0273-2289/96/5758—0039$7.25

Enzymatic Hydrolysis of Corn Starch After Extraction of Corn Oil with Ethanol

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ABSTRACT

Simultaneous corn oil extraction and alcohol dehydration, a solvent corn-milling process developed in our laboratory, was tested on a pilot scale to recover corn oil. Over 92% of the corn oil was extracted by 95% ethanol with a liquid-to-solid ratio of 0.75 at 65°C. Following the oil extraction, 95% of zein fraction was also extracted from the defatted ground corn by 65% ethanol at 65°C with a liquid-to-solid ratio of 1.0 to produce extracted ground corn. After corn oil and zein extraction by ethanol, the ability of the amylases to hydrolyze the starch fraction in extracted ground corn was studied. Results showed the starch fraction can be hydrolyzed to glucose by α-amylase and glucoamylase without separatio of fiber and residual protein. The glucose yield in hydrolysis was a function of temperature, enzyme concentration, and solid-to-liquid ratio. With a two-step heating hydrolysis, corn starch was converted to glucose with a 97.2% yield from extracted ground corn. After filtration and washing of the hydrolyzed mass, the final glucose concentration was 24.3% glucose (w/v).

Index Entries: Corn starch; enzymatic hydrolysis; oil extraction; anhydrous ethanol; corn oil.

INTRODUCTION

Glucose is the largest end use of corn starch, which can serve as a feedstock for microbial fermentation into industrial solvents and fuel ethanol *(1)*. Generally, corn is processed by either wet milling or dry milling to produce corn oil, corn starch, and corn meal to facilitate the processing of starch by hydrolysis *(2)*.

Wet milling has been the preferred method for recovering starch from corn for many years. It has experienced enormous growth since the 1970s because of the increased demand for starch as raw material for the production of high-fructose corn syrup as a sweetener and ethanol as an alternative fuel source. Because current wet-milling methods require considerable amounts of capital investment and the process involves high energy consumption, including the steeping and separation of starch from protein processes, many researchers have been searching for new low-cost approaches for fractionating corn to produce novel byproducts with higher values than those obtained by current milling processes *(3)*.

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In an effort to develop an energy-efficient alternative method to wet milling, a solvent corn milling process that simultaneously carries out oil extraction and alcohol dehydration has been developed *(4–8)*. This process has the following advantages over the current wet-milling methods:

1. Elimination of the time-consuming steps that adversely affect the functional and edible properties of the protein. As a result, a food-grade protein concentrate is produced that is expected to have a higher nutritional value than gluten meal because the germ proteins are also extracted;
2. Replacement of the ternary distillation for dehydration of asotropic ethanol with a simple water-absorption step with dried corn as the adsorbent; and
3. Realization of a higher oil yield than the wet-milling process.

In order to illustrate the large-scale feasibility of the new process, the objective of this project is to complete a series of experiments to study starch hydrolysis after extraction of corn oil and protein by ethanol. Since the use of enzymes has several advantages over acid hydrolysis of starch, such as fewer byproducts and higher yield *(9)*, we have chosen enzymatic hydrolysis as the starch hydrolysis method.

MATERIALS AND METHODS

Materials

Ground No. 2 yellow dent corn was obtained from a local feed station. Anhydrous and 95% ethanol were purchased from McCormick Distilling Co., Inc. (Weston, MO). Thermostable α-amylase (EC 3.2.1.1, Termamyl 120L) and glucoamylase (EC 3.2.1.3, AMG 200L) were generously provided by Novo Nordisk Bioindustrials, Inc. (Danbury, CT); cellulase TAP was a gift of International Enzyme Co., Inc. (Fallbrook, CA).

Methods

Characterization of Ground Corn

For the determination of the particle size distribution, dried corn was fractionated on a sieve shaker (Ro-tap, Model B, Tyler Industrial Products) and shaken for 10 min. The retained corn on each sieve was weighed. The moisture content of the ground corn was determined by drying in an air oven at 135°C for 2 h (AACC 1983). The total oil content of the ground corn was determined in accordance with a standard procedure (AACC 1983).

