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# ENZYMATIC HYDROLYSIS OF POTATO PULP*

Mariusz Lesiecki✉, Wojciech Białas, Grażyna Lewandowicz

Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences
Wojska Polskiego 48, 60-627 Poznań, **Poland**

## ABSTRACT

**Background.** Potato pulp constitutes a complicated system of four types of polysaccharides: cellulose, hemicellulose, pectin and starch. Its composition makes it a potential and attractive raw material for the production of the second generation bioethanol. The aim of this research project was to assess the usefulness of commercial enzymatic preparations for the hydrolysis of potato pulp and to evaluate the effectiveness of hydrolysates obtained in this way as raw materials for ethanol fermentation.
**Material and methods.** Sterilised potato pulp was subjected to hydrolysis with commercial enzymatic preparations. The effectiveness of the preparations declared as active towards only one fraction of potato pulp (separate amylase, pectinase and cellulase activity) and mixtures of these preparations was analysed. The monomers content in hydrolysates was determined using HPLC method.
**Results.** The application of amylolytic enzymes for potato pulp hydrolysis resulted in the release of only 18% of raw material with glucose as the dominant (77%) constituent of the formed product. In addition, 16% galactose was also determined in it. The hydrolysis of the cellulose fraction yielded up to 35% raw material and the main constituents of the obtained hydrolysate were glucose (46%) and arabinose (40%). Simultaneous application of amylolytic, cellulolytic and pectinolytic enzymes turned out to be the most effective way of carrying out the process as its efficiency in this case reached 90%. The obtained hydrolysate contained 63% glucose, 25% arabinose and 12% other simple substances.
**Conclusion.** The application of commercial enzymatic preparations made it possible to perform potato pulp hydrolysis with 90% effectiveness. This was achieved by the application of a complex of amylolytic, cellulolytic and pectinolytic enzymes and the hydrolysate obtained in this way contained, primarily, glucose making it a viable substrate for ethanol fermentation.

**Key words:** potato pulp, enzymatic hydrolysis, cellulose, starch, pectin

## INTRODUCTION

From among potential alternative energy sources, lignocellulose is perceived as the most important source for biofuel production, including bioethanol of the 2nd generation. This complex forms the main part of plant biomass and is made up, primarily, of cellulose, hemicelluloses and lignin and, to a lesser extent, of pectins, protein and ash. The lignocellulose complex composition depends on plant species, its age as well as conditions of its vegetation [Carpita and Gibeaut 1993]. Standard lignocellulose raw materials i.e. softwoods or hardwoods contain about 45-55% cellulose, 25-35% hemicellulose and 20-30% lignin [Sun and Cheng 2002]. Moreover they need cost consuming pretreatment to remove lignin and enhance the

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✉mariusz-lesiecki@wp.pl

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accessibility of cellulose fraction to cellulolytic enzymes. Potato pulp like sugar beet pulp significantly differs from other lignocellulosic materials its loose and hydrated structure, as well as significantly lower lignin content. It contains not only cellulose and hemicellulose but also considerable amount of pectin. [Sun and Hughes 1998].

Potato pulp is a waste which is produced in considerable quantities by starch manufacturing industry. It constitutes the residue which remains after washing out nearly all starch from potato mash. Its composition depends, to a considerable extent, on starch production technology. Apart from the non-starch fraction which comprises cellulose, hemicellulose and pectins, the so called 'unwashed starch' is present in significant amounts in potato pulp. However, in the process of bioethanol production, it is the hydrolysis of the cellulose fraction that constitutes the most difficult stage which has decisive impact on the efficiency of the entire technological process [Gumul et al. 2011].

