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EFFECTS OF RYE PRETREATMENT AND ENRICHMENT WITH HEMICELLULOLYTIC ENZYMES ON ETHANOL FERMENTATION EFFICIENCY

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ABSTRACT

High temperature extrusion cooking of rye was compared to conventional pressure cooking and “cold liquefaction” with commercial hemicellulase addition to improve the efficiency of ethanol fermentation. The influence of saccharification with added hemicellulases on non-starchy carbohydrates of rye as well as on some quality factors of mashes and stillages were examined. Laboratory experiments showed the significant influence of the type of pretreatment and hemicellulases enrichment on the dynamic of saccharification and efficiency of ethanol fermentation. Using of hemicellulolytic enzymes along with saccharyfing enzymes during mashing increased the speed of breakdown of long chain carbohydrates and the yield of ethanol. In conclusion we can state that the increase of ethanol yield is not connected with pentosans fermentation but with the increased availability of hexose (starch) bound with pentose chain for amylolytic enzymes.

Key words: ethanol, mashing, pentosans, hemicellulolytic enzymes

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INTRODUCTION

Rye is one of the most important starch materials for ethanol fermentation in Poland (more than 80% of bioethanol is produced from rye) and there is a considerable interest in alcohol yield increasing from this raw material. To improve the efficiency of the process, extrusion cooking is suggested since both energy and water saving could be realised.

Korn and Harper [5] used a high temperature short time extrusion (HTST extrusion) treatment for corn and showed that it requires less energy and water than conventional cooking methods, with optimal extrusion condition 160°C, 15% water. No α-amylase addition to the gelatinised starch was necessary, owing to the low initial viscosity of the extruded material. Ethanol yield improvements under these extrusion conditions were found 8.4% over autoclaved samples and 33.3% over conventionally cooked samples. Lee at al. [6] used the extrusion cooking for 7 types of flour as a pretreatment in production of Takju, a traditional Korean alcoholic beverage. In the laboratory scale experiments extruded flour produced 15-20% more alcohol than steamed one. In the pilot plant it was 10-11% more. Grossman [4] showed that extrusion was an efficient pretreatment for simultaneous saccharification and fermentation of cassava starch, resulting in ethanol conversion from 88.6 to 97.2%

Czarnecki and Nowak [3] used HTST extrusion of rye as a pretreatment for ethanol fermentation when yeast and bacteria *Zymomonas mobilis* were compared for their fermentation rates. Extrusion cooking caused, on average, a 7.5% increase in ethanol yield in comparison to autoclaved samples. The best results were achieved for grain with a moisture of 21-23% which was extruded at temperatures of 160-180°C.

In this paper high temperature extrusion cooking of rye was compared to conventional pressure cooking and ”cold liquefaction” with commercial hemicellulase addition to improve the efficiency of ethanol fermentation. The influence of mashing with added hemicellulases on non-starchy carbohydrates of rye as well as on some quality factors of mashes and stillages were examined.

MATERIALS AND METHODS

Motto rye grain (87.7% d.m., 62.3% starch in d.m., 11.2% total pentosans in d.m., 1.6% soluble in water pentosans in d.m) from August Cieszkowski Agricultural University Experimental Station in Swadzim was used.

Commercial α-amylase GAMALPHA HT 120L and amyloglucosidase GAMMYLO 300L were applied for saccharification. Additionally GAMMAZYM CPL with activity of xylanases, cellulases and ß-glucanase was used for saccharification.

Polish commercial distillery yeast D-2 was used for laboratory and large scale experiments. Industry scale experiments were carried out in the distillery plant of the Experiment Station of the Agricultural University in Poznań in Swadzim.

Laboratory experiments

3 methods of gelatinising of rye material were used:

- pressure cooking (121°C, 1h)
- "cold liquefaction" (100°C, 1h)
- HTST extrusion in a single screw extruder S-45 (METALCHEM Gliwice) (180°C in main section)

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For mashing GAMALPHA HT 120L at 75°C during 20 min and GAMMYLO 300L at 55-60°C (80 min) were used. GAMMAZYM CPL was used together with saccharyfing enzymes.

The samples were inoculated with D-2 yeast and incubated at 30°C for 72 h.

