EFFECT OF THE CORN GRAIN STORAGE METHOD ON SACCHARIFICATION AND ETHANOL FERMENTATION YIELD

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Abstract. The chemical conservation was chosen in the study as the method for preserving fresh corn grain for distilleries. Five types of preserved corn samples were prepared. The control (with no additives) and four preserved with the preparation, based on formic and propionic acids (KemiSile 2000 Plus), at different levels. All the samples were stored for two months. Ethanol fermentations of low-temperature-cooked and pressure-cooked corn starch were carried out using commercial distillery yeast. The yeast strain, after starch hydrolysis with two enzymes, was able to produce 86-93% of theoretical ethanol yield from low-temperature-cooked corn. The ethanol production was almost equal to that produced from pressure-cooked corn starch (121°C), which was 87-94% of theoretical ethanol yield. The quality of distillates was also investigated. The most common by-products found were: acetaldehyde, ethyl acetate, propanol, isobutanol and 3-metylo-1-butanol. There were no important differences of spirits chemical composition between low-temperature--cooking and pressure-cooking method as well as between the kind of corn sample.

Key words: ethanol yield, corn grain, chemical conservation

INTRODUCTION

Bioethanol is the most promising biofuel and the starting material for various chemicals production. Increase in the demand for ethanol as a fuel additive has resulted in an increase in the amount of corn used for ethanol production also in countries where corn grain was not used for this purpose so far. Corn is characterised by high crop (8.0 t·ha^{-1}) and ethanol yield (417 l·t^{-1}) from ha whereas for rye there is a crop of 2.5 t·ha^{-1} and ethanol yield about 390 l·t^{-1}. Rye was the most popular raw-material for ethanol production till the end of XX century in Poland but now corn is the most important.

Maize grain contains over 60% of starch and is easy to handle as a material for fermentation [Belyea et al. 2004, Kupczyk 2007, Lipski 2002]. Corn, as the distillery material, has achieved the highest popularity in the United States. The United States, besides Brasil, is the biggest bioethanol producer. Maize in the USA is converted into ethanol by either wet or dry milling method [Devantier et al. 2005, Kwiatkowski et al. 2006]. For industrial-scale, where ethanol is produced from starchy materials, generally starch is first hydrolysed by adding a liquefying enzyme (α-amylase) and next the liquefied starch is hydrolysed to glucose with a saccharifying enzyme (glucoamylase) [Słomińska et al. 2003]. Applying, besides amylolytic enzymes, also preparates decomposing non-starchy polysaccharides may be very beneficial, especially when rye is used as a raw-material for fermentation [Czarnecki and Nowak 2005].

The hydrolysing process must be preceded by gelatinization of the starch. Pressure-cooking (which is traditionally used in Polish distilleries) is very effective for further fermentation of starchy materials but production costs are high due to the high energy consumption in the cooking process. The processes to reduce the high production costs were required and low-temperature-cooking fermentation system has also been successfully used and reaches, in most of the distilleries, a fermentation efficiency equal to that of the conventional pressure-cooking fermentation system [Fujita et al. 2004, Shigechi et al. 2004]. The other important problem in ethanol production from corn is the storing of the grain especially during wet and short summers like in Poland. The climatic conditions in Poland determine the harvest time of corn what causes that fresh grain contains about 40% of moisture and is not proper for a longer storage. Dried corn grain is stable and can be stored for a long time in dry conditions, but the drying process is expensive. Effective post-harvest storage treatment of grain for ethanol production is crucial for distilleries. One of the most appropriate methods may be using of biological or chemical preservatives. Propionic acid is highly effective mould inhibitor, commonly used in the food and feed industry. It was shown that it controls the growth of aflatoxigenic fungi and aflatoxin production in high moisture maize kernels [Aksu 2004, Harrison 1999, Marin et al. 2000]. Preserved corn grain is reported as a valuable raw-material for distilleries and the kind of preserving has generally no negative influence on ethanol production process.

In the present study, we examined the effectiveness of ethanol production and its quality when corn grain fresh, dried and wet preserved with the formic and propionic acids based preparate KemiSile 2000 Plus (in four levels) were used for bioprocess.

