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ETHANOL FERMENTATION OF MAIZE MASHES

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Key words: distilling, ethanol, maize, extrusion cooking, fermentation

The purpose of the work was the assessment of the effect of alternative way of raw material preparation on the course and final results of maize mash ethanol fermentation.

The material for investigation was dried maize grain of KB 1902 cultivar which, after grinding and moistening up to 15% of water content, was subjected to extrusion process in single-screw extruder of DN 20 type, produced by BRABENDER firm, applying extrusion temperatures 145°C, 180°C and 215°C. The extrudates obtained in the form of granules underwent grinding and mashing with the use of different raw material concentration, as well as changeable parameters of the mashing process, *i.e*. time of the process and doses of liquefying enzyme Termamyl 120L. Fermentation testes were conducted according to periodical method at the temperature of 37°C, introducing industrial distillery yeast *Saccharomyces cerevisiae* D2. In analogous conditions there were prepared control samples from ground, not extruded maize grain of KB 1902 cultivar. In the examined and control samples there were assessed the following parameters: dynamics of ethanol fermentation process, degree of sugars consumption, ethanol yield, as well as biomass physiological condition after the fermentation process.

Fermentation time was related to mash density. It was possible to prove that the analysed changeable conditions of the process did not significantly affect the degree of sugars consumption (about 99%) by yeast. More advantageous ethanol yield was obtained for the samples prepared from mashes featuring 200 g of raw material/kg. The best final results of fermentation – the highest ethanol yield (from 0.494 to 0.509 g ethanol/g starch) and the most satisfactory physiological condition of yeast after the process resulted from fermentation of maize flour extruded mashes at the temperature of 180°C.

INTRODUCTION

Since the prices of crude oil and natural gas has been continuously growing, bioethanol is one of potential energy carriers which can be produced from standing crop or organic waste. The use of ethanol as biofuel is conditioned by lowering its production costs which, in turn, are effected by the price of raw material. In the recent years maize has become a considerable raw material for etanol production [Bortkun, 2003; Lipski, 2003; Inorowicz, 2005; Jarosz, 2005]. Due to economic reasons, classical technology of steaming starch raw material has been recently replaced by the method of non-pressurised starch release, applying enzymatic preparations of microbiological origin. Moreover, extrusion which is thermo-mechanical treatment of raw material, involving structure modification and partial starch hydrolysis, seems to be an alternative method of preparing starch raw material for fermentation [Mościcki, 2003; Linko *et al*., 1984; Grossmann *et al*., 1988; Czarnecki & Nowak, 1997 and 2001].

The aim of the work was to assess possibilities regarding extrusion application as an initial stage of preparing maize starch for fermentation, as well as an attempt of combining mashing parameters – time of mashing and the dose of liquidising enzyme.

MATERIALS AND METHODS

The material for investigation was dried maize grain of KB 1902 cultivar, coming from Agricultural Plant Production Station "Nasiona Kobierzyc" LTD Company in Kobierzyce. Commercial distillery yeast *Saccharomyces cerevisiae* D2 originating from culture collection of the Department of Food Technology and Storage of the Wrocław University of Environmental and Life Sciences was used.

Determination of maize flour and extrudates moisture. Maize flour and extrudates moisture was assessed by thermo-gravimetry method at the temperature of 105°C according to Polish Standard [PN-R-74110:1998] after grinding the material in a single-way grain mill and in a laboratory mill.

Determination of starch content. Starch content in maize flour was determined using polarimetric method by Evers, modified by Grossfeld [Sobkowicz *et al*., 1988].

Extrusion. Maize flour, whose moisture was set 15% 24 h before the process, was extruded in a single-screw extruder DN 20 by BRABENDER Company at three different temperatures applied: 145°C, 180°C and 215°C.

