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Fuel Ethanol from Raw Corn

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ABSTRACT

CRUDE amylase preparations were produced by growing Aspergillus awamori and A. niger on raw, ground, whole corn. These Koji preparations were used to hydrolyze the starch of raw, ground whole corn to sugars during simultaneous fermentation of the sugars to ethanol by distillers active dry yeast. Ethanol concentrations of the fermentation beers were determined with gas-chromatography. These fermentations yielded an average of 89.6% theoretical ethanol compared to control, conventional, fermentations that had an average of 89.9%. Carbon dioxide evolutions were determined with use of Alwood valves. The initial fermentation rate was greater for the conventional fermentation than the Koji fermentation. However, both fermentations produced 0.48 g of carbon dioxide per gram of dry substrate starch within 72 h. Koji dehydrated at 41°C had no apparent detrimental effects on theoretical ethanol yield.

INTRODUCTION

The United States and its agricultural community are heavily dependent upon liquid fuels derived from petroleum (Hall, 1981). The magnitude of this dependence was clearly demonstrated in the early 1970's when oil prices increased dramatically and the abundant supply of fuels dwindled (Doering, 1981). People, particularly farmers, realized they needed to develop dependable sources of liquid fuels. Ethanol is an attractive alternative fuel because it can be synthesized from ethylene, by fermentation and as a by-product of butanol and glycerol fermentations. The fermentation production of ethanol accounted for only 10% of all ethanol produced in the United States in 1975; this increased to 30% in 1980 and is still increasing (Sherman and Kavasmaneck, 1980; and Eveleigh, 1981). Interest in alternative fuels swept across this country almost immediately. People, particularly farmers, realized they needed to develop dependable sources of liquid fuels. Ethanol is an attractive alternative fuel because it can be produced from dependable and renewable resources, farm-grown feedstocks such as corn.

However, continued efforts to develop more efficient technology for the production of such liquid fuel from corn are needed. Presently, ethanol is produced by synthesis from ethylene, by fermentation and as a by-product of butanol and glycerol fermentations. The fermentation production of ethanol accounted for only 10% of all ethanol produced in the United States in 1975; this increased to 30% in 1980 and is still increasing (Sherman and Kavasmaneck, 1980; and Eveleigh, 1981). Most of the increase is the result of production by such wet-milling industry giants as Archer-Daniels-Midland Co., A. E. Staley Manufacturing Co. and CPC International Inc. (with Texaco Inc.). These companies normally produce corn starch from corn and then hydrolyze the starch to glucose which can be quite readily converted to ethanol. The hydrolysis, fermentation and distillation processes consume large amounts of energy. Cooking to gelatinize the starch prior to enzymatic hydrolysis consumes approximately 17% of this energy (Garcia et al., 1982). Thus, cooking energy contributes to the large processing energy demand that has brought the economic feasibility of on-farm or small-scale ethanol production into question. Recent studies have shown on-farm production of ethanol to be an exceedingly high-cost operation (Locke, 1982).

Efforts to reduce the energy costs in ethanol production, primarily cooking costs, date back to the late nineteenth century, when starch components were just beginning to be defined. Methods for hydrolyzing raw starch were being studied. In one method, malt-extract was added to ungelatinized, triturated corn and wheat to produce 2 to 25% glucose and 10 to 14% dextrins within 20 hours (Brown and Heron, 1879). Blish and his co-workers (1937) published their observations of the actions of wheat amylases on raw wheat starch; they claimed normal wheat flour contained an enzyme factor, not alpha-amylase, that accelerated the saccharification of raw untreated wheat starch. Support for this observation was obtained by others (Sandstedt and Gates, 1954; and Walker and Hope, 1963).

Balls and Schwimmer (1944) reported that ungelatinized corn, wheat and potato starches could be completely hydrolyzed by a mixture of extracts of hog pancreas and A. oryzae Koji grown on wheat bran. Koji (Milall, 1975) of this type had been first introduced into the United States by Takamine (1914) and was confirmed by Underkofler et al. (1939) as being the Aspergillus with the greatest amylolytic activity that could feasibly be produced commercially. Schwimmer (1945) postulated that this Koji contained a complimentary non-amylolytic factor that caused the complete and rapid digestion of raw starch. In 1948, Corman and Langlykke added to the understanding of the Koji amylases when they discovered a fraction of the amylases (later named glucoamylase) which was highly glucogenic. Further studies of Koji amylases by Yamazaki and Ueda (1951) revealed that Black-Koji (A. niger and A. awamori) amylases had stronger raw starch hydrolyzing activity than those of Yellow-Koji (A. oryzae) amylases and malt amylases. They also reconfirmed the earlier findings of Balls and Schwimmer (1944) that raw corn starch was more readily digested than raw wheat or potato starches. Ueda (1958a) reported that the Koji amylases from A. awamori contained two fractions but only one of which had raw starch digestive activity.