Pilot Plant Oil Extraction

The pilot-scale experiments of oil extraction were carried out in a countcurrent extractor (Crown Iron Work, Minneapolis, MN) by 95% ethanol at 65°C and 0.75 of liquid-to-solid ratio for 30–45 min retention time. Extraction residue was desolventized by Down Draft Desolventizer at 80°C to produce a defatted dried ground corn containing 4% moisture. Oil–ethanol solution was distilled to yield crude corn oil and anhydrous ethanol. Zein protein was then extracted from defatted ground corn by 65% ethanol at 65°C at an equal ratio of solid to liquid with a residence time of 30 min in the column (6 × 80 cm).

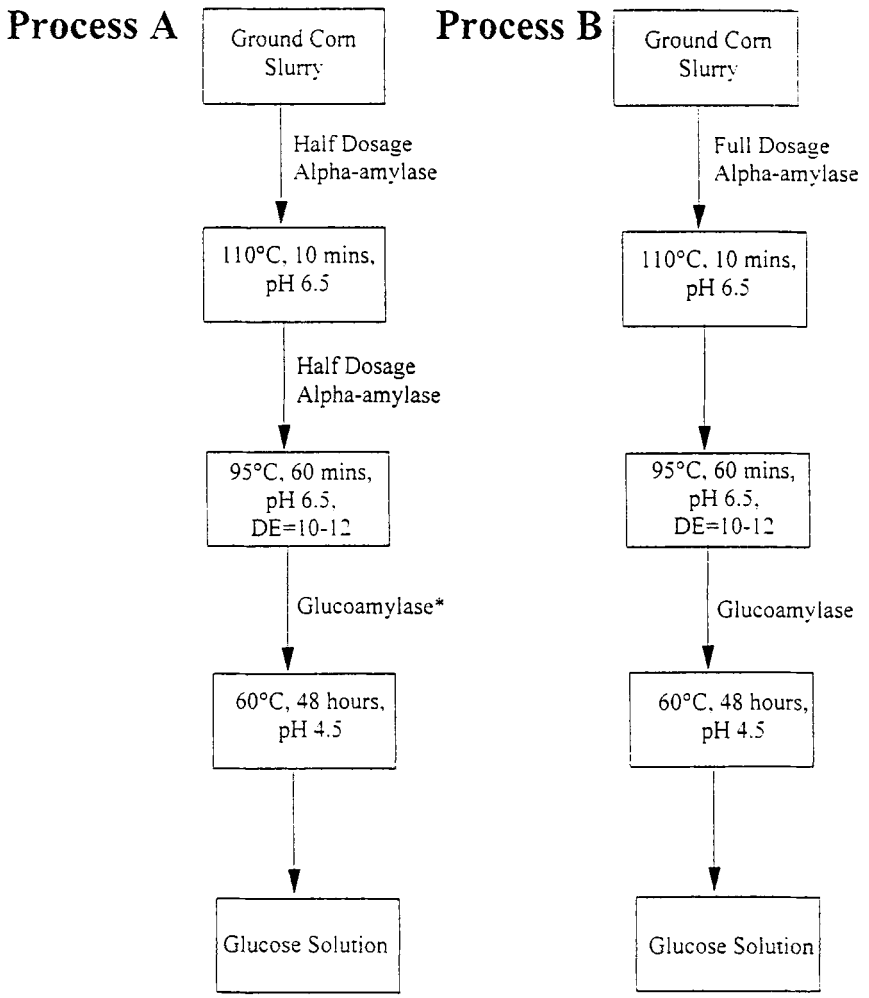

Fig. 1. Processes of defatted ground corn hydrolysis. (*) In process C an amount of cellulose was added with glucoamylase.

Gelatinization Temperature of Starch

Corn starch was prepared from blending extracted ground corn with water in a Waring Blender for 5 min with the blade turned upside down. The slurry was centrifuged in a gradually increased gravitational field. All centrifuged starch samples were combined. Corn starch (0.2%) was dispersed in a water and ethanol solution ranging from 0–60% of ethanol (v/v). Samples were determined according to the microscopic hot-stage technique described by MacMasters and Chien *(10,11)*.