Cellulases are defined as a family of enzymes which perform the process of degradation of cellulose into glucose. They are widespread in nature and are particularly common in the world of bacteria and fungi. They are manufactured, among others, by symbiotic bacteria found in multi-compartmental stomachs of ruminants (primarily in the rumen). Most animals, including humans, do not synthesise cellulases and, therefore, are incapable of utilising the entire energy contained in plant material [Kuhls and Lieckfeldt 1996]. These enzymes are extracellular and can be found in the post-culture liquid. That is why, even commercially available preparations, alongside their principle activity, also possess other properties, less documented and not indicated by the manufacturer, including pectinase or hemicellulase activity. The additional activity of a given enzymatic preparation depends, primarily, on the strain of microorganisms and, first and foremost, on the applied technology of the product isolation from the post-culture liquid [Kumar et al. 1996].

The aim of this study was to assess the usefulness of commercial enzymatic preparations for the hydrolysis of potato pulp and to estimate the usefulness of the hydrolysates obtained in this way as raw materials for ethanol fermentation.

## MATERIAL AND METHODS

The potato pulp used in these experiments was obtained in the course of potato campaign in 2009 from a manufacturing plant in Staw which constitutes part of the Wielkopolska Potato Industry Enterprise S.A. in Luboń.

The following commercial enzymatic preparations were employed in the performed investigations: Thermamyl 120L (Novozymes) which was a thermo-stable α-amylase derived from genetically modified strains of the *Bacillus* genus, Fermenzyme L-400 (Genencor) which was a glucoamylase from *Aspergillus Niger*, pectinase from *Aspergillus niger* (Sigma Aldrich) as well as cellulase from *Trichoderma reesei* ATCC 26921 (Sigma Aldrich).

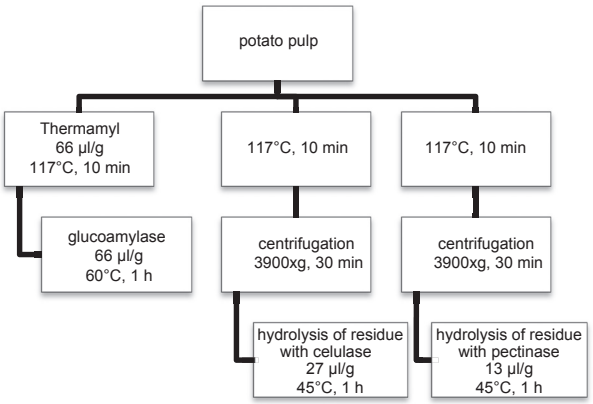


**Fig. 1.** Parameters of hydrolyses catalysed with the individual enzyme preparations

The catalytic activity of preparations assumed to be active only in relation to one fraction of the potato pulp (separate action of amylases, pectinases and cellulases – Fig. 1) as well as the activity of different mixtures of enzymatic preparations (Fig. 2) were studied. Prior to hydrolysis, the applied potato pulp was subjected to a hydrothermal treatment. In the case of experiments with amylases, the applied hydrothermal treatment was combined with hydrolysis by a thermostable α-amylase (Thermamyl). In the case of experiments conducted with pectinase and cellulase, the potato pulp was centrifuged after the applied hydrothermal treatment, the supernatant was discarded, and only the sediment was subjected to hydrolysis.