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Glucoamylases in Koji amylases of both *A. niger* and *A. awamori* were shown to be of two fractions (Pazur and Ando, 1959 and Watanabe and Fukimbara, 1965). Smiley et al. (1971) tried to determine the reason for the existence of these two fractions but were unable to come to any conclusions. Ueda et al. (1974) later confirmed the presence of two glucoamylases. They reported one of the glucoamylase fractions was highly active in raw starch digestion. This fraction likely had given the Koji amylase fraction which Ueda discovered in 1958 its raw starch degrading activity.

Based on the discovery of raw starch degrading capability, Ueda and Koba (1980) developed a continuous fermentation system that used Aspergillus glucoamylase and Psuedomonas isoamylase to hydrolyze raw corn starch. Their system produced 7% (v/v) ethanol in three days, but required periodic removal of inhibitory substances. Another continuous raw corn starch fermentation system was reported by Lutzen (1980) but it required 40% starch substrate slurries and a large amount of handling to obtain 10% (w/w) yields of ethanol. Additionally, Ueda et al. (1981) developed a simultaneous hydrolysis and fermentation process for raw, ground, whole cassava that used a dual culture of *A. awamori* and *A. niger* grown on sterile wheat bran to produce glucoamylases for hydrolysis of the raw, ground, whole cassava. They fermented the raw cassava to ethanol by simultaneous hydrolysis of starch and fermentation of sugars to ethanol. Their yields of ethanol ranged between 82.3 and 99.6% of theoretical based on starch content of the cassava. Further studies reported 89.0 to 90.0% theoretical ethanol yields from both raw corn and cassava starch (Park and Rivera, 1982). A recent simultaneous hydrolysis using amylases from Rhizopus and fermentation of raw, ground corn was developed by Matsumoto et al. (1982). They reported 88.3% theoretical ethanol yields with ethanol concentrations of 14.2% (v/v) in the beer. Also in 1982, Hayashida et al. hydrolyzed raw, ground corn using a liquid Koji (submerged culture) from *A. awamori* in conjunction with fermentation by an ethanol tolerant mutant of Sake yeast to obtain an ethanol concentration of 20.1% (v/v) in 5 days.

Previous work by Ueda et al. (1981) with a Koji amylase system acting on raw cassava showed some promise. As a result, the approach of this research was patterned after theirs with two modifications; (a) raw, ground whole corn was substituted for sterile wheat bran as the Koji substrate since it is cheaper and more readily available than wheat bran and (b) raw, ground whole corn as typically used for animal feeding was taken as fermentation substrate. Innovative use of raw, ground whole corn would help reduce production costs since (a) such technology could easily be adapted to small-scale ethanol production, (b) available equipment could be used for corn preparation and (c) management time and costs would be the same or less than in conventional production of ethanol.

Dehydration of raw corn Koji was also studied to see what effect this would have on ethanol yields. Dehydration of the Koji without losing amylase activity would allow for its large-scale production in one centralized location and distribution for use at other locations. Time, labor and operating costs at the fermentation site would be reduced by use of a dry Koji. Objectives of this work were to produce Koji on raw, ground whole corn; to test this Koji for ethanol yields in a simultaneous hydrolysis-fermentation of raw, ground whole corn; to evaluate procedures for Koji preservation. The overall objective was to develop a process for producing ethanol from corn in small-scale plants with reduced energy input and high yields.

**PROCEDURES**

**Koji Preparation**

Crude amylases used for hydrolysis in Experiment I comparing Koji fermentation ethanol yields with those of conventional fermentation were produced by a dual culture Koji of *A. niger* (isolated from spoiled bread) and *A. Awamori* (NRRL 3112) grown on raw, ground, whole Yellow No. 2 corn obtained from the University of Illinois farm feedmill. Five grams of corn (13.5% moisture), ground with a 1.27 cm hammermill, were inoculated with a 5 mL 0.1% Triton X-100 suspension containing 10^7 to 10^8 spores of *A. niger* and a similar suspension of *A. awamori* for each fermentation. The spore suspensions were well mixed with the corn which was spread in thin layers (< 5 mm) on Pyrex baking dishes. These dishes were then covered with aluminum foil to retain moisture and incubated for 72 h at 25°C in a controlled temperature chamber.

