolysis into glucose requires enzymes for all four types of bonds. If hydrolysis is carried out in four stages, it would lead to an incomplete hydrolysis, increase of stage duration together with occurrence of secondary reactions and decrease of alcohol output. Practically, enzyme hydrolysis is carried out in two stages:

- the first stage consists of fragmentation of amylose and amylopectin macro-molecules into dextrins, the starch is liquefied (the stage is called "liquefaction");
- in the second stage, dextrins are completely hydrolyzed into oligosaccharides and then into maltose and glucose (the stage is called "saccharification").

The hydrolysis has been studied using as raw material wheat and corn, ground in hammer mill, with sieves of 1 mm (Banu, 2006) to assure a complete conversion. The heat hydration of starch granules is influenced by temperature and pressure conditions under which the process takes place. Starch gelatinization is carried out efficiently in the case of under pressure boiling when the amylose is totally solubilized and amylopectin is spread into the amylose solution. The content of free sugars in cereals may increase

at under pressure boiling of almost 4 times, due to heat hydrolysis of solubilized starch.

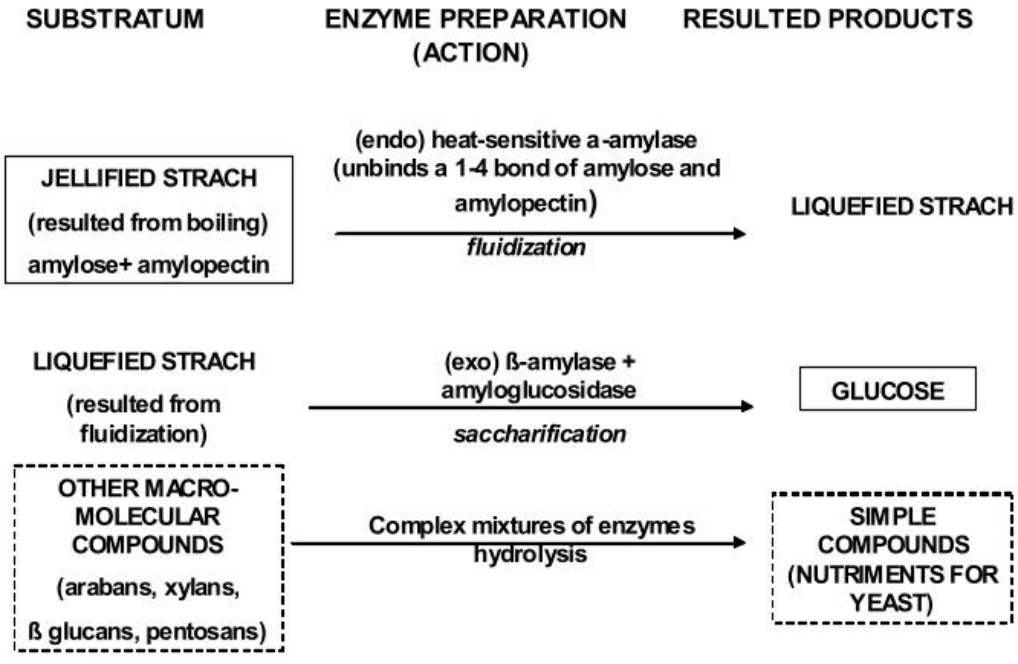

Fig. 1: Saccharification scheme of starchy raw materials

Experimental

Wheat or corn milling samples were used for hydrolysis which chemical composition is shown in the tables 1 and 2.

Table 1: Chemical composition of corn milling

No.	Corn milling	Moisture, %	Protein, %	Protein, dried substance, %	Ash, %
1.	Sample 1	9.90	6.65	7.30	1.4
2.	Sample 2	9.77	6.60	7.40	1.6
3.	Sample3	9.86	6.65	7.38	1.5

Table 2: Chemical composition of wheat milling

No.	Wheat milling	Moisture, %	Fatty substances, %	Protein, %	Protein dried substance, %
1.	Sample1	12.39	2.15	9.33	10.65
2.	Sample 2	12.38	2.12	9.32	10.62
3.	Sample 3	12.40	2.20	9.35	10.61

The boiling system was established depending on the corn quality. Thus, for healthy corn, whole grains and moisture up to 16%, the raw material used in experiment determinations, the boiling system is shown in table 3.

Table 3: Boiling conditions for corn milling

No.	Corn milling	Sample weight, g	Added water, ml	Pressure, bar	Temperature, ºC	Time, min
1.	Sample 1	2059.2	8708	1.5	105	45
2.	Sample 2	2059.2	8708	1.5	105	45
3.	Sample 3	2059.2	8708	1.5	105	45

Table 4: Boiling conditions for wheat milling

No.	Wheat milling	Sample weight, g	Added water, ml	Pressure, bar	Temperature, ºC	Time, min
1.	Sample 1	2059.2	8708	1.5	105	45
2.	Sample 2	2059.2	8708	1.5	105	45
3.	Sample 3	2059.2	8708	1.5	105	45

The starch glued under pressure and high temperature influence is easier to be attacked by liquefaction and saccharification enzymes. Two combinations of enzymes were used to carry out the two stages, enzymes which hydrolyze all types of bonds between glucose molecules and which composition is shown in tables 5 and 6.

