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RESEARCH CONCERNING THE ACTION OF SOME ENZYMATIC PREPARATIONS ON POLYSACCHARIDES FROM WHEAT GRAIN

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Abstract

In order to get a sugar base suitable for fermentation, this paper evaluates the hydrolytic process of polysaccharides from wheat grain, using a complex of amylolytic and cellulolytic enzymes. The enzymatic hydrolysis of the wheat starch require, on the one hand, the liquefaction - dextrinisation of the starch with thermo-stable α-amylase, in order to render soluble the starch macromolecules and, on the other hand, the saccharification of those molecules by means of the amyloglucosidase and an enzymes mixture of amiloglucosidase with cellulase. As a result of hydrolytic process, the highest level of glucose was obtained through a certain enzymatic combined treatment.
Keywords: *starch hydrolysis, enzymatic hydrolysis; amylolytic complex.*

Introduction

The use of a cereals biomass containing considerable amounts of starch and cellulose provide a sugar platform for the production of numerous bio-products. Pretreatment technologies have been developed to increase the bioconversion rate for both starch and cellulosic-based biomass. (Palmarola-Adrados, 2005, Vidmantiene, 2006).

The technology used for conversion of plant biomass to alcohol is well known and developed, but it could still be improved, both as technological process and utilization of secondary products. The ethanol production process is based on conversion of cereal grain starch to glucose, which is then fermented to ethanol.

In order to improve the conversion of some wheat species to ethanol, this work investigates the action of some enzymatic

preparations on polysaccharides from some wheat grain varieties cultivated in Suceava plateau.

Experimental

Eight wheat grain varieties, coming from SCDA Suceava experimental farms, whose main features are reproduced in the table 1, represented the biological material used in this work.

The enzymatic preparations used for starch hydrolysis and saccharification were Amylex 3T, Diazyme X4 and Laminex GC 440. The first one was a liquid concentrated α-amylase preparation (from *Bacillus subtilis*) with an activity of 940 amylase activity units (AU) mL^{-1}(Technical card, Amylex 3T)

This enzyme preparation also contained neutral protease and β-glucanase activity.

Table 1. The composition of the raw material

Wheat variety	Starch (%)	Cellulose (%)	Crude protein (%)	Water (%)
Flamura 85	69.15	2.10	13.90	13.20
Dropia	68.50	1.85	13.85	13.50
Gasparom	68.65	2.15	13.85	13.65
Magistral	69.40	2.15	13.85	13.70
Esential	68.75	1.90	13.70	13.90
Drobeta	67.54	1.90	13.40	13.63
Voronet	68.80	2.10	13.65	14.08
Sv 2071-00	67.69	1.98	13.30	14.15

The second enzyme was a liquid concentrated amyloglucosidase preparation (from *Aspergillus sp)* with an activity of 400 GAU. 1 GAU represents one gram of reducing sugars, resulted after an hour from 4% soluble starch, at pH = 4.2, 60°C temperature, during 60 min. (Technical card - Diazyme X4). The third one was a liquid concentrated cellulase preparation. Laminex GC 440 contains a combination of enzymes which effectively modify and hydrolyze non-starch carbohydrates, the structural material of plant cells (Technical card - Laminex GC 440).

All the enzymatic preparations were offered by S.C. Enzymes & Derivates Romania S. A.

The wheat biomass was firstly milled and cleaned on preparation stage and then digested by enzymes to release sugars, whose concentration was further analyzed.

Raw material was treated by a two-step enzymatic hydrolysis procedure consisting of liquefaction and saccharification steps. A 300 g portion of ground grain was mixed with water, in the ratio 1:3 (v/v), and kept at the same temperature (90-100°C) in a water bath for 10 min. In this manner it is accomplished the starch jellification in order to increase the enzymes accessibility for the enzymatic attack. Heat was applied at this stage to enable liquefaction. Liquefaction of starch was carried out for 60 min at a temperature of 90°C and a pH value of 5.8, with initial supplementation of a selected amount of Amylex 3T, according to the table 2 and table 3. High temperatures reduce bacteria levels within the mash. The mash heat was cooled and the secondary enzyme, Diazyme X4 (gluco-amylase) – table 2 and table 3, and the third one Laminex GC 440 (cellulase) – table 3, were added. For saccharification, into the cooled mash from mash was added Diazyme X4 to convert the liquefied starch to fermentable sugars (dextrose). The starch saccharification process in our experience was carried out for 60 min at a temperature of 60°C and a pH value of 4. Laminex GC 440 was used to increase the fermentable sugars amount by means of turning to good account of wheat grain cellulose.

The starch content in wheat grain was determined by a method based on starch hydrolysing in acid medium, the cellulose content was dosed according to Scharrer-Kurschner method and the glucose content through Auerbach-Bodlander method (Vâță, 2000).

Crude protein was determined according to micro-Kjeldahl method (Villegas, 1975), and the water content was indirectly evaluated by means a weighting method.

Results and Discussion

The table 1 reproduces the values of main carbohydrates (starch and cellulose), proteins and water in grain belonging to eight wheat varieties. As seen, there were small differences between samples, especially as to values of cellulose, protein and water content.

Although not even starch content generally registered great differences between samples, its values ranged between 67.54 and 69.21%.

The efficiency of combined enzyme treatment on starch in eight wheat grain samples is rendered in the table 2.