Four Kojis were prepared as above for Experiment II on Koji preservation. The control was given no further treatment. For the second, Koji was dehydrated at room temperature for 18 h in an evacuated desiccator containing activated alumina. For the third, Koji was placed in a covered dish and heated for 18 h in a forced air oven (Blue M Electric Co., Blue Island, Illinois) at 41°C. For the fourth, Koji was placed in an open dish and simultaneously heated and dehydrated for 18 h in the forced air oven at 41°C.

**Koji Hydrolysis and Fermentation**

All Koji hydrolyses and fermentations took place in 500 mL Erlenmeyer flasks and followed a standardized procedure. This procedure was developed to better simulate field conditions of a small-scale ethanol production facility were reduced labor and management would likely take greater priority than maintaining optimum operational parameters. Ingredients for each trial were 45 g of 13.5% moisture raw, ground whole corn, 15 g of Koji, 200 mL of tap water, 10 drops of 85% phosphoric acid to adjust pH to the range of 4.0 to 4.2 and 0.05 g of distilled active dry yeast (Universal Foods, Inc., Milwaukee, Wisconsin). All ingredients were added at once and mixed well. Starch concentrations, including starch present in the Koji prior to propagation, were held at 13 to 14% (w/w) to facilitate wetting the Koji mat which forms during the Koji incubation. Mixing, provided by a magnetic stirring bar was continued at intervals of one minute every 15 min. The incubations were carried out for 72 h in a controlled environment cabinet at 32°C.

**Conventional Fermentation**

Conventional fermentations using heat-gelatinized starch took place in 500 mL Erlenmeyer flasks and followed a representative small-scale ethanol production
process (Weller, 1983). Initially, 45 g of 13.5% moisture raw ground whole corn, 85 mL of tap water, 0.03 mL of alpha-amylase, Termamyl 50L (Novo Laboratories, Wilton, Connecticut) and one drop of concentrated ammonium hydroxide were mixed together in the flasks. The flasks were heated to 85°C and held for two hours with constant stirring. They were then cooled to 60°C by adding 45 mL of tap water and bathing the flask in a controlled environment chamber.

**Carbon Dioxide Determination**

The metabolic pathway that produces ethanol also produces carbon dioxide on an equimolar basis. Therefore, carbon dioxide production was recorded during the incubation period to provide an estimate of the ethanol production rate. Alwood valves (Rascher and Betzold, Inc., Chicago, IL) containing concentrated sulfuric acid were used to trap moisture from the evolved carbon dioxide stream. Weight of the flask-valve assembly was recorded over time; loss in weight was taken as carbon dioxide evolution.

**Ethanol Determination**

Ethanol concentrations of the beers were determined using a Kemmerer-Hallett lab-scale steam distillation system and a Varian 3700 gas-chromatograph (Varian Associates, Inc., Palo Alto, CA) interfaced with a Hewlett-Packard 3390A Integrator (Avondale, PA) and equipped with a Flame Ionization Detector (FID). Representative 25 mL samples of each beer were first distilled to separate the ethanol from solubles and suspended solids. Then 0.5 μL of 1:10 dilutions of the beers were injected into the 80/120 Carbopack B/5% Carbowax 20M gas-chromatograph column (Suppleco, Inc., Bellafonte, PA) for comparison with 1% (v/v) ethanol external standard solutions. The column temperature was 135°C with a carrier gas (helium) flow of 20 mL/min. The injector temperature was 150°C and the FID temperature was 250°C. Air and hydrogen were used as detector gases.

**RESULTS AND DISCUSSION**

**Koji on Corn**

Suitability of raw corn for Koji enzyme development and as a fermentation substrate was determined by comparing theoretical yields of experimental fermentations to those from conventional fermentations. Conventional fermentations used the same corn fermentation substrate as used in the Koji hydrolyses and as a fermentation substrate was determined by comparing theoretical yields of experimental fermentations to those from conventional fermentations. Theoretical yields for the Koji and conventional fermentations were based on the ethanol obtainable if 100% of the starch present in the mash was used (see Appendix).