Table 5: Composition of 1st enzyme batch used at starch liquefaction and saccharification

No.	Enzyme	Generating microorganism	Optimum pH	Temp, ºC	Action time, min	Enzyme activity	Dose used, g/sample
1.	SPEZYME (α -amylase)	Bacillus licheniformis	5.4-5.8	105-110	5-7	6.700-7.300 AAU/g	1.08
2.	OPTIMASH (endo-glucanase)	Geosmithia emersoni)	3-6.5	75	60	5625 u/g	1.292
3.	DISTILLASE L 500 (amyloglucosidase)	Aspergillus niger	4-4.4	65	220	450 GAU/g	1.08
4.	FERMGEN (protease)	Trichoderma reesei	3.5-5.0	60	80	1000 SAPU/g	1.8

Spezyme is a fungus α-amylase, very stable, which hydrolyzes 1, 4 α-glucosidic bonds reducing the viscosity of jellified starch. Optimash is an endogluconase which catalyzes the hydrolysis of 1, 3 or 1,5 bonds of β-glucans (Belitz, 1999). Distillase is 1, 4 α-D-glucan glucohydrolase, a saccharification enzyme which catalyzes successive unbinding of glucose molecules from the non-

reducing ends of dextrins and oligosaccharides. Fermgen is a fungus protease which hydrolyzes easily and efficiently the proteins from milling, bringing contribution to decrease of medium viscosity, being a nutriment source for yeasts.

Table 6: Composition of 2nd enzyme batch used at starch liquefaction and saccharification

No.	Enzyme	Generating microorganism	Optimum pH	Temp, ºC	Action time, min.	Enzyme activity	Dose used, g/sample
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 (amyloglucosidase)	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

Two types of raw materials were analyzed in this study, corn flour and wheat flour respectively, which have been subjected in turn to the action of the two different enzyme preparations previously presented.

The experiment results regarding sugar content, fermentescent sugar and alcohol which correspond to fermentescent sugar, calculated on the basis of refractometric extract (dried substance) of non-fermented mashes (STAS 6180-60 accordingly) are shown in tables 7, 8, 9 and 10.

In the case of non-fermented corn mashes using the first enzyme packet presented, we obtained the results shown in table 7.

Table 7: Sugar content, fermentescent sugar and alcohol in non-fermented corn mashes

No.	Corn mash	Dried substance read by refractometer %	Sugar g/l	Fermentescent sugar, g/l	Alcohol resulted from fermentescent sugar, % vol
1	Sample 1	18.81	174.6	169.6	9.9
2	Sample2	21.37	201.2	196.2	11.5
3	Sample3	18.3	169.2	164.3	9.6

In the case of non-fermented corn mashes using the second enzyme packet presented, we obtained the results shown in table 8.

In the case of non-fermented wheat mashes using the first enzyme packet presented, we obtained the results shown in table 9.

Table 8: Sugar content, fermentescent sugar and alcohol in non-fermented corn mashes

No.	Corn mash	Dried substance, %	Sugar, g/l	Fermentescent sugar, g/l	Alcohol resulted from fermentescent sugar, %vol
1	Sample 1	16.6	151.2	146.2	8.6
2	Sample 2	17.3	158.7	153.7	9
3	Sample3	15.99	144.9	139.9	8.2

Table 9: Sugar content, fermentescent sugar and alcohol in non-fermented wheat mashes

No.	Wheat mash	Dried substance read by refractometer, %	Sugar, g/l	Fermentescent sugar, g/l	Alcohol resulted from fermentescent sugar, %vol
1	Sample 1	16.6	151.2	146.2	8.6
2	Sample 2	17.3	158.7	153.7	9
3	Sample 3	17.6	161.9	156.9	9.2

In the case of non-fermented wheat mashes using the second enzyme packet presented, we obtained the results shown in table 10.

Table 10: Sugar content, fermentescent sugar and alcohol in non-fermented wheat mashes

No.	Wheat mash	Dried substance read by refractometer, %	Sugar, g/l	Fermentescent sugar, g/l	Alcohol resulted from fermentescent sugar, %vol
1	Sample 1	17.5	160.8	155.8	9.1
2	Sample 2	18.6	172.5	167.5	9.8
3	Sample 3	118.1	167.2	162.2	9.5

Conclusion

In the case of corn flour mashes it was noticed that the use of the first enzyme packet is more efficient, since higher values of fermentescent sugar content and consequently of alcohol were obtained. Instead, in the case of wheat flour mashes, a higher efficiency was noticed in the second enzyme packet regarding the parameters analyzed.

Acknowledgments

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References

Banu, C., *Bioalcoolul combustibilul viitorului*, Editura AGIR, Bucuresti, 2006
Belitz, H.D., *Food Chemistry, Springer Verlag*, Berlin, 1999, p.149-155
Jurcoane, St., *Tratat de biotehnologie*, Editura Tehnica, Bucuresti, 2004, p.360-364
Niculita, P., Popa M., Belc N., *Bioinginerie si Biotehnologii Alimentare*, Editura Academiei Romane, Bucuresti, 2006
Rotaru, V., *Tehnologia fabricarii spirtului si a drojdiei comprimate*, Editura Didactica si Pedagogica, Bucuresti, 1989,