Table 2. Glucose (%) obtained as a result of combined action of varied doses of enyzmatic preparations (Amylex 3T & Diazyme X4)

Wheat variety	Amylex 3T (kg/t starch)	Diazyme X4 (g/hl)	Glucose (%)
Flamura 85	0.6	10	17.20
		15	21.15
		20	34.80
		25	35.20
Dropia	0.6	10	15.34
		15	19.80
		20	30.15
		25	30.45
Gasparom	0.6	10	18.35
		15	22.90
		20	33.85
		25	34.00
Magistral	0.6	10	17.30
		15	22.40
		20	36.15
		25	35.90
Esential	0.6	10	14.80
		15	18.36
		20	30.08
		25	29.85
Drobeta	0.6	10	18.05
		15	24.55
		20	35.70
		25	36.10
Voronet	0.6	10	18.50
		15	21.09
		20	35.34
		25	34.90
SV 2071-00	0.6	10	15.03
		15	20.45
		20	31.70
		25	30.40

There was used a combination of Amylex 3 T (0.6 kg/t starch) with various doses of Dyazime X4 (10, 15. 20 and 25 g/hl starch).

Evaluating the glucose content resulted from enzymatic action; one can ascertain that for each variety have been four varied values, corresponding to the combination between Amylex 3T and the four doses of Dyazime X4. In each variety, the highest glucose values were as a result of the combination action of 0.6 kg/t starch (Amylex) with 20 or 25 g/hl starch (Dyazime). It has to be mentioned that the difference between glucose, obtained through combination of 0.6 Amylex with 20 Dyazime, and glucose obtained through combination of 0.6 Amylex with 25 Dyazime was insignificant for the most analysed varieties. The glucose values obtained by enzymatic combination of above mentioned doses (0.6 Amylex 3T + 20 or 25 Dyazime X4) ranged between 29.86 and 36.15 %.

In the table 3 are reproduced the glucose values, resulted from the combined action of starch hydrolyzing enzyme (Amylex 3T and Diazyme X4) with a cellulose hydrolyzing enzyme (Laminex GC 440). As seen, in this variant of experience it has used a combination of Amylex 3 T (0,6 kg/t starch) with Laminex GC (0,3 kg/t d.m.) and Dyazime X4 (10, 15. 20 and 25 g/hl starch).

The adding of Laminex GC, having a cellulolytic action, has modified a little the amount of glucose resulted from combined action of Amylex 3 T and Dyazime X4. In all analysed wheat grain samples, one can see increase of the glucose amount more obvious, at combinations using 20 and 25 g/hl starch Dyazime X4.

The glucose values obtained by treble enzymatic combination (0.6 Amylex 3T + 0,3 Laminex GC + 20 or 25 Dyazime X4) ranged between 30,70 and 37,04 %.

The hydrolysis of wheat grain starch, achieved with a combination of α-amylase (Amylex 3 T – 0.6 kg/t starch), and glucoamylase (Dyazime X4 - four various doses (10, 15, 20 and 25 g/hl starch), has evidenced a greater efficiency of glucose by using 20 and 25 g/hl Dyazime X4, with differences between grain varieties, depending on initial grain starch content. There were no significant differences between glucose amounts obtained by using 20 or 25 g/hl Dyazime X4.

Table 3. Glucose (%) obtained as a result of combined action of varied doses of enzymatic preparations (Amylex 3T & Laminex GC & Diazyme X4)

Wheat variety	Amylex 3T (kg/t starch)	Laminex GC (kg/t d.m*.)	Diazyme X4 (g/hl)	Glucose (%)
Flamura 85	0.6	0.3	10	17.20
			15	22.25
			20	35.18
			25	36.20
Dropia	0.6	0.3	10	15.75
			15	19.75
			20	30.85
			25	30.70
Gasparom	0.6	0.3	10	18.85
			15	23.10
			20	34.55
			25	34.30
Magistral	0.6	0.3	10	18.00
			15	22.95
			20	37.04
			25	36.85
Esential	0.6	0.3	10	15.30
			15	18.70
			20	31.20
			25	30.75
Drobeta	0.6	0.3	10	18.25
			15	24.90
			20	36.06
			25	36.30
Voronet	0.6	0.3	10	19.20
			15	22.10
			20	35.84
			25	35.70
SV 2071-00	0.6	0.3	10	17.36
			15	21.25
			20	32.45
			25	32.09

*d.m. = dry matter

The enzymatic combination of α-amylase, glucoamylase and cellulase has led to an increase of efficiency of glucose obtaining from wheat grain pollysaccharides. Using Amylex 3 T (0.6 kg/t starch), Dyazime X4(four various doses - 10, 15, 20 and 25 g/hl starch) and Laminex GC 440 (0.3 kg/t d.m.) - the last one with cellulolytic activity - it also has obtained an increasing of glucose efficiency especially by using doses of 20 and 25 g/hl Dyazime X4.

Conclusions

The hydrolysis of polysaccharides from eight wheat grain varieties, carried out with a combination of enzymatic preparations, has evidenced an increased glucose obtaining efficiency at certain doses of enzymatic preparation combinations. Even if there were no great differences between using of double combinations (starch hydrolytic enzymes) and treble combinations (starch and cellulolytic enzymes) however it has evidenced, in the last case, a certain increasing of fermentable sugars biomass, which could be taken into account under large scale production conditions.

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*** *Technical card – Amylex 3T,* S.C. Enzymes & Derivates Romania S. A

*** *Technical card – Diazyme X4,* S.C. Enzymes & Derivates Romania S. A
*** *Technical card – Laminex GC 440,* S.C. Enzymes & Derivates Romania S. A