Starch Hydrolysis

Slurry was prepared by mixing the extracted ground corn with 100 ppm calcium chloride aqueous solution (pH 6.5) at different liquid-to-solid ratios *(12)*. Different dosages of α-amylase were added in different processes (Fig. 1). The slurry was heated to 110°C and kept for 10 min in an autoclave. After flash cooling, the slurry was kept at 95°C until no blue color reaction occurred with iodine

Table 1
Composition of Ground Corn, dry base, g/100 g

Main component	Whole corn	Extracted ground corn
Lipids	4.9	0.35
Proteins	10.3	4.15
Starch and sugars	74.6	84.2
Fiber	10.2	11.27

solution. After liquefaction, the pH of the slurry was adjusted to pH 4.5 using 2.0*N* HCl solution. The saccharification step was completed by adding 4 AGU of glucoamylase (AMG 200L)/g corn starch in processes A and B, or 4 AGU of glucoamylase (AMG 200L)/g corn starch and 5 U of cellulase (TAP)/g corn starch in process C. The reactions were kept at 60°C for 48 h. The slurry was filtered and washed to collect glucose hydrolysate. Glucose concentration was determined by high-performance liquid chromatography (HPLC) methods (Aminex HPX-87C column, Bio-Rad Laboratories, Hercules, CA), and dextrose equivalent (DE) value was calculated on a dry wt basis by determining the relative reducing power of the liquefact with anhydrous dextrose given the value 100.

Enzyme Activity

α-Amylase activity was determined by the Novo analytical method AF-9. One KNU was the amount of enzyme that breaks down 5.26 g starch/h at pH 5.6 at 37°C *(13)*. Glucoamylase activity was determined by the Novo analytical method AF-22. One AGU was defined as the amount of enzyme that hydrolyzes 1 μmol maltose/min at pH 4.3 at 25°C *(13)*. One cellulase unit was defined as that amount of activity liberating 1.0 μmol of glucose from α-cellulose in 1 h at pH 5.0 at 37°C.

RESULTS AND DISCUSSION

Pilot Plant Oil Extraction

In this pilot-scale testing, over 92% of the corn oil was recovered, which was about 15–17% over the oil yield of a typical wet-milling process *(12)*. When the cracked corn was dried to about 1% moisture, each kilogram of the cracked corn was able to absorb water in 95% ethanol to produce 500 mL 99.6% (w/w) ethanol. Table 1 shows the composition change of ground corn before and after extraction. Compared with industrial corn starch containing 0.65% of fat, the extraction residue of the current method contained only 0.35% of fat. Furthermore, with 95% of zein protein removal, the residue after desolventization exhibited a very loose structure that facilitated corn starch hydrolysis without the need for further separation of starch and fiber. A glucose yield of 95% was obtained by enzymatic hydrolysis of defatted ground corn.

Gelatinization and Liquefaction

Extracted ground corn was subjected to enzymatic hydrolysis to study the effect of ethanol treatment on starch hydrolysis. Starch gelatinization is a precondition of efficient hydrolysis. Once the starch has been gelatinized, the molecule must be

Table 2
Effect of Ethanol Treatment
on Initial and Final Gelatinization Temperature of Corn Starch

	Gelatinization temperature, °C[a]			
	Sample 1		Sample 2	
% EtOH, v/v	Initial	Final	Initial	Final
0	59.3	66.0	61.0	68.0
20	59.3	66.1	61.0	68.0
40	68.0	75.5	70.0	77.5
45	70.5	78.8	68.0	77.0
50	76.4	81.0	78.5	>81.0
60	>81.0	>85.0	>81.0	>85.0

[a]Sample 1, the undefatted ground corn; sample 2, extracted ground corn; deviation of temperature = ±0.5°C.

rapidly hydrolyzed or retrogradation will occur, particularly if the substrate is cooled during liquefaction. On cooling, the starch chains reassociate with each other, with the linear outer branches of amylopectin, or with lipids to reform the lipid complex, forming semicrystalline precipitate, which is highly resistant to hydrolysis, leading to filtration problems as well as loss of yield *(2)*. Table 2 shows the initial and final gelatinization temperatures of extracted ground corn. This indicates that gelatinization of starch granules can be affected by high concentration of ethanol and the gelatinization does not occur during the extraction and desolventization processes.

