

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Progress in ethanol production from corn kernel by applying cooking pre-treatment

Neven Voca^{a,*}, Boris Varga^b, Tajana Kricka^a, Duska Curic^c, Vanja Jurisic^a, Ana Matin^a^a Faculty of Agriculture University of Zagreb, Department for Agricultural Technology, Storing & Transport, Svetosimunska cesta 25, HR-10000 Zagreb, Croatia^b Department for Grassland and Forage, Svetosimunska cesta 25, Zagreb, Croatia^c Faculty of Food Technology and Biotechnology, Department of Food Engineering, Pierottijeva 6, Zagreb, Croatia

ARTICLE INFO

Article history:

Received 23 October 2008

Received in revised form 11 December 2008

Accepted 16 December 2008

Available online 5 February 2009

Keywords:

Corn kernel

Cooking

Pre-treatment

Ethanol production

ABSTRACT

In order to improve technological properties of corn kernel for ethanol production, samples were treated with a hydrothermal pre-treatment of cooking (steaming), prior to drying. Two types of cooking process parameters were applied; steam pressure of 0.5 bars during a 10 min period, and steam pressure of 1.5 bars during a 30 min period. Afterwards, samples were dried at four different temperatures, 70, 90, 110 and 130 °C. Control sample was also submitted to the afore-mentioned drying parameters. Since the results showed that starch utilization, due to the gelatinization process, was considerably higher in the samples pre-treated before the ethanol production process, it was found that the cooking treatment had a positive effect on ethanol yield from corn kernel. Therefore, the highest ethanol yield was found in the corn kernel samples cooked for 30 min at steam pressure 1.5 bars and dried at 130 °C. Due to the similarity of processes used for starch fermentation, introduction of cooking pre-treatment will not significantly increase the overall ethanol production costs, whereas it will result in significantly higher ethanol yield.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Ethanol (ethyl alcohol, C₂H₅OH) is a liquid biofuel which can be produced from several different biomass feedstock and conversion technologies. Ethanol is an attractive alternative fuel because it is a renewable bio-based resource and it is oxygenated thereby provides the potential to reduce particulate emissions in compression-ignition engines (Hansen et al., 2005).

World ethanol production in 2006 reached 51 billion liters, up from 46 billion liters in 2005 (RFA, 2007). The major producers are Brazil and the USA, which together account for about 70% of the world production. Around 60% of the ethanol is produced by fermentation. Main feedstock for ethanol production are sugar cane (in Brazil) and corn grain (USA), while many other agricultural raw materials rich in fermentable carbohydrates, or those locally available that could be converted to yield the fermentable sugars, are used worldwide (Whittington, 2006).

Nearly all ethanol is produced by fermentation of corn glucose in the United States or sucrose in Brazil, but any country with a significant agronomic-based economy can use current technology for ethanol fermentation. This is possible because, during the last two decades, technology for ethanol production from non-food-plant

sources has been developed to the point at which large-scale production will be a reality in the next few years (Lin and Tanaka, 2006). In the United States, 90% of ethanol is derived from corn (de Oliveria et al., 2005).

Concerning the EU, in November 2001 a Directive was accepted that requests member states to establish legislation about utilization of fuels from renewable resources. In 2008, this utilization should cover 4.25% of total fuel consumption. This quota is expected to increase to 5.75% in 2010 and to rise further. Some member states like Finland, Sweden or Austria have already fulfilled the set quota (Mojovic et al., 2006). The potential demand for ethanol as fuel for transportation in the EU countries, calculated on the basis of the Directive 2003/30/EC, is estimated at about 12.7 billion liters in 2010. In Europe, the feedstock used for ethanol is predominantly wheat, sugar beet and corn. It is estimated that full implementation of the Directive regarding domestically produced biofuels will require between 4% and 13% of total agricultural land in the EU (Lombardi, 2003; Larsson, 2006).

Biological feedstock that contain substantial amounts of sugar – or materials that can be converted into sugar, such as starch or cellulose – can be fermented to produce ethanol to be used in gasoline engines (Malça and Freire, 2006). One major problem with ethanol production is the availability of raw materials for the production. The availability of feedstock for ethanol can vary considerably from season to season and depends on geographic locations. The price of the raw materials can also highly affect the costs of ethanol production (Yoosin and Sorapipatana, 2007). Because feedstock

* Corresponding author. Tel.: +385 1 2393 625; fax: +385 1 2393 919.
E-mail address: nvoca@agr.hr (N. Voca).

typically account for more than one-third of the production costs, maximizing ethanol yield is imperative (Dien et al., 2003).

Corn kernels for ethanol production are usually dried after the harvest in order to reduce kernel moisture until hygroscopic equilibrium is achieved so that it can be stored for a longer period of time. Depending on the air temperature applied, there is a decrease in the amino acid or protein content as well as an increase in starch gelatinization degree. During the last few years, extensive researches have been conducted on different treatments that could improve the quality of corn kernel. A pre-treatment process, applied prior to drying, is usually carried out in order to improve acceptability of the products. Several studies have been carried out to investigate the effect of pre-treatment and hot air on the quality of processed corn kernels. One of such treatments is the cooking process, namely steaming of the whole corn kernel immediately after harvesting and prior to drying (Pulier, 1993; Mujumdar, 2000). The cooking process is one of essential steps in the production of starchy whole grains. The main purpose of the cooking procedure is to gelatinize the starch *in situ* while keeping the grain integrity. During cooking of whole grains, starch granules gelatinize in their natural environment as embedded in the endosperm (Sagol et al., 2006). The purpose of cooking process is to convert the raw grain into palatable, digestible and workable form through gelatinization of starch. This will result in a better utilization of corn kernel during ethanol production.