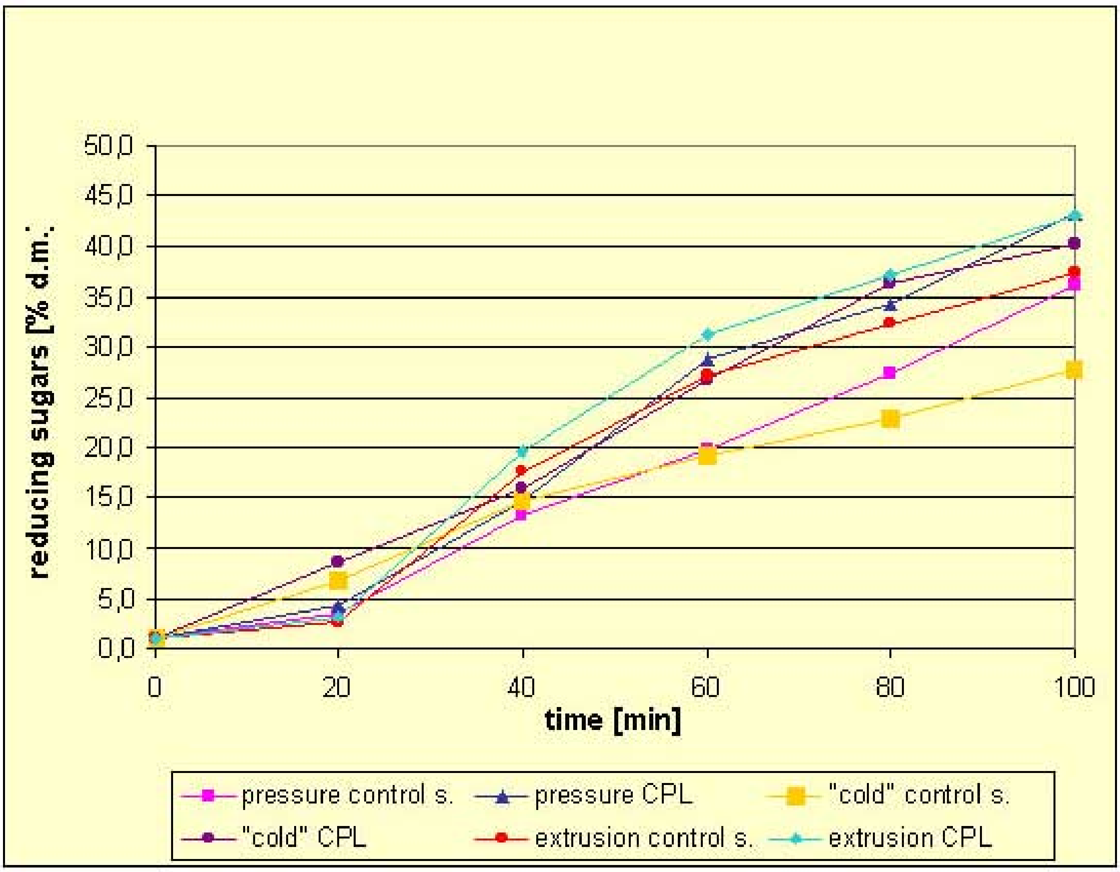
Experiments in the distillery plant were carried out during 5 following days for experimental control batches and the results were tested everyday. These experiments were aimed only for estimation of the influence of GAMMAZYM CPL addition.

- Dynamic of saccharyfing process was controlled after 20, 40, 60 and 100 min. Total pentosans (by method of Czarnecki et al. [2]) and soluble in water pentosans [2] as well as relative viscosity according Antoniou et al. [1] were tested in the mash
- Ethanol (by distillation method) as well as extract (areometric method), reducing sugars [7], pentosans and viscosity were estimated after fermentation
- In the large scale experiments sedimentation of stillage were also controlled by visual estimation of clarifying zone in measuring tubes

RESULTS AND DISCUSSION

Laboratory experiments showed the significant influence of the type of liquefying and enrichment with hemicellulase enzymes on the dynamic of saccharification of rye mashes. Dynamic of saccharification of extruded samples expressed as the increase of level of reducing sugars in dry matter of rye material was better than that of pressure cooked samples and significantly ($\alpha = 0.05$) than ”cold mashed” samples. Addition of GAMMAZYM CPL improved the process of sacharification for all the three kind of pre-treatment (Fig. 1).

Fig. 1. Effect of liquefaction methode and CPL xylanase action on course of saccharification



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Using of hemicellulolytic enzymes along with saccharyfing enzymes during mashing increased the speed of long chain carbohydrates breakdown. This is especially important in the case of "cold liquefaction" because this type of pretretment is highly used in polish distilleries and it needs less energy than traditional pressure cooking [9]. Addition of hemicellulases not only improved saccharification process but also significantly decreased the viscosity of sweet mash produced by this method and ethanol yield (Tab. 1).