The liquefaction of extracted ground corn is a function of α-amylase concentration. The progress of liquefaction can be monitored by measuring the DE value of the slurry and its ability to develop a blue color with iodine. Increasing enzyme concentration can accelerate the liquefaction rate in the same condition (Fig. 2, process A). Comparing the effect of three different enzyme concentrations, the DE values for two lower enzyme dosages were approximately linear with time in the first hour at 95°C. When the enzyme dosage was over 0.8 KNU/g ground corn, which was slightly higher than the amount used in the commercial practice of 0.7 KNU/g *(13)*, the DE went up to a value of about 12, which was ideal for the subsequent saccharification process. Therefore, a 0.8 KNU/g corn of α-amylase dosage was used in all the following experiments.

Substrate composition also affected the DE progress curve of liquefaction (Fig. 3). Using 30% starch slurry as control, which was equal to the starch content in extracted ground corn in a liquid-to-solid ratio of 3.5, results showed that the removal of oil and zein protein enhanced the enzyme's capability of accessing starch molecules in the extracted residue. The hydrolysis characteristic of extracted ground corn is similar to industrial starch. Because fat exerts a considerable influence on the starch hydrolysis process, whole ground corn shows a totally different hydrolysis characteristic. Ethanol extraction reduced the effect of fat on starch hydrolysis.

Dextrose Yield

High yield and high concentration of glucose are the key issues for an economically viable practice to utilize corn starch. Although extracted ground corn had a characteristic of loose structure after oil and zein protein extraction, the hydrolysis

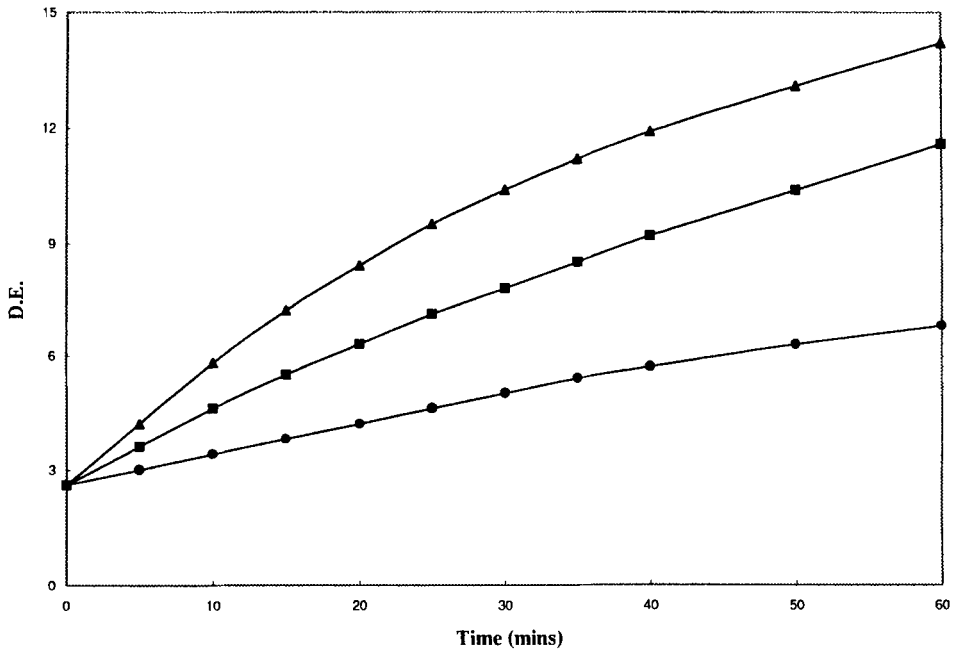

Fig. 2. Liquefaction time-course of defatted ground corn at different amylase concentration. Temperature: 95°C; pH = 6.5; liquid-to-solid ratio: 3.5; size of ground corn: 32–60 mesh, process A. ●, 0.4 KNU/g corn; ■, 0.8 KNU/g corn; ▲, 1.6 KNU/g corn.