The aim of this paper was to determine the possibility of improvements in ethanol production technology, by introducing cooking process as a hydrothermal processing of corn kernel applied before the drying process itself. For that purpose, the main objective was to determine the optimum cooking parameters, which would result in better starch utilization of the raw material.

2. Methods

2.1. Corn

The corn hybrids of FAO Group 400, Bc 462 (semi flint), Bc 4982 (dent), Bc Jumbo 48 (dent), Florencia (dent), and Stefania (hard dent) were grown in the experimental field of the Faculty of Agriculture University of Zagreb, in a standard rotation corn–soy–wheat pattern and with standard plantation measures applied. The three studied hybrids, Bc Jumbo 48, Bc 4982 and Bc 462, are of Croatian origin and are produced by Croatian Bc Institute, while Florencia and Stefania are introduced hybrids, produced by the Pioneer company.

2.1.1. Physico-chemical properties of corn

Hectoliter mass of corn kernel was determined by use of the standard method (Schopper scale). The absolute mass (mass of 1000 kernels) was determined according to Mujumdar (2000). To determine the average kernel size, a sample of 100 kernels was randomly picked and their three major dimensions, length, width and thickness, were measured using a micrometer, with accuracy of 0.01 mm. The sphericity coefficient shows the properties of an object relative to a sphere, and is determined based on kernel dimensions according the equation Mohsenin (1970), Strohshine et al. (1986).

Moisture, starch, protein and oil content analyses were conducted on healthy corn kernels cleaned from all impurities. Moisture content was determined according to ISO 712, 1998. Starch content was determined by the Ewers method (ISO 10520, 1997). Protein and oil contents were determined according to AOAC methods (AOAC, 1990).

2.2. Cooking experiments

Firstly, corn kernels were rehydrated up to approximately 32% of moisture content, which is the average moisture content during the field harvest of kernel, in order to obtain the comparability of kernels for the investigation purpose. After rehydration, the hydrothermal cooking treatment was applied.

Lab-scale cooking device, used in this investigation, consisted of a high-pressure hermetically closed vessel instead of industrial cooking jet. The difference is that the steam was not introduced externally, but the kernel was steamed with water from the bottom of the vessel. The vessel contained an additional porous-soiled container, beneath which an aluminum-made perforated pad was placed, preventing direct contact of kernel with water and high temperatures. There were no barriers to the steam flow through the kernel and the steaming of the sample was enabled. The vessel was hermetically sealed so that the preferable temperature and pressure values in the system could be obtained. The vessel received the heat from electric heater and contained thermometer and manometer for temperature and pressure control. It also contained a steam valve and a safety valve. Dimensions of the lab-scale cooking device were 600 × 790 × 650 mm (Fig. 1).

In this high-pressure vessel, the samples were treated with steam in modified cooking treatment with two different ways: for 10 min at pressure of 0.5 bar (C1) and 30 min at pressure of 1.5 bar (C2).

2.3. Drying process

Both, treated and untreated corn kernels (the latter was control) were dried in a lab-scale dryer at air temperatures of 70, 90, 110 and 130 °C, and air flow of 1.0 m/s. Dryer consisted of a centrifugal fan to supply the air flow, an electric heater, air filter and an electronic proportional controller. Air temperature was controlled by

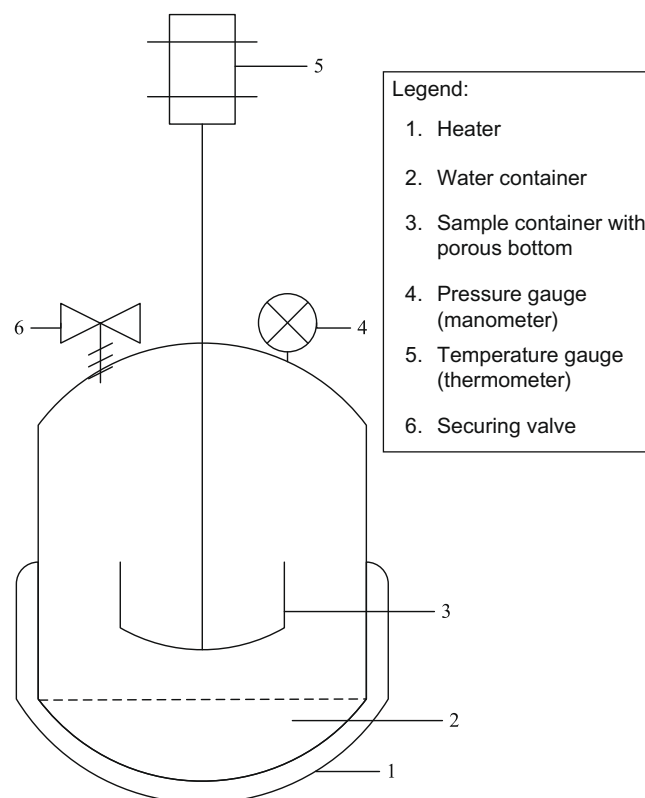


Fig. 1. Lab-scale cooking device.

means of a proportional controller and air flow-rate for all drying runs was measured with anemometer (Edra five). Drying was conducted until corn kernel moisture content was reduced to 14%. Then, the sample was cooled for 10 min and kept in air glass jars for further analyses. All measurements were carried out in triplicates and the average moisture ratio at each value was used for calculation of drying equations.