**Ethanol Yields:** Two control trials of conventional fermentation were performed. Theoretical yields (Table 1) were 89.7 and 90.1%, for an average of 89.9%. These yields were slightly less than the 90.0 to 94.0% expected by industry (Paturau, 1982 and Schopmeyer, 1982); but were consistent with those reported by Yang et al. (1982) and Garcia et al. (1982). However, the use of a standard literature value for the amount of starch in the corn, 70% (dry solids basis) (Inglett, 1970), may have affected the theoretical yields. Another major factor that undoubtedly decreased yields below the industry

### TABLE 1. THEORETICAL AND OBSERVED ETHANOL CONCENTRATIONS AND THEORETICAL ETHANOL YIELD FOR ALL FERMENTATIONS

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>Theoretical</th>
<th>Theoretical</th>
<th>Observed</th>
<th>Observed</th>
<th>Theoretical</th>
<th>Theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ethanol, g</td>
<td>ethanol, %</td>
<td>ethanol, g</td>
<td>ethanol, %</td>
<td>yield, %</td>
<td>yield, %</td>
</tr>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>8.10</td>
<td>15.30</td>
<td>13.72</td>
<td>89.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>8.43</td>
<td>15.30</td>
<td>13.78</td>
<td>90.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw ground</td>
<td>8.67</td>
<td>18.64</td>
<td>16.40</td>
<td>88.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>whole corn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koji 1</td>
<td>8.79</td>
<td>18.84</td>
<td>16.75</td>
<td>88.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koji 2</td>
<td>8.77</td>
<td>18.78</td>
<td>16.94</td>
<td>90.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koji 3</td>
<td>8.89</td>
<td>18.75</td>
<td>17.09</td>
<td>91.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koji 4</td>
<td>8.89</td>
<td>18.75</td>
<td>17.09</td>
<td>91.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Koji</td>
<td>8.63</td>
<td>19.62</td>
<td>16.58</td>
<td>84.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Koji</td>
<td>8.83</td>
<td>19.78</td>
<td>16.85</td>
<td>85.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room temperature</td>
<td>8.54</td>
<td>19.36</td>
<td>15.62</td>
<td>80.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dehydrated Koji 1</td>
<td>8.67</td>
<td>19.47</td>
<td>15.78</td>
<td>81.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room temperature</td>
<td>8.67</td>
<td>19.47</td>
<td>15.78</td>
<td>81.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41°C dehydrated</td>
<td>8.28</td>
<td>19.49</td>
<td>16.10</td>
<td>82.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koji 1</td>
<td>8.48</td>
<td>19.36</td>
<td>16.49</td>
<td>85.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41°C heated</td>
<td>8.77</td>
<td>19.78</td>
<td>16.80</td>
<td>84.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koji 2</td>
<td>8.60</td>
<td>19.60</td>
<td>16.94</td>
<td>86.4</td>
<td></td>
<td></td>
</tr>
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</table>
standard was the size of the corn particles used as substrate. The corn particles varied in size as can be seen in Table 2. The larger particles, with smaller surface to volume ratios, resulted in reduced yields due to incomplete hydrolysis. The germ of the kernel, in some instances, was still attached to endosperm prior to fermentation and this reduced the surface area for amylase activity. Furthermore, yeast growth and metabolism and the production of glycerol and fusel oils, ethanol fermentation by-products as noted by Yang et al. (1982), may also have affected the yields.

Four replicate trials using raw corn for both Koji preparation and hydrolysis resulted in 88.0, 88.9, 90.2, and 91.2% theoretical yields (Table 1) for an average of 89.6%. Analysis of variance indicated the Koji fermentation average yield is not significantly different from the 89.9% average yield for conventional fermentation at the 5% level of probability. The Koji fermentations were affected not only by the same factors as conventional fermentation but also by assimilation of carbon from the starch by the Aspergilli for cell growth and metabolism, so ultimately less sugar was available for conversion to ethanol and carbon dioxide. Furthermore, the reduced yield may have been caused by a lower yeast inoculum; the Koji fermentations contained a lower yeast inoculum; the Koji fermentations contained additional starch from the corn used to propagate the mold.

Another factor that may have affected the fermentations is the initial level of glucose in each mash at the time of yeast inoculation. Conventional fermentation started hydrolysis 3 h prior to yeast addition whereas Koji fermentation simultaneously began hydrolysis and fermentation; thereby allowing initial glucose levels in conventional fermentation to be greater than those in Koji fermentation.

Nevertheless, no definite conclusions can be drawn to clearly account for the rate difference. Opportunities for further research abound and would most likely center on the aforementioned factors.

Koji Preservation

Criteria for evaluation of the four Koji preservation methods were the theoretical ethanol yields from subsequent fermentations. Holding time for each respective preservation treatment was 18 h with the Koji used immediately thereafter. Moisture variations between the dehydrated and undehydrated Koji were compensated for by adding additional water during initial mixing prior to hydrolysis and fermentation.

Theoretical ethanol yields from Kojis dehydrated at room temperature and at 41% were compared with those from fermentations using a heated but not dehydrated Koji and a control (unheated and undehydrated) Koji (Table 1). The heated, undehydrated Koji was included to determine whether heating alone affected ethanol yields. Two replicates using room temperature dehydrated Koji gave an average yield of 80.9% while replicates using Koji dehydrated at 40°C averaged 83.9%. The undehydrated Koji heated to 41°C yielded
an average of 85.7% theoretical ethanol and the control yielded 84.9%.