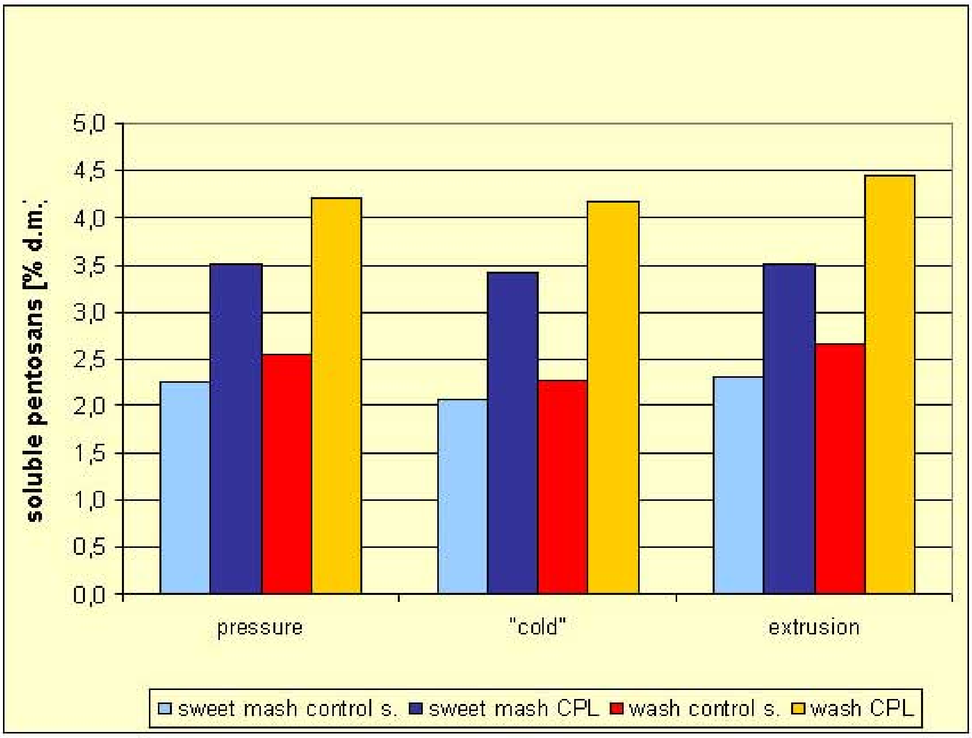
Table 1. Effect of CPL xylanase content on saccharification and fermentation course changes ("cold" liquefaction).

Enzyme dose (% of starch)	Sweet mash			Wash		Spent wash		Ethanol yield (% of theoret.)
	Sacchar. factor	Soluble pentosans (% d.m.)	Relative viscosity	Ethanol content (% v/v)	Soluble pentosans (% d.m)	Extract (° Blg)	Reducing sugars (% d.m.)	
0	44.6 a	2.07 a	30.1 c	6.4 a	2.27 a	4.6 b	2.39 a	82.1 a
0.005	47.5 a	2.47 ab	22.6 b	6.6 ab	3.10 ab	4.4 ab	3.84 b	84.6 ab
0.015	64.5b	3.43 b	11.3 a	6.9 b	4.18 bc	4.2 a	4.30 b	88.8 b
0.045	68.9 b	3.61 b	10.9 a	7.0 b	4.45 c	4.2 a	4.18 b	90.1 b

mean values in columns with different letters are significantly different ($\alpha = 0.05$)

The amount of pentosans in sweet mashes increased when GAMMAZYM CPL was added, but the level of pentosans was even higher in fermented mash (Fig. 2). There were no changes in total pentosans during fermentation in all the three kind of differently prepared rye and only the increase of soluble pentosans was evident. It is to conclude that the increase of ethanol yield in samples prepared with GAMMAZYM CPL addition is connected with the increased availability of hexose (starch) bound with pentose chain for amylolytic enzymes and more complete bioconversion to ethanol.