2.4. Enzymes and microorganisms

α -Amylase and amyloglucosidase enzymes were used for the starch hydrolysis process. α -amylase (endo-1,4- α -D-glucan glucohydrolase, EC 3.2.1.1) stock solution was obtained by dissolving lyophilized enzyme (*Bacillus subtilis*, ~380 U/mg, Sigma Aldrich) and amyloglucosidase (exo-1,4- α -D-glucan glucohydrolase, EC 3.2.1.3) enzyme was already prepared for the hydrolysis (*Aspergillus niger*, Roche, 500 U).

Saccharomyces cerevisiae (Old Herold Hefe s.r.o., Slovakia) was used for the fermentation of corn meal. A 6 g/L (on dry basis) yeast solution was prepared and kept at 4 °C.

2.5. Starch hydrolysis

Starch hydrolysis was conducted in 500 mL-flask in a water bath with shaker. Corn meal was hydrolyzed in a two-step process. Firstly, 70 ppm of $\text{Ca}_{(\text{aq})}^{2+}$ were added to corn meal, pH was adjusted to 6.0–6.5 with the addition of 0.2 M $\text{NaOH}_{(\text{aq})}$ and 0.2 mL/L of α -amylase was added. In the first step, liquefaction, the mixture was heated for 30 min at 90 °C. The samples were then cooled to 60 °C, pH was adjusted to 4.0–5.0 with 5% $\text{H}_2\text{SO}_{4(\text{aq})}$, and 0.4 mL/L of amyloglucosidase was added. In the second step, saccharification, the mixture was heated for 50 min at 60 °C. Saccharification process was monitored by putting a small drop of corn hydrolyzate on filter paper together with a drop of 0.01 M $\text{I}_{2(\text{aq})}$ solution. If there was no change in color, the process was considered as finalized. Approximately 25 mL of corn hydrolyzate was then centrifuged at 3000 rpm during 10 min. The supernatant was partly used for reducing sugar determination, and partly for glucose determination.

2.6. Ethanol fermentation of starch hydrolyzates

Hydrolyzed corn meal was prepared by suspending 40 g of corn kernel, milled in a laboratory mill (IKA, MF 10 basic), with water in 1:7 ratio, in a 500 mL Erlenmeyer flask. Samples were then steamed in autoclave at 121 °C for 20 min to obtain better gelatinization degree and then hydrolyzed. Fermentation of hydrolyzates was conducted in 300 mL Erlenmeyer flasks, in water bath at 30 °C for 16 h, after addition of *S. cerevisiae*, 2 g/L $(\text{NH}_4)_2\text{SO}_{4(\text{aq})}$ and 1 g/L $(\text{NH}_4)_2\text{HPO}_{4(\text{aq})}$.

2.7. Analytical methods

2.7.1. Determination of starch gelatinization degree

An amylose/iodine method was used for determination of starch gelatinization degree according to Birch and Priestley (1973) method, modified by Cai and Diosady (1993). The method is based on the reaction of amylose (which is released during gelatinization process) with iodine which results in blue coloration of solution. The intensity of the staining was determined on spectrophotometer (Lambda 25, UV/VIS Spectrometer), at $\lambda = 600$ nm.

2.7.2. Determination of reducing sugars content

The sugar content was determined according to AACC Method 80–68, Reducing Sugars–Schoorl Method, i.e. by methods developed for determination of reducing sugars content.

2.7.3. Determination of glucose content

Glucose content was determined by color-enzyme method (Glucose PAP) on spectrophotometer at $\lambda = 500$ nm, and its concentration was determined indirectly by applying previously defined calibration.

2.7.4. Determination of ethanol content

The gas chromatography (GC) was performed using the capillary gas chromatograph (Varian CP-3800 Series) equipped with flame ionization detector (FID) and capillary column (Varian, CP-Sil 88, 25 m \times 0.32 mm; 0.2 μm).

The chromatography was conducted under the following conditions:

- Oven temperature: 155 °C (isothermal).
- Inlet temperature: 175 °C.
- Detector temperature: 250 °C.
- Run time: 5.5 min.
- Ethanol retention time: about 1.4 min.
- Carrier gas: He.
- Make up: He, flow-rate 28 mL/min.
- H_2 flow: 30 mL/min.
- Air flow: 30 mL/min.
- Injection volume: 50 μL .
- Split ratio: 20:1.
- Column temperature program: 75 °C, isothermal for 4 min.

Data were collected and the gas chromatography controlled on a PC with Galaxie Workstation Data Handling System (supplied by the GC manufacturer).