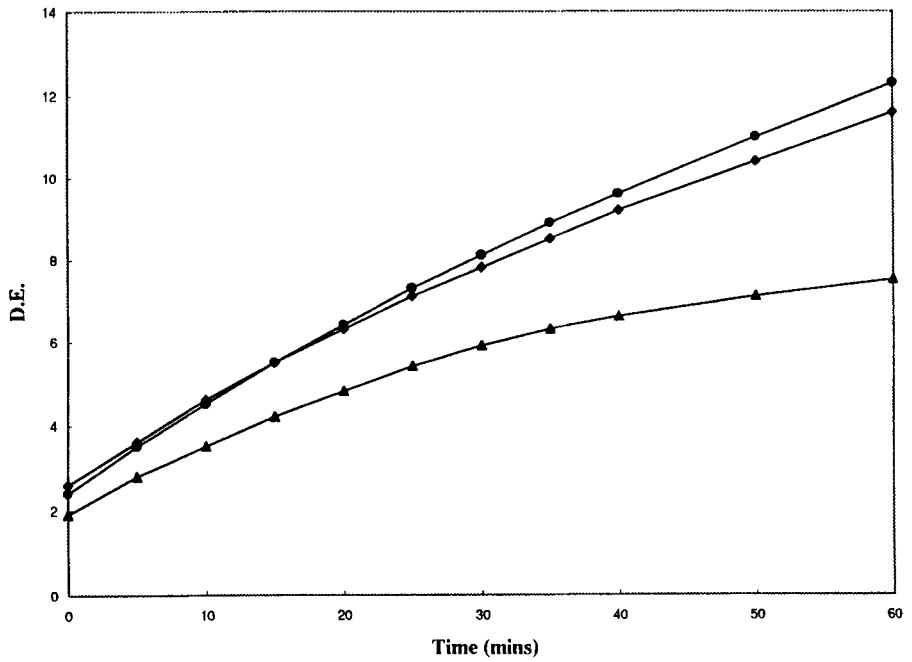

Fig. 3. Liquefaction time course of different substrates. Temperature: 95°C; pH = 6.5; liquid-to-solid ratio: 3.5; size of ground corn: 32–60 mesh; α-amylase concentration: 0.8 KNU/g corn; control: 30% starch slurry. ◆, defatted ground corn; ●, 30% starch control; ▲, ground corn.