MATERIAL AND METHODS

Raw material

Seven samples of corn grain were used in the research: one fresh, one dried and five samples kept for two months at room temperature with formic and propionic acids based preservative preparation KemiSile 2000 Plus from Kemira (Finland). The stored samples were inoculated with four different levels of the preparation and one sample was kept without any additives. The tested samples were signed as follows: "0" – without additives, "I-IV" – with increasing dosage (1, 2, 3, 4 ml·kg^{-1}) of preservative.

Corn grain was milled before all the analysis.

Yeast and enzymes

Distillery yeast *Saccharomyces cerevisiae* (preparate "Fermiol") was used in this work for fermentation experiments (0.3 g of the preparate·l^{-1} of mash). Commercial α--amylase SPEZYME ETHYL and amyloglucosidase SPIRYZYME FUEL (Genencor International) were applied for saccharification in the amounts according to the producer recommendation.

Fermentation process

Low-temperature-cooking (100°C, 1 h) and pressure-cooking (121°C, 1 h, 1 atm.) were used for gelatinisation the corn starch before mashing. Mashing experiments were carried out in 250 ml Erlenmeyer flasks placed in a shaker (150 rpm). The mashing media were prepared by mixing 25 g of milled corn grain and 150 ml of distilled water. For liquefying SPEZYME ETHYL at 80°C during 20 minutes and SPIRYZYME FUEL for saccharification at 60°C during 100 minutes were used. The media after saccharification were inoculated with distillery yeast and incubated at 30°C for 72 h.

Analytical methods

Samples of corn grain were analysed for dry matter, reducing sugars, starch and pH value. Dry matter was estimated directly by drying at 130°C for 90 minutes. Carbohydrates were measured directly and after enzymatic hydrolysis (calculated as starch) as reducing sugars by DNS-method [Miller 1959]. Ethanol was assayed by distillation using areometric method. The composition and purity of the obtained distillates were checked on a Hewlett Packard HP gas chromatograph, using a Supelcowax-10 (60 m × 0.53 mm × 1.0 µm) column and a FID detector. The by-products in the medium were determined using the retention times of the peaks and normalized using the retention time of the internal standard (2-heptanon).

Statistical analysis

The analysis of variations was used to determine the relationship between corn samples concerning general characteristic and fermentation efficiency ($p < 0.05$).

RESULTS AND DISCUSSION

Dry matter, carbohydrates composition, starch and pH value of analysed corn samples are presented in Table 1. The samples of corn were characterised by very similar dry matter (fresh sample and samples 0-IV) by 70% except of dried corn which reached 87% of dry matter. The kind of storage and the level of applied preservative preparation did not change the starch content, which was estimated for 62-63% of d.m. and for fresh sample 76% of d.m. The difference appeared when analysing reducing sugars content, which increased following the dosage of the preservative preparation from 8 to 18 mg·g^{-1} (Table 1). pH value was also different for analysed samples and was much lower for "0" sample (4.5) than for other samples (5.3 to 5.9) and 6.0 for fresh and dry corn (Table 1). No negative results were observed about sensory analysis in colour and odour properties of silages.

Table 1. Characteristic of raw-material
Tabela 1. Charakterystyka surowca

Sample Próba	Preservative preparation additive ml·kg^{-1} of corn Dodatek preparatu konserwującego ml·kg^{-1} kukurydzy	d.m. % s.s. %	Reducing sugars directly Cukry redukujące wprost		Starch Skrobia		pH
			mg·g^{-1}	% d.m. % s.s.	mg·g^{-1}	% d.m. % s.s.	
Fresh Świeża	–	70.3	1.0	1.47	535.7	76.2	5.9
Dried Suszona	–	87.2	4.0	0.46	545.9	62.6	6.1
0	–	70.7	4.6	0.65	440.8	62.3	4.6
I	1.0	69.7	3.0	0.43	441.5	63.4	5.8
II	2.0	70.3	4.8	0.68	441.5	62.8	5.9
III	3.0	71.1	5.8	0.82	437.4	61.5	5.5
IV	4.0	70.8	6.4	0.91	448.5	63.3	5.3

The main objective of this study was to determine the effect of the preservative preparation and its dosage on further corn ethanol fermentation for bioethanol production. There were no problems to mash (hydrolysed by enzymes) all the samples – no inhibiting effects on the used enzymes were noticed (Table 2).