Fig. 2. Effect of liquefaction methode and CPL xylanase addition on soluble pentosans changes in mashes



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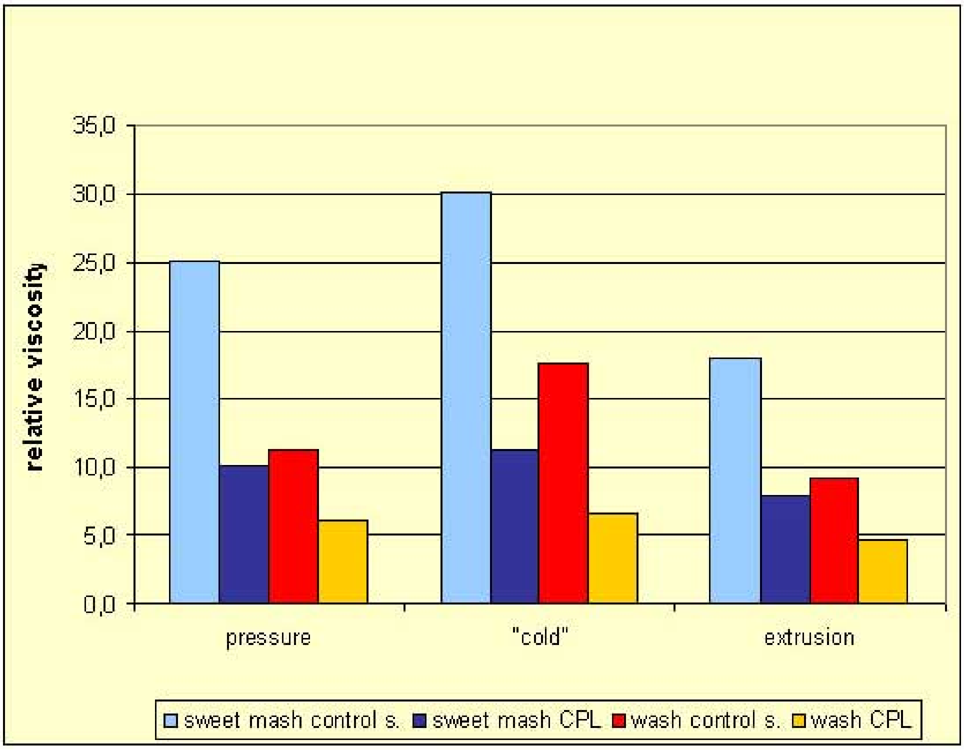
The addition of hemicellulases resulted in decrease of the relative viscosity of sweet mashes and spent washes for all three kind of liquefaction methods (Fig. 3). The viscosity was the lowest after extrusion of rye material both for samples saccharified with and without hemicellulases. The decrease of viscosity of sweet mashes and fermented mashes is more important for rye than for other starch material as rye mashes are more viscous which cause problems during fermentation and distillation [8].

Fig. 3. Effect of liquefaction methode and CPL xylanase addition on on relative viscosity of mashes



The yield of ethanol during laboratory experiments were shown on Fig. 4. Extrusion process gave the best ethanol yields close to 90% (calculated as the percentage of theoretical). This yield was significantly higher than those got from samples pressure cooked and ”cold mashed”. The addition of hemicellulase enzymes during saccharification of rye material caused the increase of ethanol yield from 3-6% (Fig. 4). Fortifying of enzymes by hemicellulases in ”cold mashing” process gave also the positive results during experiments made earlier by Stecka and Milewski [10].