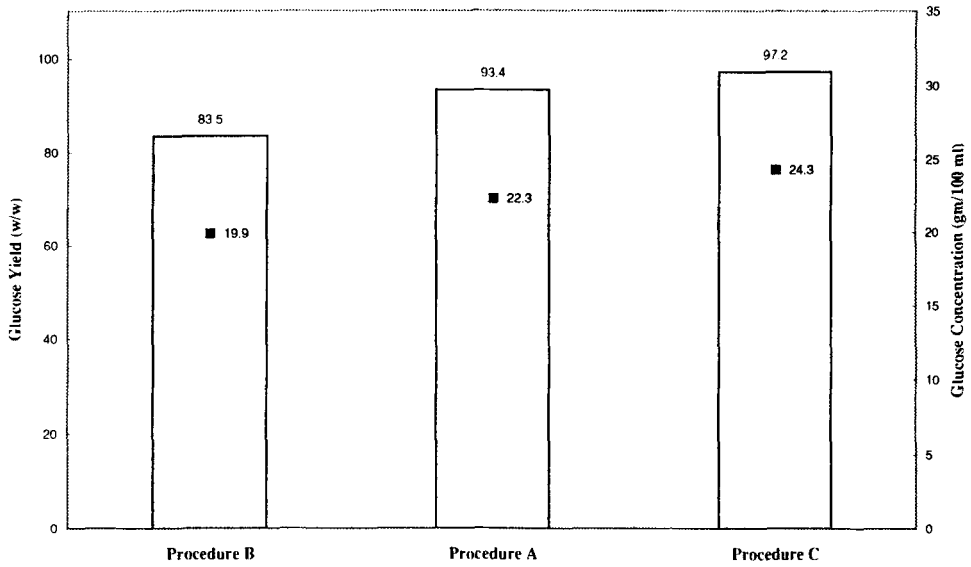

Fig. 4. Effect of processes on glucose yield (bar) and glucose concentration (■). Liquefaction condition: temperature: 95°C; pH = 6.5; Liquid-to-solid ratio: 3.5; size of ground corn: 32–60 mesh; α-amylase concentration: 0.8 KNU/g corn; time: 60 min. Saccharification condition: temperature: 60°C; pH = 4.5; enzyme concentration: glucoamylase, 4 AGU/g corn for processes A and B, and 4 AGU/g corn plus 5 U cellulase/g corn for process C; time: 48 h.

condition of starch fraction is not very different from the commercial starch process. The existence of cellulose fiber and residual tissue structure may hinder the diffusion of enzyme to starch. We have observed that starch can be hydrolyzed more efficiently at a temperature of 110°C than at 105°C. Since 110°C is the maximum limit of thermostable temperature for α-amylase Termamyl 120L, a relatively large part of activity has been lost by the process. In process B, therefore, only an 83.5% glucose yield was achieved (Fig. 4). To avoid enzyme deactivation at this temperature, enzyme was added in two stages: one-half of the enzyme dosage was added before heating at 110°C and the rest after the first step and before the second step of heating of 95°C (process A). As a result, 93.4% of the glucose yield was obtained. A modified process suggested by the Novo company *(13)*, process C, was used to enhance the hydrolysis of extracted ground corn. In this process, 5 IU of cellulase/g of corn were added with glucoamylase during the saccharification stage at 60°C, and the glucose yield was increased to 97.2%. After filtering and washing of the hydrolysis residue, a glucose concentration of 24.3% (w/v) was obtained from process C.

Particle size affects the yield of oil extraction as well as glucose yield in the subsequent starch hydrolysis. Figure 5 shows that glucose yield increased from 80 to 98% following the particle size changes from 16 to 60 mesh. Decreasing particle size of ground corn can enhance the diffusion rate of extraction solvent, water, and enzyme.

Adsorption of water is the most important factor in controlling the rate of starch hydrolysis. The nonstarch materials, such as cellulose fiber, are hydrophilic and exist in the exterior of the extracted ground corn. It holds water and competes with starch for water; therefore, the amount of water added to the residue has to be compensated. It is expected that the liquid-to-solid ratio will affect the glucose yield as well as glucose concentration of hydrolysate (Fig. 6). In our experiments, a liquid-to-solid