Pressure-cooking contributed faster enzyme action in all the samples, which is typical. It was observed that pressure-cooked samples let obtain significantly ($p < 0.05$) higher starch saccharification (76-91%) compared to gelatinisation by low-temperature-

Table 2. Mashing of corn samples after gelatinisation by low-temperature cooking (100°C, 1 h) and pressure-cooking (121°C, 1 h, 1 atm), %
Tabela 2. Zacieranie ziarna kukurydzy po procesie obróbki metodą bezciśnieniową (100°C, 1 h) i ciśnieniową (121°C, 1 h, 1 atm), %

Sample Próba	Starch saccharification after gelatinisation by Scukrzenie skrobi po obróbce metodą	
	low-temperature-cooking – bezciśnieniową	pressure-cooking – ciśnieniową
Fresh Świeża	74.9	83.1
Dried Suszona	73.5	76.2
0	72.4	86.1
I	74.3	84.5
II	72.1	87.3
III	72.9	90.8
IV	71.3	76.4

-cooking (71-75%; Table 2). When gelatinisation by pressure-cooking was applied, the starch saccharification after 120 minutes of enzymes action was even too high because some yeast do not accept very high level of glucose and maltose in the early stages of fermentation. In the further research we stated that the kind of corn starch gelatinisation had no significant influence on ethanol yield.

The ethanol yield obtained in the experiments was very high: 82-93% of theoretical yield for low-temperature-cooking method and 85-94% of theoretical yield for pressure-cooking method (Table 3). These results let us conclude that low-temperature-cooking fermentation system can be successfully used both for fresh, dried and preserved corn material for bioethanol production. Results from that study showed that from the point

Table 3. Ethanol fermentation of corn grain with *S. cerevisiae* at 30°C and 72 h
Tabela 3. Fermentacja etanolowa ziarna kukurydzy z użyciem drożdży *S. cerevisiae* w 30°C i czasie 72 h

Sample Próba	Gelatinisation Metoda obróbki	Remaining sugars Pozostałe cukry $mg \cdot ml^{-1}$	Ethanol yield Wydajność etanolu		
			% theoreth. yield % wyd. teoretycznej	$l \cdot 100 \ kg^{-1}$ of starch $l \cdot 100 \ kg^{-1}$ skrobi	$l \cdot 100 \ kg^{-1}$ of grain $l \cdot 100 \ kg^{-1}$ ziarna
Fresh Świeża	low-temperature-cooking bezciśnieniowa	0.69	82.1	59.0	31.6
	pressure-cooking ciśnieniowa	0.63	85.2	61.2	32.8
Dried Suszona	low-temperature-cooking bezciśnieniowa	1.21	89.2	64.1	35.4
	pressure-cooking ciśnieniowa	1.16	92.6	66.6	36.3
0	low-temperature-cooking bezciśnieniowa	1.05	89.9	64.6	28.5
	pressure-cooking ciśnieniowa	0.99	91.1	65.5	28.9
I	low-temperature-cooking bezciśnieniowa	0.85	87.4	62.8	27.8
	pressure-cooking ciśnieniowa	0.88	87.3	62.8	27.7
II	low-temperature-cooking bezciśnieniowa	0.83	86.2	61.9	27.4
	pressure-cooking ciśnieniowa	0.80	90.9	65.3	28.8
III	low-temperature-cooking bezciśnieniowa	0.68	93.4	67.1	28.9
	pressure-cooking ciśnieniowa	0.76	94.1	67.6	29.6
IV	low-temperature-cooking bezciśnieniowa	0.72	91.9	66.1	29.6
	pressure-cooking ciśnieniowa	0.83	91.8	66.0	29.6

of view of enzymatic hydrolysis process all the samples might be prepared by less energy consuming low-temperature-cooking method of gelatinisation (Table 3). Shigechi et al. [2004] reported the same conclusion about corn starch gelatinisation and reached 97.2-98.0% of theoretical ethanol yield.