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Fermentation tests. Fermentation media made of ground extrudates of maize flour were prepared in 12-cup-laboratory mashing device ZL 1 type E. The mashes were made using non-pressurised starch liquefaction, based on enzymatic preparations Termamyl 120L and San Ultra (Novozymes), following the instructions by the producer [Kapela & Solarek, 2004].

The examinations were conducted in two stages. The first stage involved the assessment of mash concentration and extrusion conditions on dynamics and final effects of ethanol fermentation. The material subjected to experiments, extruded at the temperatures of 145°C, 180°C and 215°C, was applied in two concentration doses: 160 and 200 g of raw material per 1 kg of the sample. In analogous conditions there were prepared control samples from ground, not extruded maize grain. The second stage of investigation consisted in an attempt to shorten mashing time by 15 min and diminishing α-amylase dose recommended by the producer for selected conditions of extrusion, *i.e.* temperatures 180°C and 215°C and mash concentration of 200 g of raw material per 1 kg of the sample.

Saccharomyces cerevisiae D2 yeast inoculum was standardized according to the curve of relation between absorbance and yeast dry mater concentration (not published data) and was dosed into mashes in the amount of 2 g yeast dry matter/kg of the sample. Fermentation was carried out at the temperature of 37°C.

Fermentation dynamics assessment. The amount of CO_2 (g) released in the course of the process was assumed as a criterion assessing fermentation dynamics. The samples were weighed every 3 h until the difference in mass measurements was less than 0.05 g. There was compared percentage contribution of CO_2 released after 3, 21 and 42 h of fermentation in relation to total amount of released CO_2 (g).

Assaying final effects of fermentation. After fermentation was completed the samples underwent double direct distillation. After first distillation, the samples were neutralized by using 30% NaOH. There was determined reducing sugars content, according to the method by Nizowkin and Jemielianowa, modified by Soczyński, in the decoctions previously clarified and subjected to hydrolysis [Lisińska *et al*., 2002]. The degree of sugar consumption (%) was calculated from the difference in starch content before and after fermentation.

Ethanol content (g/L) was assayed pycnometrically in distillates. There was determined ethanol yield- $Y_{(g\ ethanol/\ g\ starch)}$, counted over 1 g of starch.

Post-fermentation liquids were subjected to the assessment of yeast cells physiological condition (% contribution of inactive cells and budding cells), using survival preparation stained with methylene blue. Yeast cells were counted in Thom hematocrite camera, using Biolar 2308 microscope.

Statistical analysis. In order to determinate significance of differences between the results obtained, regarding the degree of sugar consumption and ethanol yield there were done analysis of variance at unidirectional classification for one variable, as well as Duncan test. Statistica 6.0 program was applied to statistically work out the results. Statistically homogenous groups were denoted in Figures with small letters.

RESULTS

Fermentation dynamics, determined by the amount of CO_2 released in time, is one of the determinants of yeast fermentation activity in the environment. Figure 1 showed compared dynamics of CO_2 released in selected hours of fermentation, *i.e.* 3, 21 and 42, described as% of total CO_2 amount (g) released in the course of the process. Fermentation of mashes featuring lower concentration (A) – 160 g of raw material/kg of the sample, regardless the temperature of extrudates preparation, lasted 48 h. Fermentation of mashes of concentration 200 g of raw material/kg of the sample (B) was completed after 64 h. The most significant differences in the quantity of CO_2 released between the samples of different density were observed within the first 24 h of fermentation.

The degree of sugars consumption from fermentation medium (Figure 2), regardless mash density and extrusion temperature, exceeded 99%. The highest degree of sugars consumption was observed in the control sample (C) of