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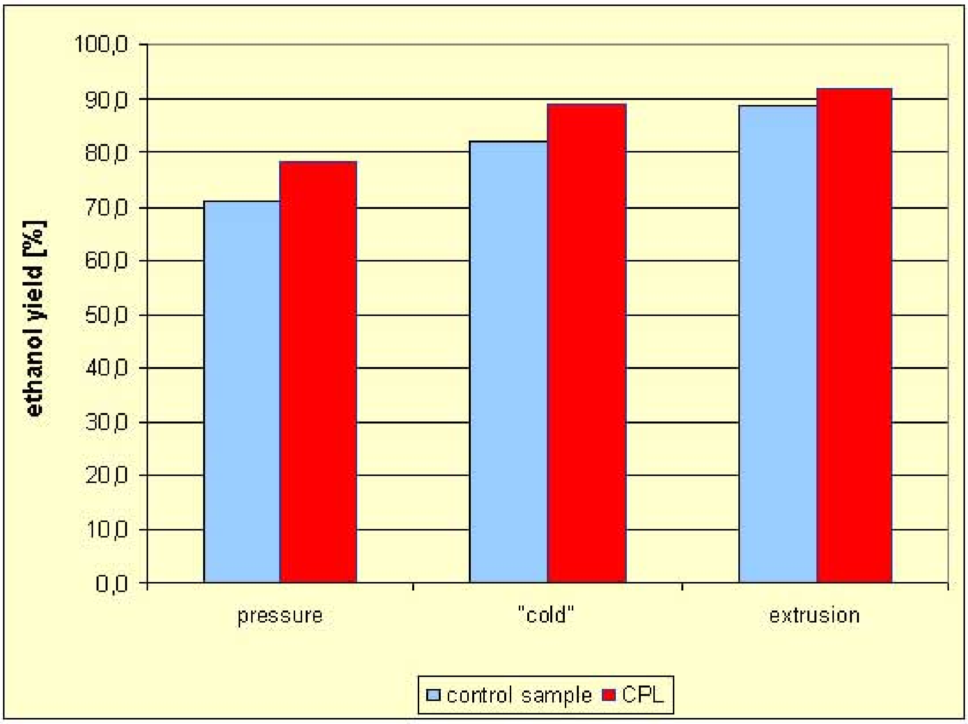
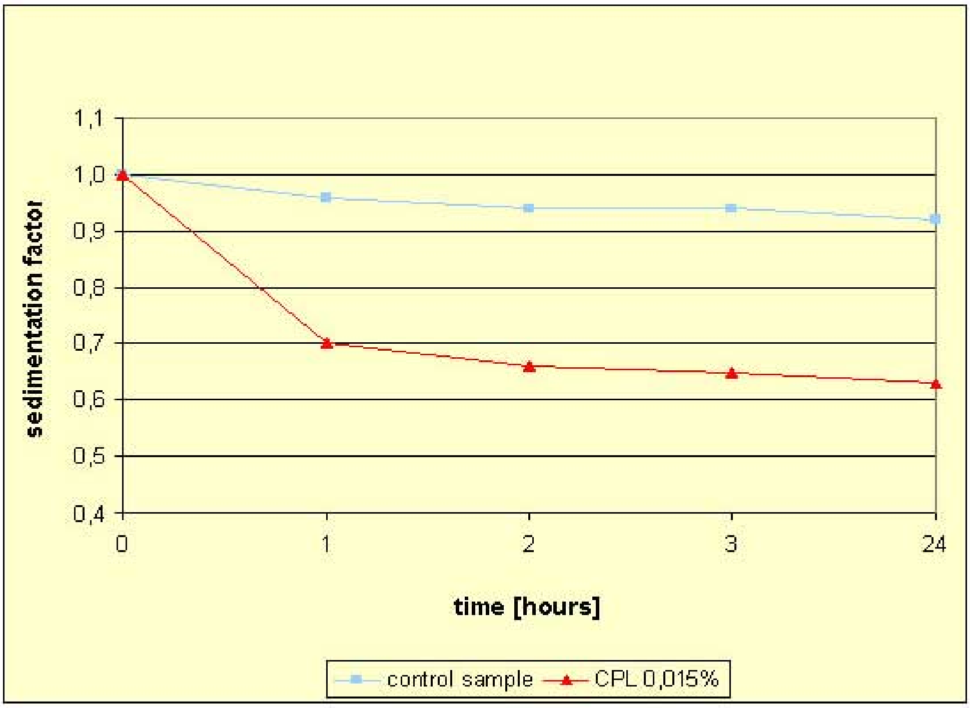
Fig. 4. Effect of liquefaction methode and CPL xylanase addition on ethanol yield



Our experiments made in the distillery plant confirmed the laboratory results. Addition of hemicellulase enzymes increased ethanol yield for more than 5% (calculated as the percentage of theoretical) with relatively low cost of added GAMMAZYM CPL. The addition of hemicellulase preparate resulted additionally the better sedimentation of spent wash (Fig. 5) which is important for its farther utilization.

Fig. 5. Effect of liquefaction methode and CPL xylanase addition on course of stillage sedimentation



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CONCLUSIONS

1. Laboratory experiments showed the significant influence of the type of pretreatment and hemicellulases enrichment on the dynamic and efficiency of ethanol fermentation of rye. Extrusion cooking is especially effective method of rye preatreatment for its saccharification rate and ethanol fermentation yield.
2. Using of hemicellulolytic enzymes along with saccharyfing enzymes during rye mashing increased the speed of breakdown of long chain carbohydrates and caused 3-6% increase of ethanol yield.
3. The increase of ethanol yield is not connected with pentosans fermentation but with the increased availability of hexose (starch) bound with pentose chain for amylolytic enzymes which effects its more complete bioconversion to ethanol.
4. The experiments made in the distillery plants confirmed the laboratory results. Addition of hemicellulase increased ethanol yield for more than 5% (calculated as % of theoretical) with relatively low cost of added hemicellulolytic enzymes.
5. Addition of hemicellulase enzymes decreased the viscosity of mashes and improved the sedimentation of spent wash.

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