2.8. Statistical analysis

All data were analyzed according to GLM procedure in the SAS system package version 8.00 (SAS Institute, 1997). Since the design of the experiment was not specifically defined (the allocation was made on absolutely random basis with various numbers of observations), the average values for each investigated factor were calculated and interaction was included in the relevant analysis. All measurements were carried out in triplicates. Data were expressed as mean \pm SD using ANOVA, and if justified by the statistical probability ($P < 0.05$), subjected to Duncan's new multiple range test. Differences were considered statistically significant if $P < 0.05$.

3. Results and discussion

Chemical composition of corn samples was determined and is shown in Table 1.

After rehydration, the corn samples were treated in two cooking conditions; for 10 min at 0.5 bar pressure (C1) and for 30 min at a higher pressure of 1.5 bars (C2). Fig. 2 shows the results of average kernel moisture values after applying cooking as a treatment. It was observed that all investigated hybrids cooked for 10 min at 0.5 bar, had increased kernel moisture content by 3.19% in average, while those cooked for 30 min at 1.5 bar had increased kernel moisture content by 5.25% in average. Thus it can be determined that application of cooking treatment will increase initial moisture content in corn kernel, which will certainly result in extended time of water release from the hybrids observed.

In order to determine the differences in water release rate from untreated and treated corn kernels, they were dried in convection dryer at four different air temperatures. Based on the measurements on mass loss, corn kernel water release coefficient was determined for all applied temperatures and each hybrid up to the corn moisture equilibrium reached (14%), which is shown in

Table 1
Composition of corn kernels used in the investigation.

Hybrid	Moisture (%)	Hectoliter weight (kg/hL)	1000 Kernel mass (g)	Sphericity	Starch (% on dry basis)	Protein (% on dry basis)	Oil content (% on dry basis)
Bc 462	15.25 ± 1.23 ^a	76.42 ± 1.97 ^a	325.88 ± 34.89 ^d	0.67 ± 0.04 ^a	75.92 ± 1.96 ^b	12.40 ± 1.85 ^b	7.04 ± 0.12 ^a
Bc 4982	15.94 ± 0.85 ^a	70.78 ± 2.15 ^b	372.09 ± 43.35 ^c	0.64 ± 0.05 ^b	76.44 ± 2.13 ^b	12.47 ± 1.24 ^b	6.96 ± 0.32 ^a
Bc Jumbo 48	14.76 ± 1.68 ^{ab}	69.30 ± 2.18 ^b	410.10 ± 54.18 ^a	0.66 ± 0.06 ^a	76.26 ± 1.65 ^b	12.38 ± 1.58 ^b	6.70 ± 0.25 ^a
Florencia	14.78 ± 2.12 ^{ab}	66.62 ± 1.86 ^c	401.85 ± 35.16 ^b	0.59 ± 0.04 ^c	78.47 ± 2.47 ^a	13.27 ± 1.63 ^{ab}	6.49 ± 0.29 ^a
Stefania	13.88 ± 1.37 ^b	69.87 ± 2.65 ^b	360.29 ± 41.43 ^c	0.62 ± 0.05 ^b	76.86 ± 3.52 ^b	13.76 ± 2.42 ^a	6.53 ± 0.14 ^a
<i>p</i> value	**	***	***	***	***	*	NS

Data are averages ± SD of three determinations. Different letters within a column indicate significant differences at the 5% level by Duncan test. n.s. = nonsignificant.

* Significant with $P \leq 0.05$.

** Significant with $P < 0.01$.

*** Significant with $P < 0.001$.

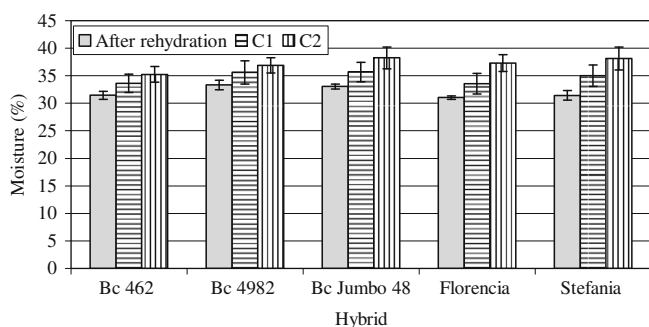


Fig. 2. Increase of initial moisture after cooking treatment.

Table 2. It was found that in addition to differences in dry down rate of various drying temperatures, there were also differences between kernels regardless of whether it was treated with cooking procedure. This is related to increased levels of initial moisture and the fact that water is retained in the area of pericarp's tubulous cells, as corroborated by literature references (Putier, 1993). Comparison of mean values of kernel water release rate, no matter to which treatment was undertaken, showed that hybrid Stefania had the slowest water release, while Bc 462 had the highest water

release rate. However, there are no significant differences between the two hybrids.

Table 2 shows that with increase in drying temperature, irrespective of presence of cooking treatment, the hectoliter mass decreased. It can be determined that drying temperature had significant influence on hectoliter mass reduction, which was according to literature data (Mujumdar, 2000). Moreover, there were significant differences between samples which were cooked before drying process and those which were not treated. Because of the latter statement, it can be determined that cooking treatment also affects the reduction of corn kernel hectoliter mass. Furthermore, cooking treatment also resulted in reduction of absolute kernel mass, but the differences were not substantial. Significant decrease in both, hectoliter mass and 1000-kernel mass in treated samples was a result of the kernel surface shrinkage due to the rapid water loss during the drying process; water was then substituted during the hydrothermal pre-treatment. Moreover, the decrease in absolute kernel mass was a result of high drying temperature or longer kernel exposure to the pre-treatment itself; namely, the kernel was of poorer quality due to the cracking of its surface as a result of high temperature during the cooking pre-treatment.