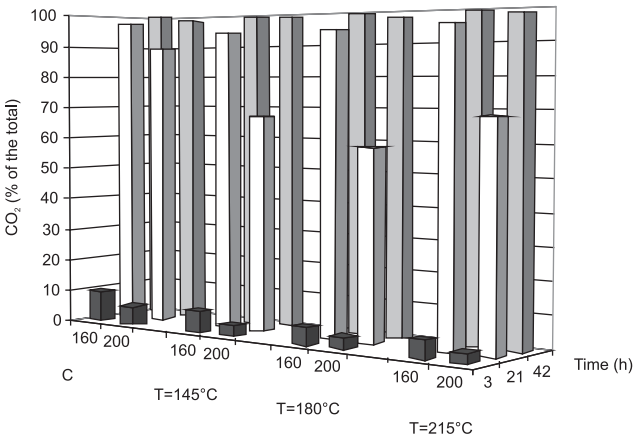

FIGURE 1. Dynamics of CO_2 release during maize mashes fermentation (160, 200- mashes concentration (g/kg); C- control sample from non-extruded material; 145, 180, 215- samples from maize extruded at the temperature of 145, 180 and 215°C, respectively).

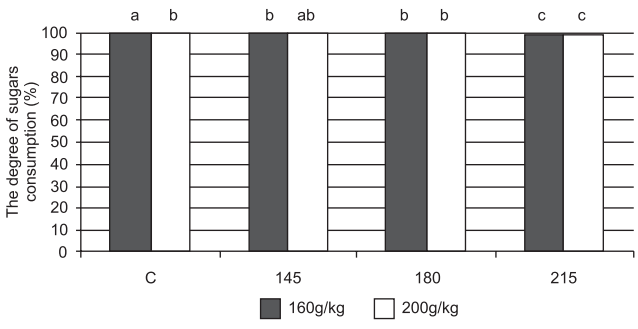

FIGURE 2. The degree of sugars consumption by yeast *Saccharomyces cerevisiae* D2 during mashes fermentation (160, 200 – mashes concentration (g/kg); C – control sample from non-extruded material; 145, 180, 215 – samples from maize extruded at the temperature of 145, 180 and 215°C respectively, a÷c – statistically homogenous groups).

the density 160 g/kg – 99.63%. The lowest degree of sugars consumption was recorded for fermentation of mashes prepared from extrudates prepared at the temperature of 215°C – 99.03% for the mash of 160 g/kg and 99.09% for the mash of 200 g/kg density. The remaining samples, as far as degree of sugars consumption was concerned, formed statistically homogenous group, whose value of the parameter in question was about 99.30%.

There were not stated any statistically significant differences between ethanol yield in the samples of higher concentration (200 g/kg), regardless the temperature of extrusion (Figure 3). The mentioned yield ranged from 0.494 to 0.509 g ethanol/g starch and it was the highest among the yields discussed. The mashes of the lowest density fermented with lower yield than the ones of higher concentration. The lowest yield was obtained for control sample (C) featuring the lowest density values – 0.454 g ethanol/g starch.

The most satisfactory physiological condition after fermentation (Figure 4) was reported for *Saccharomyces cerevisiae* D2 yeast cells colonizing control sample of lower density. Among the mashes prepared from extrudates the best environment for yeast proved to be the medium extruded at the temperature of 180°C. In spite of considerable amount of inactive cells (from 28.2 to 31.6%, depending on mash density),

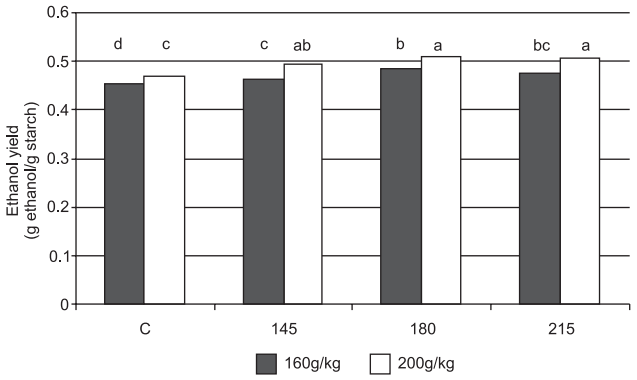

FIGURE 3. Ethanol yield after maize mashes fermentation (160, 200 – mashes concentration (g/kg); C – control sample from non-extruded material; 145, 180, 215 – samples from maize extruded at the temperature of 145, 180 and 215°C respectively, a(d-statistically homogenous groups).