Analysis of variance indicated that the room temperature dehydrated Koji gave a yield that was significantly lower than the others at the 5% level of probability. This yield was about 81% as compared to an average of approximately 85% for the other three. However, all four values were lower than those for the conventional control which averaged almost 90% yield (Table 1); it should be noted that incubation temperature dropped below 25°C for the four preservation trials and this might have caused the reduced yields. However, the important point is that Koji dehydration at 41°C gave results statistically equivalent to the untreated Koji within the same experiment; the yield was reduced only one percent. Storage studies should be made to test for shelf life of this dehydrated Koji.

GENERAL IMPLICATIONS

The overall objective was to develop a process for fermenting the starch in raw corn, so that the cooking step presently used could be eliminated to save energy. In addition to reducing energy requirements and maintaining high ethanol yields, the new process may produce high-value by-products not available from present conventional small-scale processes. Thus, stillage presently obtained from conventional fermentation of cooked corn represents a disposal problem. It can be dried at tremendous energy cost by evaporating nine kg of water to obtain one kg of solids for sale as a feed ingredient (Solar Energy Research Institute, 1982), or it can be fed wet, but this would require a large animal feeding operation. For instance, a plant with an annual capacity of 308,890 L of anhydrous ethanol would require a feedlot holding at least 76 head of cattle (Day and Chen, 1980). A waste disposal plant would be as large as the fermentation-distillation operation. Disposal on the land is also costly.

By the new process, the beer contains raw gluten and germ in addition to ethanol. If separated, the by-products could be of the same quantity and quality as obtained by corn wet-milling which results in three major products, crude corn oil, corn gluten feed (21% protein) and corn gluten meal (60% protein) (Corn Refiners Association, Inc. 1982). Representative by-product values, based on prices for corn wet-milling products on three dates along with corn feedstock prices on those dates, are presented in Table 3. However, before the values can be realized, further investigations must be made. Included are methods for separating the individual by-product components from the beer, the effects of fermentation upon the nutritional value of by-products and the economics of any proposed by-product recovery system for small-scale ethanol operations.

SUMMARY AND CONCLUSIONS

This study shows that raw corn can be used to produce ethanol at reduced energy and other costs with yields comparable to the conventional process. This new approach should enable high quality protein and oil recovery.

Raw whole corn ground in a hammermill typical of those on farms can be used as the substrate for production of enzymes (Koji) as well as the fermentation to obtain an average 89.6% of theoretical ethanol yield. Success with using corn of this grind indicated that it may readily be used in both large- and small-scale production operations with a limited investment in grinding equipment.

Gelatinization of raw ground whole corn was not necessary prior to hydrolysis by Koji fermentations. The amylases produced by the Koji were able to degrade the raw starch to fermentable sugars. Elimination of the cooking step lowers energy inputs of ethanol production and eliminates the need for cookers. Thus, operating costs and capital costs can be reduced for both large- and small-scale production facilities when compared to present operations.

All the ingredients were combined in one vessel so fermentation occurred simultaneously with the hydrolysis. Therefore, as soon as sugars were formed, the yeast utilized them for ethanol production. Combining all of these processes into one vessel reduces management. Thus, costs for time, labor and management can be reduced for both large- and small-scale production facilities over those for existing operations.

Carbon dioxide production values indicated that ethanol production for the Koji fermentations were not initially as rapid as for the conventional fermentations. However, both processes eventually produced the same amount of carbon dioxide within 72 hours.

Raw corn Koji can be preserved by dehydration at 41°C without any apparent detrimental effects. Nevertheless, shelf-life studies are needed to determine stability of the crude amylases.

References


![Table 3. Cash Market Prices for Corn and Corn Wet-Milling By-Products and Total Value of By-Products Based on a Cubic Meter of Corn Feedstock](image)

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Sample Calculation of Theoretical Ethanol Yield

Starch wt = [(45.3 g wet fermentation substrate corn x (1.0-0.135) g dry corn/g wet corn) + (15.0 g wet Koji x (1.0-0.43)) g dry Koji/g wet Koji] x 0.70 g starch/g dry corn = 33.42 g starch

Theoretical ethanol wt = 33.42 g starch x 1.1 g dextrose/g starch x 0.51 g ethanol/g dextrose = 18.75 g

Theoretical yield = (17.09 g observed ethanol/18.75 g theoretical ethanol) x 100% = 91.2%