It is also important to notice that there were no contamination problems observed (which sometimes appears in low-temperature-cooking method) and that system enabled to reach the fermentation efficiency equal to that of the pressure-cooking fermentation system.

Taking into account the influence of preservative preparation dosage on ethanol production we observed statistically important ($p < 0.05$) differences between the samples. The highest ethanol yield was obtained from "III" and "IV" sample (93% and 92% of theoretical yield) and this was compared to ethanol amounts produced from dried sample (Table 3). The preparate from Kemira showed to be successfully used as the additive to preserve wet corn material during storing before fermentation process. The ethanol yield obtained from preserved samples was even higher (93% theoretical yield from sample "III") than from dried (89% theoretical yield) and fresh (82% theoretical yield) samples ($p < 0.05$).

Table 4. The composition of spirits obtained from fermented dried corn after gelatinisation by low-temperature-cooking (100°C, 1 h), mg·l^{-1} of 100% spirit
Tabela 4. Skład spirytusów uzyskanych z fermentacji ziarna kukurydzy po obróbce metodą bezciśnieniową (100°C, 1 h), mg·l^{-1} 100% spirytusu

Name of the compound Nazwa związku	Fresh grain Ziarno świeże	Dried grain Ziarno suszone	Samples – Próby				
			0	I	II	III	IV
Acetaldehyde Aldehyd octowy	–	159.42	221.22	207.61	242.26	342.50	399.56
Ethyl acetate Octan etylu	–	162.78	500.30	241.15	272.10	265.04	219.06
Propanol Propanol	–	507.27	1 389.29	1 416.18	1 898.17	1 773.01	1 193.43
Izobutanol Izobutanol	718.97	841.48	980.15	1 073.14	1 097.43	1 042.02	1 018.33
Butanol Butanol	3.83	4.94	13.77	24.21	8.91	8.83	9.17
3-methyl-1-butanol 3-metylo-1-butanol	846.92	2 989.73	1 480.34	1 598.19	1 433.03	1 399.49	1 631.04
Ethyl caproate Kapronian etylu	–	1.50	2.71	3.52	1.95	1.97	1.36
1-pentanol 1-pentanol	1.25	1.04	2.93	2.17	2.44	2.11	1.50
Furfural – Furfural	4.06	3.75	8.04	3.98	5.48	2.79	4.90
Methanol – Metanol	84.98	105.69	172.16	225.18	217.30	173.71	136.22
Acrolein – Akroleina	–	–	0.88	1.07	2.26	1.87	1.76

When analysing the quality of distillates, one has to take into account that any kind of rectification process was used. The percentage of ethanol in the distillates was always higher than 99% of all volatile compounds detected, still the amount of by-products was very high. The most common by-products found in high quantities were acetaldehyde (120-340 mg·l^{-1} of 100% spirit), ethyl acetate (150-500 mg·l^{-1} of 100% spirit), propanol, isobutanol and 3-metylo-1-butanol (as the sum of 3-methyl-1-butanol and 2-methyl-1--buthanol). The last one in a very high quantities up to 4.5 g (the average amount close to 1.5 g·l^{-1} of 100% spirit; Table 4 and 5). Furfural and acrolein were found in most of the samples, which is undesirable in commercial scale. When the high level of higher alcohols is not a problem to exclude in rectification process, aldehydes and acrolein might be a problem for the commercial consumable spirit products as well as methanol in some samples. However such ethanol can be definitely used for biofuel production.

Table 5. The composition of spirits obtained from fermented corn after gelatinisation by pressure-cooking (121°C, 1 h), mg·l^{-1} of 100% spirit

Tabela 5. Skład spirytusów uzyskanych z fermentacji ziarna kukurydzy po obróbce metodą ciśnieniową (121°C, 1 h), mg·l^{-1} 100% spirytusu