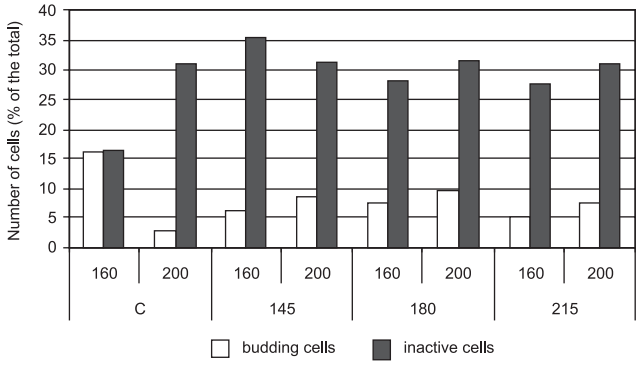

FIGURE 4. The physiological condition of yeast cells after maize mashes fermentation (160, 200 – mashes concentration (g/kg); C – control sample from non-extruded material; 145, 180, 215 – samples from maize extruded at the temperature of 145, 180 and 215°C, respectively).

the mentioned medium provided the best conditions for biomass proliferation (from 7.5 to 9.8% budding cells).

Subsequent stage of work involved conditions modification of mashing process, *i.e.* the time of the process was shortened by 15 min (no gelatinizing at the temperature of 95°C) – alternative II and, additionally, the dose of liquidizing enzyme Termamyl 120L recommended by the producer was diminished by half (alternative experiment III) or the mentioned preparation was not added at all (alternative IV). For that stage of investigation there were selected mashes featuring the highest concentration of raw material in the sample, *i.e.* 200 g/kg and extrudates were not prepared at the temperature of 145°C.

Applied modifications of mashing process did not affect the rate of CO_2 release from fermenting mashes (Figure 5). Fermentation of extrudates prepared according to all alternative ways of mashing presented, lasted for 64 h. Better dynamics of the process featured control sample.

The degree of sugars consumption by yeast *Saccharomyces cerevisiae* D2 during mash fermentation of concentration 200 g of extruded raw material per 1 kg of the sample, slightly decreased in the course of the mashing process amounted from 99.35% to 99.06% in the case of mashes extruded at the temperature of 180°C and from 98.85% to 99.09% – for mashes from row material extruded at the temperature of 215°C (not published data).

The shortening of mashing time by 15 min caused the decrease in ethanol yield by 0.015 and 0.017 g ethanol/g starch respectively in the samples extruded at the temperature 180°C and 215°C (Figure 6). Decreased by half dose of enzymatic preparation Termamyl 120L did not significantly statistically effected ethanol yield. The samples mashed without α-amylase addition featured better ethanol yield than the control sample.

As far as physiological state of yeast *Saccharomyces cerevisiae* D2 after fermentation of maize extrudates was concerned, no significant differences were proved for diversified mashing conditions (not published data). Better physiological state featured yeast cells fermenting mashes from extrudates prepared at the temperature of 180°C. Yeast cells fermenting material extruded at 215°C contained in their population from 7 to 8.2% more inactive cells as compared to the cells fermenting material extruded at 180°C.

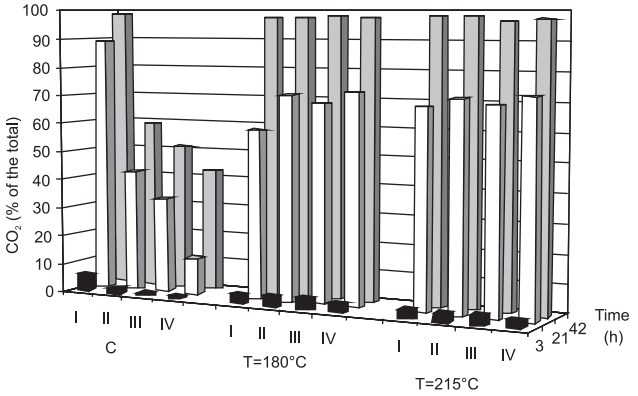

FIGURE 5. Dynamics of CO_2 release during fermentation of mashes prepared in different alternatives (C – control sample from non-extruded material; I, II, III, IV – alternatives of mashing process).