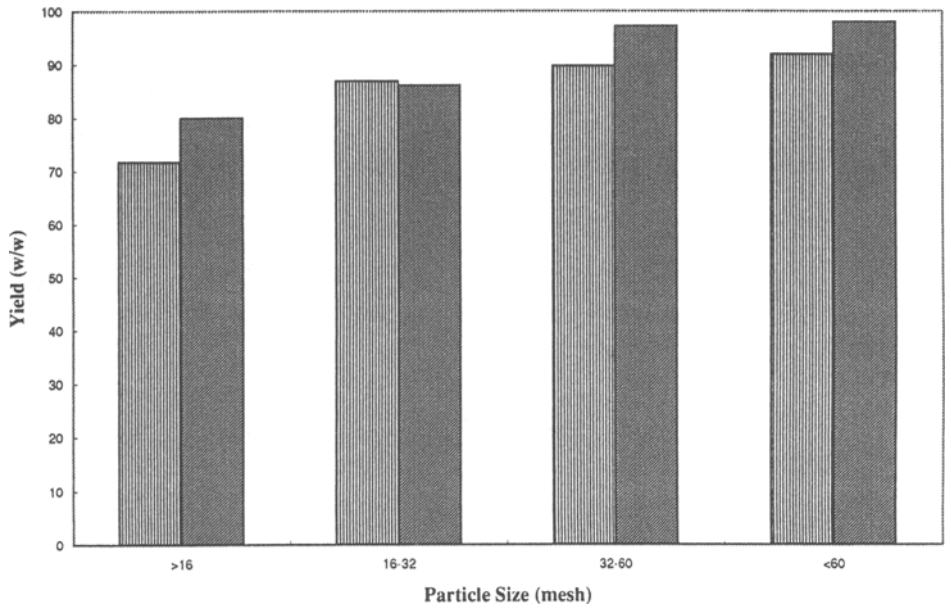

Fig. 5. Effect of particle size of ground corn on oil extraction yield (▥) and glucose yield (■). Condition of oil extraction with countcurrent extractor: extraction solvent: 95% ethanol; temperature: 65°C; retention time: 30–45 min; ratio of liquid to solid: 0.75; hydrolysis condition: same as Fig. 4.

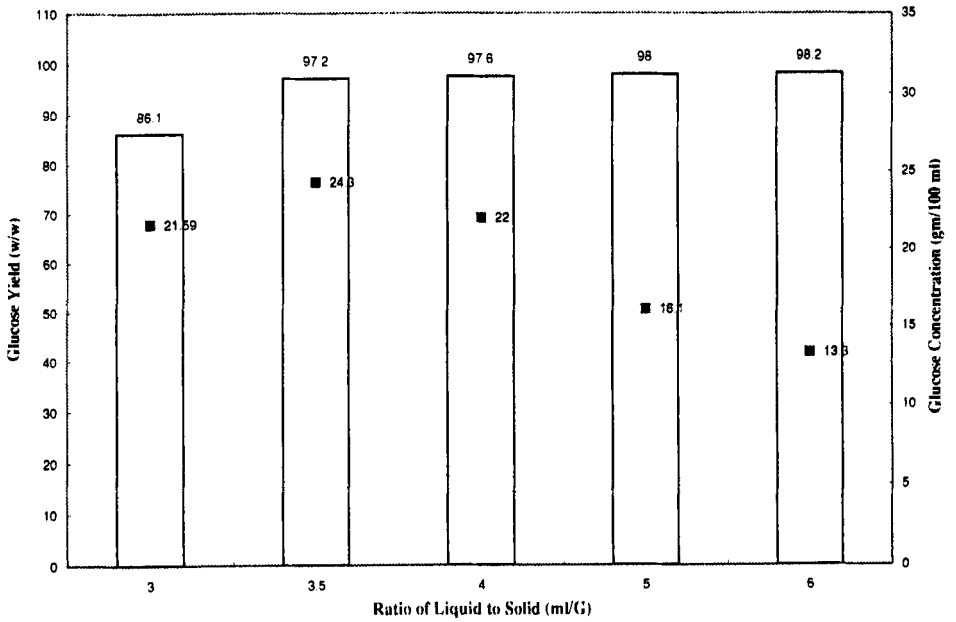

Fig. 6. Effect of liquid-to-solid ratio on glucose yield (bar). Liquefaction condition: temperature: 95°C; pH = 6.5; Size of ground corn: 32–60 mesh; α-amylase concentration: 0.8 KNU/g corn; time: 60 min. Saccharification condition: temperature: 60°C; pH = 4.5; enzyme concentration: 4 AGU/g corn plus 5 U cellulase/g corn; time: 48 h. ■, glucose concentration.

ratio of 3.5 gave a 97.2% glucose yield and 24.3% of glucose hydrolysate. This glucose concentration is suitable for commercial fermentation processes.

CONCLUSION

A new solvent corn-milling process could be an economical alternative process to utilize corn in the production of high fructose corn syrup and ethanol. It not only produced up to 92% oil recovery and 500 mL anhydrous ethanol/kg dried corn, but also provided a kind of extracted ground corn having loose matrix structure. The removal of corn oil can reduce the amount of oil present on starch hydrolysis, and it would simplify the liquefaction and saccharification processes. The defatted ground corn produced can be efficiently hydrolyzed by thermostable α-amylase and glucoamylase combined with a small amount of cellulase. At optimum conditions, without separation and purification of starch, a hydrolysate has been produced with 97.2% yield of glucose.

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