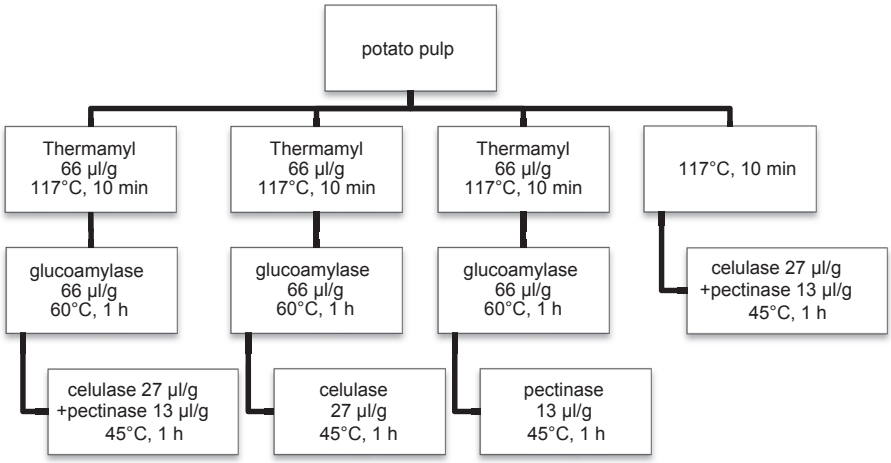


**Fig. 2.** Parameters of the hydrolyses catalysed with the mixtures of enzymes

The process of hydrolysis was carried out in 250 ml flasks and the weight of each analytical sample was always 15 g. The applied enzyme doses and hydrolysis parameters are presented in diagrams (Figs 1 and 2). Samples for analyses were collected at definite time intervals, centrifuged (3900×g, 30 min) and the obtained supernatant was analysed for the content of simple sugars and uronic acids with the use of high performance liquid chromatography (HPLC).

Dry matter content was determined in three replications at the temperature of 105°C by the gravimetric method in accordance with the PN-75/C-04616.01 standard.

Glucose, arabinose, galactose, galacturonic acid and rhamnose were determined by HPLC method with the assistance of the MERCK-HITACHI chromatograph (automatic sample injector MERCK HITACHI L-7250, MERCK-HITACHI L-7100 pump with a RI MERCK-HITACHI L-7490 detector, Aminex HPX-87H 300×7.8 mm column from BIORAD) under the following conditions: sample volume 30 µl; mobile phase 0.001 N $H_2SO_4$; flow rate 0.5 ml/min; column temperature 50°C.

The yield of hydrolysis was defined as the ratio of the quantity of the liberated simple substances from the fibrous fraction of the raw material to the amount of these substances theoretically possible to obtain determined on the basis of the dry matter content of the potato pulp. The efficiency of hydrolysis was calculated as a ratio of dry mass (g) contained in the supernatant of centrifuged sample of hydrolysate to the dry mass (g) contained in the sample of potato pulp subjected to hydrolysis and expressed as percentage.

All data are expressed as means ±SD (n = 3). Analysis of variance for measures was performed to study differences in efficiency of hydrolysis catalyzed by different mixtures of enzymes. A Tukey post hoc test was applied in case of a significant F-ratio to locate the differences. Simple linear regression analysis was also performed to estimate relationship between the efficiency of hydrolysis and the different enzyme dose. Statistical significance was set at $p < 0.05$.

## RESULTS AND DISCUSSION

The analysed potato pulp contained in its dry matter 15 to 20% of the so called "unwashed starch". This value is characterised by a considerable variability associated, primarily, with changing technological parameters of the starch plant. Unwashed starch appears to be a significant and relatively easily available source of fermenting sugars and, therefore, the hydrolysis efficiency of this potato pulp fraction can be important for the cost-effectiveness of the entire process of bioethanol production.

When only the Thermamyl 120L preparation was applied, the output of the hydrolysis process of the starch fraction present in the potato pulp was determined at the level of 8% (Fig. 3). When alongside the thermostable α-amylase also the Fermenzyme L400

preparation containing glucoamylase was used, the hydrolysis output increased over two times to 18%. Glucoamylase, also known as γ-amylase, affords an effective hydrolysis reaction of both α-1,4-glycoside as well as α-1,6-glycoside bonds with glucose released as the final product [Sadeghi et al. 2008]. When the duration of the process was prolonged, the output of the process was not found to increase and, in individual cases, it was even observed to decrease. It is presumed that at high glucose or enzyme concentrations, as in the case of acid hydrolysis, glucose repolymerisation (reversal) is possible accompanied by formation of maltose and isomaltose [Mosier et al. 2005].