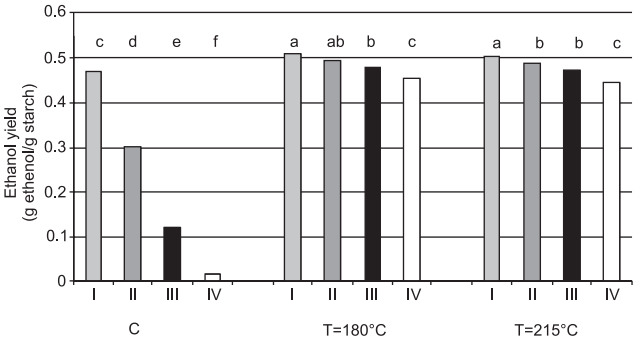

FIGURE 6. Ethanol yield after fermentation of mashes prepared in different alternatives (C – control sample from non-extruded material; I, II, III, IV – alternatives of mashing process, a(f-statistically homogenous groups).

DISCUSSION

According to Govindasama *et al*. [1997] and Czarnecki & Nowak [1997] application of the process of extrusion (extrusion-cooking) enables appropriate preparation of starch raw material to conducting fermentation process and eliminating the necessity of using energy-consuming pressurised process of starch digestion. At the same time it allows to introduce economically-justified modifications in technologies using non-pressurised starch hydrolysis. Intensive and long-lasting heating of plant raw materials containing protein and carbohydrates generally leads to the decrease in value due to the occurrence of Maillard reaction products. There are formed bonds between free amino acids and aldehyde groups which are resistant to enzymatic activity. Therefore, temperature is a factor effecting the biological value of raw material [Mościcki, 2002]. Investigation carried out by Miladinov & Hanna [2001] proved that temperature and moisture content of the material subjected to extrusion are the most important factors affecting the degree of gelatinization and partial starch saccharification, which significantly influences mash density. Observations of starch texture proved that too low temperature of the process results in the fact that starch does not undergo any physical-chemical alternations. In the case of extrusion at the temperature of 155°C or higher, there was recorded more noticeable deformation of starch particles and better solubility of extruded starch in water. In the presented work, an increase in extrusion temperature up to 215°C resulted in extrudates darkening and partial caramelisation of sugars.

One of the ways leading to intensification of ethanol production can be high gravity mashes fermentation. This parameter determines, to a high degree, the costs of alcohol production [Rzepka *et al.*, 2000]. Considerable limit to mash density increase is their increased viscosity. Presented in this work increased mash density to more than 200 g of raw material/kg appeared to be impossible due to too high viscosity at the initial stages of mashing in the conditions assumed for this experiment.

According to Czarnecki & Nowak [2001], initial preparation of starch raw material through extrusion enables to diminish enzyme doses needed for liquidising mashes, as well as improves obtained ethanol yields. The authors compared ethanol yield from rye prepared in the process of pressure cooking, non-pressurised enzymatic hydrolysis end extrusion and than hydrolysis with different α-amylase doses. The lowest ethanol yield (72% of theoretical) was obtained after fermentation of mashes prepared according to traditional method, through steaming. In the case of "cold liquefaction" that parameter increased to 82% of the theoretical yield, while ethanol yield for the process with the use of extrusion increased up to 88% of the theoretical yield. Moreover, tree times diminished dose of liquidising enzymatic preparation did not considerably affect ethanol yield decrease.