Conversely, drying temperature did not have significant affect on absolute mass. The highest kernel sphericity value was found in the samples which were dried at 130 °C, both treated and un-

Table 2
Effect of cooking treatment and drying temperature on kernel physical properties during its processing for ethanol production.

Parameter	Kernel water release coefficient (1/s)	Hectoliter mass (kg/hL)	1000 Kernel mass (g)	Sphericity
<i>Drying temperature (TEMP)</i>	***	***	NS	***
70 °C	0.0415 ± 0.0023 ^b	66.41 ± 2.86 ^a	326.97 ± 48.89 ^a	0.66 ± 0.05 ^b
90 °C	0.0508 ± 0.0028 ^b	64.97 ± 3.14 ^b	322.04 ± 47.32 ^a	0.65 ± 0.05 ^c
110 °C	0.05338 ± 0.0026 ^b	63.96 ± 2.59 ^c	330.00 ± 47.82 ^a	0.66 ± 0.06 ^b
130 °C	0.07228 ± 0.0035 ^a	62.24 ± 2.97 ^d	323.62 ± 45.75 ^a	0.68 ± 0.04 ^a
<i>Treatment (TRE)</i>	***	***	NS	***
Drying	0.07198 ± 0.0042 ^a	65.50 ± 3.26 ^a	328.10 ± 43.36 ^a	0.67 ± 0.05 ^a
C1 + drying	0.04892 ± 0.0037 ^b	64.11 ± 3.19 ^b	326.41 ± 42.59 ^a	0.66 ± 0.05 ^b
C2 + drying	0.04262 ± 0.0044 ^b	63.57 ± 3.23 ^c	322.47 ± 38.52 ^a	0.66 ± 0.05 ^b
<i>Hybrid (HYB)</i>	NS	***	***	***
Bc 462	0.05863 ± 0.0038 ^a	68.52 ± 2.19 ^a	303.44 ± 37.02 ^c	0.68 ± 0.06 ^a
Bc 4982	0.05179 ± 0.0027 ^a	64.50 ± 2.34 ^b	305.15 ± 46.12 ^c	0.64 ± 0.05 ^b
Bc Jumbo 48	0.05511 ± 0.0051 ^a	62.91 ± 1.81 ^c	349.78 ± 50.94 ^a	0.67 ± 0.04 ^a
Florencia	0.05785 ± 0.0036 ^a	61.85 ± 1.77 ^d	354.19 ± 38.31 ^a	0.65 ± 0.05 ^b
Stefania	0.04911 ± 0.0038 ^a	64.19 ± 3.34 ^b	315.73 ± 44.95 ^b	0.68 ± 0.05 ^a
Int TEMP × HYB	NS	***	NS	***
Int TEMP × TRE	NS	NS	NS	NS
Int HYB × TRE	NS	NS	NS	**
Int TEMP × HYB × TRE	NS	*	NS	**

Data are averages ± SD of three determinations. Different letters within a column indicate significant differences at the 5% level by Duncan test. n.s. = nonsignificant.

* Significant with $P \leq 0.05$.

** Significant with $P < 0.01$.

*** Significant with $P < 0.001$.

Table 3
Activation energy for all investigated hybrids and all treatments.

Treatment	Activation energy (kJ/mol)				
	Bc 462	Bc 4982	Bc Jumbo 48	Florenzia	Stefania
Drying	11.63 ± 0.85 ^b	13.80 ± 2.20 ^b	11.01 ± 1.96 ^a	13.43 ± 1.24 ^a	12.06 ± 1.80 ^b
C1 + drying	16.19 ± 1.58 ^a	15.38 ± 1.59 ^{ab}	13.45 ± 1.70 ^a	13.72 ± 1.88 ^a	13.58 ± 2.02 ^b
C2 + drying	16.45 ± 1.20 ^a	16.80 ± 1.80 ^a	13.63 ± 1.46 ^a	14.45 ± 1.17 ^a	15.38 ± 1.32 ^a
<i>p</i> value	***	*	NS	NS	**

Data are averages ± SD of three determinations. Different letters within a column indicate significant differences at the 5% level by Duncan test. n.s. = nonsignificant.

* Significant with $P \leq 0.05$.

** Significant with $P < 0.01$.

*** Significant with $P < 0.001$.

treated ones. The cooking treatment also had a significant influence on modifying shape and, thus, on dimension of corn kernels. Hence, it can be determined that cooking treatment significantly changed physical properties of kernels during the ethanol production.