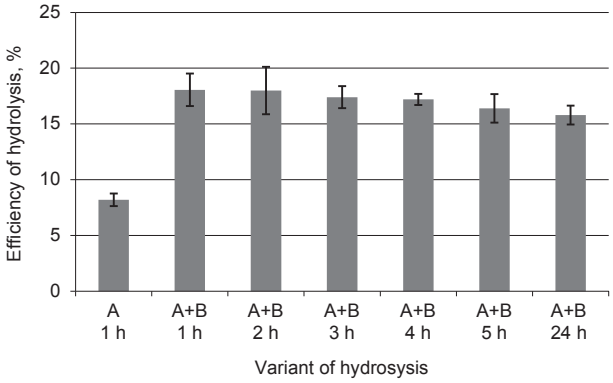


**Fig. 3.** Efficiency of hydrolysis catalysed by amylases: A – Thermamyl 120L, A+B – Fermenzyme L400 after prehydrolysis with Thermamyl 120L

The analysis of the composition of supernatant fraction proved that it was made up, primarily, of glucose (77%) exerting a positive impact on the assessment of this hydrolysate as a substrate for ethanol fermentation. Furthermore, the presence of other simple substance, including galactose (16%) and galacturonic acid (4%), was determined indicating that during the hydrothermal treatment also the pectin fraction of the potato pulp was susceptible to hydrolysis. The observed quantities of released glucose indicated effective hydrolysis of the starch fraction.

The process of hydrolysis with the assistance of cellulase isolated from *Trichoderma reesei* ATCC 26921 was carried out for 24 hour following hydrothermal treatment which caused removal of the entire gelatinised starch fraction. Maximal yield values were reached already after 1 hour (Fig. 4) and prolongation of the time of hydrolysis over 1 hour failed to exert a significant impact on the process output (data not given). The dose of 26 µl/g of the enzyme appeared to be the adequate addition. It should be said that the highest yield of hydrolysis was achieved after the addition of 46 µl/g of the enzymatic preparation (nearly 35%) but differences in the output of simple substances were negligible with $p > 0.05$.

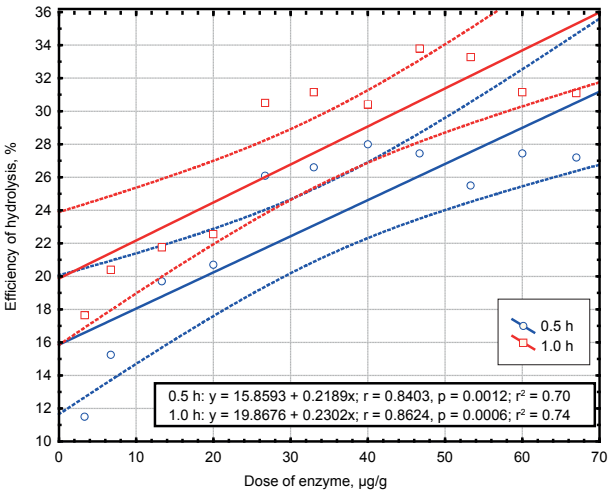


**Fig. 4.** Efficiency of the hydrolysis catalysed by cellulase preparation from *Trichoderma reesei* ATCC 26921

The analysis of the composition of the hydrolysate obtained after the treatment with cellulase proved that it was dominated by glucose (46%) and non-fermenting arabinose (40%). Galactose made up 5%, galacturonic acid 1% and other non-fermentable substances 8%. Thus obtained hydrolysate consisted half in fermentable sugars and half in non-fermentable substances. Therefore, despite the fact that over 30% of potato pulp dry matter passed into the liquid fraction as a result of the action with this enzyme (against barely 20% following the treatment with amylases), the participation of this enzyme in the manufacture of raw materials for ethanol fermentation turned out to be not dominant.