Name of the compound Nazwa związku	Fresh grain Ziarno świeże	Dried grain Ziarno suszone	Samples – Próby				
			0	I	II	III	IV
Acetaldehyde Aldehyd octowy	144.68	109.87	254.06	112.57	151.69	196.26	306.86
Ethyl acetate Octan etylu	342.89	246.58	319.87	270.16	214.00	188.84	153.37
Propanol Propanol	1 352.04	702.26	1 409.20	1 943.56	1 694.00	1 542.35	1 080.83
Izobutanol Izobutanol	571.74	1 594.94	1 075.62	920.52	974.36	1 027.07	1 044.95
Butanol Butanol	3.24	5.44	8.67	13.84	7.74	8.47	11.13
3-methyl-1-butanol 3-metylo-1-butanol	578.51	4 752.58	1 410.69	1 331.42	1 293.34	1 369.13	1 729.47
Ethyl caproate Kapronian etylu	–	2.83	2.88	2.95	2.04	2.31	1.66
1-pentanol 1-pentanol	–	1.33	1.38	1.31	1.24	1.02	1.02
Furfural – Furfural	16.82	7.93	6.15	3.64	5.47	7.97	3.96
Methanol – Metanol	140.68	176.19	157.29	239.66	241.15	191.47	134.43
Acrolein – Akroleina	–	0.87	1.40	1.28	1.09	1.10	1.15

No profound differences were found between pressure and low-temperature-cooking method in spirits chemical composition (Table 6).

In conclusion it should be stress that there is the possibility to efficiently produce ethanol both from fresh, dried corn grain and preserved with KemiSile 2000 Plus preparation using commercial distillery yeast and two enzymes for starch hydrolysis. A low-

Table 6. Ethanol and by-products content of corn distillates, percentage of all detected volatile compounds

Tabela 6. Zawartość etanolu i związków ubocznych destylatów kukurydzianych, procent wszystkich wykrytych związków lotnych

Name of the compound Nazwa związku	Fresh grain Ziarno świeże	Dried grain Ziarno suszone	Samples – Próby				
			0	I	II	III	IV
Low-temperature-cooking (100°C, 1 h) Metoda bezcisnieniowa (100°C, 1 h)							
Ethanol Etanol	99.22	98.98	99.40	99.32	99.35	99.37	99.42
By-products Związki uboczne	0.78	1.02	0.60	0.68	0.65	0.63	0.58
Pressure-cooking (121°C, 1 h) Metoda ciśnieniowa (121°C, 1 h)							
Ethanol Etanol	98.92	99.05	99.41	99.40	99.42	99.43	99.44
By-products Związki uboczne	1.08	0.95	0.59	0.60	0.58	0.57	0.56

-temperature-cooking system for gelatinisation corn starch for ethanol fermentation was as effective as the pressure-cooking system.

The analysis of our study proved that raw harvested corn grain does not have to be directly processed into ethanol and the expensive process of drying can be substituted by cheaper preserve preparations.

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WPŁYW METODY PRZECHOWYWANIA ZIARNA KUKURYDZY NA PROCES SCUKRZANIA SKROBI I WYDAJNOŚĆ FERMENTACJI ETANOLOWEJ

Streszczenie. W pracy zastosowano metodę chemicznej konserwacji ziarna kukurydzy z użyciem preparatu na bazie kwasu mrówkowego i propionowego (KemiSile 2000 Plus). Przygotowano pięć prób: kontrolną (bez dodatków) oraz cztery próby z różnymi ilościami preparatu. Wszystkie próby przechowywano dwa miesiące. Zastosowane do fermentacji drożdże gorzelnicze pozwoliły na uzyskiwanie wydajności etanolu 86-93% w stosunku do teoretycznej z kukurydzy poddawanej bezciśnieniowemu parowaniu przed procesem zacierania. Produkcja etanolu była porównywalna z wydajnością po ciśnieniowym parowaniu ziarna (87-94% w stosunku do wydajności teoretycznej). Badano także jakość uzyskanych destylatów. Związkami ubocznymi występującymi w największych ilościach okazały się: aldehyd octowy, octan etylu, propanol, izobutanol i 3-metylo-1-butanol. Nie stwierdzono istotnych różnic w jakości destylatów, zarówno pomiędzy zastosowaną metodą bezciśnieniową i ciśnieniową parowania surowca, jak i dla poszczególnych prób konserwowanego ziarna.

Słowa kluczowe: wydajność etanolu, ziarno kukurydzy, chemiczna konserwacja