Based on the linear relation and the Arrhenius equation, the curves of kernel water release rate and drying temperature constants were determined for each investigated treatment (Henderson and Pabis, 1961; Bala, 1997). Slope line was the basis for determining the activation energy value of investigated corn hybrids kernels, both treated and untreated. The determination of activation energy values enables to define the energy that has to be introduced in the kernel in the convection dryer with the aim to excite water molecules to interact and to initiate the drying process. Namely, the higher activation energy the slower reaction occurs, and drying itself takes longer time. Since the initial moisture of the cooked samples was higher because of the steam received by kernel during cooking treatment, it can be expected that the activation energy of steamed kernels will be higher. This assumption is corroborated in Table 2 which shows significantly longer drying time of cooked kernels in comparison with those kernels which were not cooked at all drying temperatures. Table 3 gives the activation energy values for all investigated hybrids with and without cooking treatment, which were obtained from the analysis of water release rates at four drying temperatures.

Table 3 shows that activation energy was significantly higher in kernels treated in both hydrothermal conditions when compared

to the untreated kernels. Since the drying time values showed the same tendency, it can be concluded that the activation energy is directly related to the kernel water release rate (Bala, 1997). There was also somewhat higher difference between cooking methods, but these differences were not significant. It can be stated that when certain amount of water enters into kernel during the cooking process, it results in significant increase of activation energy needed for initiating the kernel water release and in extending of drying period.

After rehydration of corn kernel to approximately 32% moisture content, which is usual moisture content at the time of corn harvest, corn kernel samples were dry milled without previous separation of the germ. After milling, native starch content was determined in the samples (Table 1). During the investigation, the highest starch content mean value was found in hybrid Florenzia (78.47%). The lowest starch content, in relation to other observed hybrids, was found in Bc Jumbo 48 (75.92%). Effect of cooking treatment on starch gelatinization in corn kernel is shown in Table 4. Furthermore, it gives the starch/ethanol conversion in relation to the treatment, drying temperature and hybrid used in the investigation.

Until recently, industrial corn-derived ethanol was produced only from dried corn kernels. However, starch gelatinization in such corn kernel was very low. Starch gelatinization, i.e., the change in native structure of starch, in starch-rich cereal grains is important because it enables better usability of kernel starch

Table 4
Effect of cooking treatment and drying temperature on starch to ethanol conversion process.

Parameter	Starch gelatinization (%)	Reducing sugars (g/L)	Glucose (g/L)	Ethanol (g/L)
<i>Drying temperature (TEMP)</i>	-	***	NS	NS
70 °C	12.32 ± 1.12 ^c	104.36 ± 8.79 ^b	9.52 ± 1.32 ^a	53.35 ± 6.26 ^a
90 °C	12.87 ± 0.80 ^{bc}	104.01 ± 7.57 ^b	9.61 ± 1.34 ^a	54.26 ± 8.86 ^a
110 °C	14.17 ± 0.75 ^{ab}	104.89 ± 6.31 ^b	9.56 ± 1.29 ^a	55.39 ± 7.72 ^a
130 °C	14.82 ± 0.79 ^a	106.58 ± 7.07 ^a	9.71 ± 1.28 ^a	57.38 ± 6.89 ^a
<i>Treatment (TRE)</i>	***	NS	NS	*
Drying	6.78 ± 1.08 ^c	104.51 ± 5.18 ^a	9.57 ± 1.43 ^a	54.22 ± 5.57 ^b
C1 and drying	9.62 ± 1.17 ^b	105.24 ± 4.99 ^a	9.59 ± 1.35 ^a	54.58 ± 5.89 ^b
C2 and drying	24.28 ± 1.42 ^a	105.15 ± 5.30 ^a	9.64 ± 1.25 ^a	57.23 ± 7.53 ^a
<i>Hybrid (HYB)</i>	***	***	**	**
Bc 462	18.47 ± 1.29 ^a	104.14 ± 5.33 ^c	9.75 ± 1.15 ^a	50.69 ± 6.89 ^c
Bc 4982	10.23 ± 1.89 ^d	106.80 ± 5.02 ^b	9.49 ± 1.29 ^b	55.37 ± 7.55 ^b
Bc Jumbo 48	10.64 ± 0.43 ^d	99.74 ± 6.53 ^e	9.60 ± 0.86 ^{ab}	54.79 ± 8.83 ^b
Florenzia	15.59 ± 1.33 ^b	112.80 ± 6.62 ^a	9.78 ± 0.91 ^a	59.00 ± 5.67 ^a
Stefania	12.74 ± 1.06 ^c	101.34 ± 5.95 ^d	9.39 ± 1.08 ^b	54.88 ± 5.96 ^b
Int TEMP × HYB	*	NS	NS	NS
Int TEMP × TRE	***	NS	NS	NS
Int HYB × TRE	***	NS	NS	NS
Int TEMP × HYB × TRE	NS	NS	NS	NS

Data are averages ± SD of three determinations. Different letters within a column indicate significant differences at the 5% level by Duncan test. n.s. = nonsignificant.

* Significant with $P \leq 0.05$.