Selective hydrolysis of the pectin fraction potentially appears to provide the most attractive method of initial processing of potato pulp because it should lead to selective removal of the galacturonic acid as well

as other simple substances dispensable in ethanol fermentation. It was demonstrated in the course of the performed experiments that the yield of the hydrolysis process with pectinases barely changed significantly with time of its duration ($p < 0.05$), however yielded relatively low results ranging from 1% to 2% (Fig. 5). The observed very low hydrolysis output of the potato pulp with the assistance of pectinase can be attributed to the fact that pectin – which is a biopolymer easily undergoing hydrolysis – was removed during the hydrothermal process. This was also confirmed by the results obtained during the hydrolysis with amylolytic enzymes during which more than 20% of simple substances other than glucose were obtained.

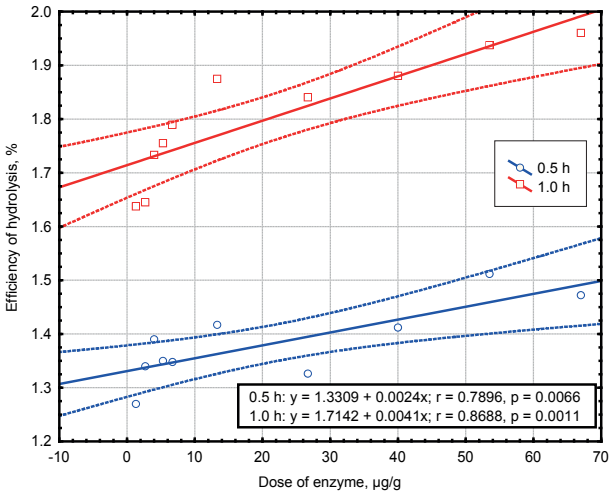


**Fig. 5.** Efficiency of the hydrolysis with different doses of pectinase

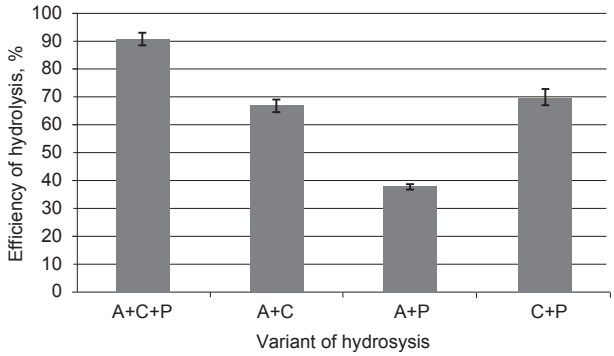


**Fig. 6.** Efficiency of hydrolysis catalysed by different mixtures of enzymes: A – amylases, C – cellulase, P – pectinase

Investigations of the catalytic efficiency of different mixtures of hydrolytic enzymes revealed that the application of amylase and pectinase was the least effective system causing liquefaction of only 38% of the potato pulp dry matter (Fig. 6). The mixture of amylases and cellulases turned out to be as effective as the mixture of amylases and pectinases because the recorded yields of hydrolysis amounted to 68% and 72%, respectively. The highest degree of hydrolysis (over 90%) was recorded in the case of the application of all the examined enzymes (Fig. 6). According to Spagnuolo et al. [1997] synergistic effect by using cellulases in combination with pectinases in process of hydrolysis of sugar beet pulp is observed. The amount of glucose released is two- fivefold higher when the pulp is hydrolysed by the mixture of enzymes than if the enzymes are used individually.

The composition of the hydrolysate obtained using this method was dominated by glucose (63%) confirming its usefulness for bioethanol production. Other fermenting sugar – galactose made only 2% of composition. The part of arabinose was 25%, galacturonic acid 1% and other non-fermentable substances 9%. In case of enzymatic hydrolysis of sugar beet pulp 21% of glucose, 5% od galactose 20% of arabinose, and 20% of galacturonic acid is realsed [Micard et al. 1997], whereas acid hydrolysis results in production of 24% of glucose, 4% od galactose 19% of arabinose, 15% of galacturonic acid, as well as 1% of xylose, 1% of mannose and 1% of rhamnose [Spagnuolo et al. 1997]. These observations proves that potato pulp competitive with sugar beet pulp as a raw material in bioethanol production. However a considerable proportion (35%) of the monomers unavailable to microorganisms carrying out the process of ethanol fermentation confirmed the need to carry our further investigations aiming at the elaboration of methods allowing effective management of these constituents.