Krzyżaniak *et al*. [2003] stated that extruded starch hydrolysis was slower than that of autoclaved starch, but extruded starch was deeper depolymerised. According to Thymi *et al*. [2005], it is possible to shorten mashing time after extrusion conducted, as starch is gelatinised and much easily dissolves in water than the initial material. De Pilli *et al*. [2004] and Słomińska *et al*. [2003] also suggested that α-amylase dose, indispensable from the economical point of view, should be experimentally determined as extrusion process, especially at higher temperatures, affects starch chains causing their degradation.

CONCLUSIONS

1. Out of the examined concentrations of raw material 160 and 200 g raw material/kg more advantageous effects of ethanol fermentation were obtained when higher concentration of raw material in the sample was applied. For mashes of higher density there were obtained higher ethanol yields (up to 0.509 g ethanol/g starch).

2. Shortened mashing time did not affect fermentation dynamics nor the degree of sugars consumption from fermenting medium, yet slightly lower ethanol yield could be recorded. There were not stated any significant differences in yeast physiological condition. Moreover, lowered by half recommended by the producer dose of enzymatic preparation Termamyl 120L did not worsen fermentation dynamics, degree of sugars consumption or ethanol yield. As the amount of applied liquidising enzyme decreased, slight improvement of yeast cells physiological state was observed.

3. The use of extrusion at the initial stage of preparing starch raw material for fermentation enabled to improve ethanol yield. The temperature of 180°C used to extrusion maize flour, proved to be optimal out of all the examined temperatures. The mashes prepared from raw material extruded at 180°C featured the best final effects of fermentation, including considerably higher ethanol yield as compared to not extruded control sample.

4. The best alternative preparation of extruded maize flour for fermentation proved to be the mash of concentration 200 g raw material/kg, liquidised for 120 min by the dose of liquidising preparation Termamyl 120L and saccharising San Ultra recommended by the producer.

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FERMENTACJA ETANOLOWA ZACIERÓW KUKURYDZIANYCH

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Celem pracy była ocena wpływu alternatywnego sposobu przygotowania surowca na przebieg i efekty końcowe fermentacji etanolowej zacierów kukurydzianych.

Materiał badawczy stanowiło suszone ziarno kukurydzy odmiany KB 1902, które po rozdrobnieniu i nawilżeniu do zawartości wody 15% poddano procesowi ekstruzji w jednoślimakowym ekstruderze typu DN 20 firmy BRABENDER przy zastosowaniu temperatury ekstruzji 145°C, 180°C i 215°C. Otrzymane w postaci chrupek ekstrudaty rozdrabniano i zacierano, stosując różne stężenia surowca oraz zmienne parametry procesu zacierania, tj. czas procesu i dawki enzymu upłynniającego Termamyl 120L. Testy fermentacyjne prowadzono metodą okresową w temperaturze 37°C z udziałem przemysłowych drożdży gorzelniczych *Saccharomyces cerevisiae* D2. W analogicznych warunkach przygotowano próby kontrolne z rozdrobnionego nieekstrudowanego ziarna kukurydzy odmiany KB 1902. W próbach właściwych i kontrolnych oceniano dynamikę procesu fermentacji etanolowej, stopień wykorzystania cukrów, wydajność etanolu oraz stan fizjologiczny biomasy po zakończeniu procesu fermentacji. Czas fermentacji uzależniony był od gęstości zacierów. Stwierdzono, iż analizowane zmienne warunki procesu nie wpływały w istotny sposób na stopień wykorzystania cukrów przez drożdże (około 99%). Lepsze wydajności etanolu uzyskiwano w próbach przygotowanych z zacierów o stężeniu 200 g surowca/kg próby. Najlepsze efekty końcowe fermentacji – najlepsze wydajności etanolu (od 0,494 do 0,509 g etanolu/g skrobi) oraz najlepszy stan fizjologiczny drożdży po zakończeniu procesu osiągnięto po fermentacji zacierów z mąki kukurydzianej ekstrudowanej w temperaturze 180°C.