** Significant with $P < 0.01$.

*** Significant with $P < 0.001$.

(Putier, 1993; Brouillet-Fourmann et al., 2003; Nayouf et al., 2003). Gelatinization is defined as irreversible breakage of crystalline structure of starch where the surface of each starch molecule becomes susceptible to solvents and reagents; this means that it has the same effect in ethanol production since it enables starch conversion into a form which can receive larger amount of water and allows faster breaking of complex chains in the presence of enzymes (Hauck et al., 1994). Since the objective of this investigation was to improve the kernel quality, prior to drying, kernel was cooked in order to obtain higher starch gelatinization degree without breaking the kernel structure. The investigation showed that with an increase in drying temperature, gelatinization degree also increased, resulting with the highest gelatinization value at temperature of 130 °C. Moreover, further significant differences were obtained in increased gelatinization degree if the kernel was cooked before drying. Accordingly, the highest values were obtained in the samples that underwent cooking treatment of 30 min at steam pressure of 1.5 bars and then were dried at 130 °C. The highest gelatinization degree was found in hybrid Bc 462, whose value was 18.47%, which was significantly higher than in all other studied hybrids. The lowest starch gelatinization degree was found in Bc 4982 hybrid with the mean value of only 10.23%.

Starch hydrolysis up to a low molecule mass product, catalyzed by α -amylase is one of the most important commercial enzymatic processes. Carbohydrates which can be decomposed by hydrolysis into simpler molecules are monosaccharide or reducing sugars, such as sucrose, cellobiose, maltose, lactose, rhamnose, ribose, mannose, arabinose, galactose, isomaltose, xylose and glucose (Ellis et al., 1998).

In this investigation, a somewhat higher value of reducing sugars was obtained among the samples that underwent the cooking treatment. However, if compared to the untreated samples that were only dried, this difference was not significant. A significant difference was obtained in the samples dried at the highest drying temperature in relation to the samples dried at other temperatures. Therefore, significantly the highest reducing sugar quantity was determined in the samples dried at 130 °C no matter if the pre-treatment was applied. Although, the highest value of reducing sugars was found in the samples cooked for 30 min at pressure of 1.5 bars and dried at 130 °C. Similar results were obtained by Arasaratnam et al. (1998) in determination of the reducing sugars content in corn hydrolyzates after starch hydrolysis by α -amylase. Reducing sugar values after hydrolysis in the latter investigation were ranging from 102.6–115.0 g/L, and in this investigation similar results were found. The highest reducing sugar value was found in Florencia hybrid and the lowest in Bc Jumbo 48 hybrid.

Investigation of the effect of hydrothermal treatments on glucose content was also conducted and the highest value was present in the samples that underwent the cooking pre-treatment of 30 min at 1.5 bars and dried at 130 °C. However, the differences were not significant. The highest glucose content, which was significantly above the ones found in other hybrids, was in Florencia hybrid and the lowest in Bc 4982 hybrid.

Since the latter results showed that the level of degradation of starch to glucose in different hydrothermal treatments was almost identical for all hybrids, it was expected that ethanol yields will not differ significantly, and that hydrothermal treatment will give the positive effect. This was also corroborated by investigations, since it was found that ethanol yield was the highest in corn kernel samples cooked for 30 min at 1.5 bars and dried at 130 °C. Moreover, it was determined that cooking treatment significantly affected the ethanol yield, while drying temperature did not show important interaction.

Finally, from the above given data, it can be stated that eventual investment in a facility for cooking treatment of kernels will generate economic benefit through higher ethanol yield. Furthermore,

drying of kernels at 130 °C did not affect the ethanol yield, but it showed a growing tendency in relation to the lower temperatures observed.

Fig. 2 displays chromatograms obtained from different corn hybrids after fermentation. As can be seen, all the peaks shared the same retention profile. The highest ethanol yield was found in Florencia hybrid sample (59.00 g/L). This hybrid had the highest starch (77.41%), reducing sugar (112.80 g/L) and glucose (9.78 g/L) content, and it also proved to be the hybrid that gave the best results when starch hydrolysis and sugar fermentation processes are concerned. The lowest yield was found in Bc 462 hybrid (50.69 g/L), even though this was a hybrid with high starch gelatinization degree and high starch hydrolysis rate in the investigations so far.

4. Conclusion

Two conditions of hydrothermal treatment of corn meal, and its subsequent enzymatic hydrolysis by α -amylase and amyloglucosidase and fermentation by *S. cerevisiae* were studied in this investigation. Several parameters were monitored, namely moisture, starch, glucose, reducing sugar and ethanol content. Moreover, starch gelatinization was determined after the treatment applied.

The results showed that hybrids cooked for 10 min at 0.5 bar had increased moisture content by 3.19%, while those cooked for 30 min at 1.5 bars had increased moisture by 5.25%, which resulted in extended water release time of the hybrids observed. In addition, when certain amount of water was introduced in the kernel by cooking, it resulted in significant increase of activation energy needed to start the kernel water release process.

Since the results showed that starch utilization was considerably higher in the samples steamed before the ethanol production process, it can be concluded that the cooking treatment had a positive effect on ethanol yield from corn kernel. Therefore, the highest ethanol yield was found in the corn kernel samples cooked for 30 min at 1.5 bars and dried at 130 °C. Moreover, it can be concluded that cooking treatment significantly affected ethanol yield, while drying temperature did not have a significant interaction. The highest ethanol yield was found in Florencia hybrid regardless of whether it was treated with cooking procedure. The lowest yield was found in domestic hybrid Bc 462.

Drying of kernels at 130 °C did not affect on the production of ethanol. For this reason, and for the purpose of shortening the kernel drying period for the purpose of ethanol production, kernel can be dried at high temperatures without affecting the further hydrolysis, fermentation and ethanol production processes.