## CONCLUSION

The performed investigations demonstrated that the applied commercial enzymatic preparations made it possible to carry out the hydrolysis of potato pulp with 90% efficiency. This can be achieved by the application of a complex of amylolytic, cellulolytic and pectinolytic enzymes. The hydrolysate obtained

in this way contained over 60% fermenting sugars making it a substrate suitable for ethanol fermentation. The exclusive application of amylases for the process of potato pulp hydrolysis resulted in nearly 20% liquefaction of the raw material, although the hydrolysate obtained in this way contained almost 80% glucose. The application of cellulase alone hydrolysed nearly 40% of the potato pulp but the obtained hydrolysate contained over 50% of non-fermenting substances. The application of mixtures of hydrolytic enzymes, i.e. amylases with cellulase, amylases with pectinase or cellulase with pectinase liquefied up to 70% of the applied potato pulp.

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## HYDROLIZA ENZYMATYCZNA WYCIERKI ZIEMNIACZANEJ

### STRESZCZENIE

**Wstęp.** Wycierka ziemniaczana jest skomplikowanym układem czterech typów polisacharydów: celulozy, hemicelulozy, pektyny i skrobi. Skład czyni ją surowcem potencjalnie atrakcyjnym do produkcji bioetanolu II generacji, czyli takiego, który na przykład jest pozyskiwany z surowców odpadowych. Warunkiem opłacalności każdego procesu technologicznego jest wykorzystanie wszystkich składników surowca bez generowania produktów ubocznych lub przynajmniej ich minimalizacji. Celem pracy była ocena efektywności obróbki enzymatycznej wycierki ziemniaczanej, będącej pierwszym etapem w procesie produkcji bioetanolu II generacji.

**Materiał i metody.** Wysterylizowaną wycierkę ziemniaczaną poddawano hydrolizie z zastosowaniem komercyjnych preparatów enzymatycznych. Badano efektywność preparatów w założeniu aktywnych tylko w stosunku do jednej frakcji wycierki ziemniaczanej (osobno działanie amylaz, pektynazy i celulazy), jak również działanie różnych mieszanin preparatów enzymatycznych. Zawartość monomerów w hydrolizatach oznaczano metodą HPLC.

**Wyniki.** Zastosowanie enzymów amylolitycznych do hydrolizy wycierki powodowało uwolnienie jedynie 18% surowca. W powstałym produkcie stwierdzono zawartość 77% glukozy i około 16% galaktozy.

Hydroliza frakcji celulozowej skutkowała uwolnieniem do 35% surowca, a w hydrolizacie była zawarta głównie glukoza (46%) i arabinoza (40%). Najbardziej korzystnym sposobem prowadzenia procesu było jednoczesne wykorzystanie enzymów amylolitycznych, celulolitycznych i pektynolitycznych, w którym to wariancie osiągnięto wydajność 90-procentową. Otrzymany hydrolizat zawierał 63% glukozy, 25% arabinozy oraz 12% innych substancji prostych.

**Wnioski.** Zastosowanie komercyjnych preparatów enzymatycznych umożliwia przeprowadzenie hydrolizy wycierki ziemniaczanej z wydajnością 90-procentową. Osiąga się to z użyciem kompleksu enzymów amylolitycznych, celulolitycznych oraz pektynolitycznych. Tak otrzymany hydrolizat w przeważającej części zawiera glukozę, co czyni go substratem przydatnym do fermentacji etanolowej.

**Słowa kluczowe:** wycierka ziemniaczana, hydroliza enzymatyczna, celuloza, skrobia, pektyny

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