References

- American Association of Cereal Chemists, 2000. Approved Methods of the AACC, tenth ed. Method 80–68. The Association: St. Paul, MN.
- AOAC, 1990. Official Methods of Analysis, fifteenth ed. Association of Official Analytical Chemists, Washington DC.
- Arasaratnam, V., Thayanathan, K., Kokuvil, K.B., 1998. Sugar syrup from corn flour. *Starch* 50 (2–3), 95–98.
- Bala, B.K., 1997. *Drying and Storage of Cereal Grains*. Science Publisher, New Hampshire, SAD.
- Birch, G.G., Priestley, R.J., 1973. Degree of starch gelatinization of cooked rice. *Starch* 25, 98–100.
- Brouillet-Fourmann, S., Carrot, C., Mignard, N., 2003. Gelatinization and gelatinization of corn starch followed by dynamic mechanical spectroscopy analysis. *Rheol. Acta* 42 (1–2), 110–117.
- Cai, W., Diosady, L.L., 1993. Model of gelatinization of wheat starch in a twin-screw extruder. *J. Food Sci.* 68 (5), 873–876.
- de Oliveria, M.E.D., Vaughan, B.E., Rykiel Jr., E.J., 2005. Ethanol as fuel: energy, carbon dioxide balances, and ecological footprint. *BioScience* 55, 593–602.
- Dien, B.S., Cotta, M.A., Jeffries, T.W., 2003. Bacteria engineered for fuel ethanol production: current status. *Appl. Microbiol. Biotechnol.* 63, 258–266.
- Ellis, R.P., Cochrane, M.P., Dale, M.F.B., Duffus, C.M., Lynn, A., Morrison, I.M., Prentice, R.D.M., Swanston, J.S., Tiller, S.A., 1998. Starch production and industrial use. *J. Sci. Food Agric.* 77, 289–311.

- Hansen, A.C., Zhang, Q., Lyne, P.W.L., 2005. Ethanol – diesel fuel blends – a review. *Bioresource Technol.* 96, 277–285.
- Hauck, B., Rokey, G., Smith, O., Herbester, J., Sunderland, R., 1994. Extrusion Cooking System, Feed Manufacturing Technology. American Feed Industry Association Inc., Arlington, SAD.
- Henderson, S.M., Pabis, S., 1961. Grain drying theory: I temperature effect on drying coefficient. *J. Agr. Eng. Res.* 6 (3), 169–174.
- ISO 10520, 1997. Native starch – determination of starch content – ewers polarimetric method.
- ISO 712, 1998. Cereals and cereal products – determination of moisture content – routine reference method.
- Larsson, J.A., 2006. Cost benefit analysis of bioethanol production from cereals in Sweden – a case study approach. MSc Thesis, Cranfield University.
- Lin, Y., Tanaka, S., 2006. Ethanol fermentation from biomass resources: current state and prospects. *Appl. Microbiol. Biotechnol.* 69, 627–642.
- Lombardi, L., 2003. Life cycle assessment comparison of technical solutions for CO₂ emissions reduction in power generation. *Energy Convers. Manage.* 44, 93–108.
- Malça, J., Freire, F., 2006. Renewability and life-cycle energy efficiency bioethanol and bio-ethyl tertiary butyl ether (bioETBE): the implications of allocation. *Energy* 31, 3362–3380.
- Mohsenin, H.J., 1970. *Physical Properties of Plant and Animal Materials*. Gordon & Breach Science Publisher, New York, SAD.
- Mojovic, L.J., Nikolic, S., Rakin, M., Vukasinovic, M., 2006. Production of bioethanol from corn meal hydrolyzates. *Fuel* 85, 1750–1755.
- Mujumdar, A., 2000. *Drying Technology in Agriculture and Food Science*. Science Publisher, Plymouth, UK.
- Nayouf, M., Loisel, C., Doublier, J.L., 2003. Effect of thermomechanical treatment on the rheological properties of crosslinked waxy corn starch. *J. Food Eng.* 59 (2–3), 209–219.
- Putier, F., 1993. Product quality and thermal treatment, Feed mix. 1(2). The American Association of Cereal Chemists Inc., St. Paul Minnesota, USA.
- Renewable Fuels Association (RFA), 2007. Ethanol industry statistics, Washington, DC, USA, [cited; available from: <www.ethanolrfa.org>].
- Sagol, S., Turhan, M., Sayar, S., 2006. A potential method for determining in situ gelatinization temperature of starch using initial water transfer rate in whole cereals. *J. Food Eng.* 76, 427–432.
- SAS Institute, 1997. SAS/STAT Software: Changes and Enhancements Through Rel. 6.12. Sas Inst., Cary, NC, USA.
- Stroshine, R., Tuite, J., Kirleis, A., Baumann, L., Emam, A., 1986. Differences in grain quality among selected corn hybrids. *Cereal Foods World* 31 (4), 311–316.
- Yoosin, S., Sorapipatana, C.A., 2007. Study of ethanol production cost for gasoline substitution in Thailand and its competitiveness. *Thammasat Int. J. Sci. Technol.* 12, 69–80.
- Whittington, T., 2006. Ethanol: International Overview of Production and Policies Department of Agriculture and Food, Government of Western Australia, [cited; available from <<http://www.agric.wa.gov.au/biofuels>>].