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# Ultrasonication of Sugary -2 Corn for Enhanced Enzymatic Hydrolysis

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**Abstract.** *This study investigates the potential application of high powered ultrasonics as a liquefaction pretreatment of sugary-2 corn slurry. Ground sugary-2 corn (Zea Mays L.) slurry was treated with ultrasonics at 20kHz and amplitudes of 192-320µm$_{pp\ (peak\text{-}to\text{-}peak)}$ for 5, 10, 15, 20 and 40 seconds. After sonication, enzymes (Stargen$^{TM}$001) were added to the samples to hydrolyze the starch into fermentable sugars. It was found that the reducing sugar released in the treated samples were 6-fold higher than in the non-treated samples. Scanning electron microscopy images revealed that the sugary starch was partially gelatinized during sonication. This observation was confirmed by polarized-light microscopic images, where deformed "Maltese crosses" were found. The swelling rate of sonicated samples was nearly 66 times higher than when applying conventional heating. This result confirms better gelatinization capability of ultrasonics compared to conventional heating. The maximum relative net energy gain (additional chemically released energy) of the sonicated samples was at 5s of sonication time with a power setting between 248-330W. The findings in this study indicated ultrasonics as a promising pretreatment step in sugary-2 corn hydrolysis.*

**Keywords.** Sugary-2 corn, ultrasound, glucose, enzyme, ethanol, saccharification, liquefaction, relative energy gain.

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## Introduction

Ethanol production based on fermentation is a rapidly growing industry. Relatively poor overall gains in energy require research to improve the production output and efficiency. One aspect that warrants studies for improvement is the source of fermentable sugars. In the United States, corn is the main source of fermentable sugars for ethanol production. There are many variants of corn that may be identified by its altered endosperm carbohydrate composition (Creech, 1968; Nelson and Burr, 1973; Gonzales, et al., 1976). Sugary maize, commonly known as sweet corn, has been categorized to have a lower starch content but higher level of sucrose (Nelson and Pan, 1995). Takeda and Preiss (1993) reported that sugary (B90) starch contains 30.6% amylose while normal (W64A) starch contains 18.5%. It is hypothesized that due to the higher amylose content, sugary maize could easily be hydrolyzed upon pretreatment. Additionally, sugary maize has smaller starch granules than starch from normal dent corn and is considered suitable for application in starch-thinned acidic foodstuffs (White et al., 1994). Most studies on sugary maize applications have been limited to food application. The novelty of this research is that it focuses on the potential of sugary corn for ethanol production and the utilization of a simple pretreatment procedure, i.e. ultrasonication, to enhance sugar production from sugary corn.

Two consequential effects of ultrasonication of corn slurry, e. g. cavitation and acoustic streaming, are considered as beneficial to the improvement of ethanol production. Ultrasound is defined as sound waves at a frequency above the upper range of the normal human hearing (>15-20 kHz). When ultrasound waves propagate through a liquid medium, these cause oscillations in pressure. The negative component of the ultrasonic pressures produces microbubbles though the phenomenon called cavitation (Suslick, 1988, Mason, 1999, Kardos and Luche, 2001). Because of surface tension, the presence of other bubbles, foreign bodies, and gradients in the pressure waves, each bubble becomes unstable beyond a critical size and eventually collapses violently. As the bubbles collapse, localized temperatures of up to 5000°K are achieved (Flint and Suslick, 1991). Ultrasound waves in liquid media also produce acoustic streaming, which facilitates the uniform distribution of ultrasound energy within the medium, convection of the liquid and dissipation of any heating that occurs (Faraday, 1831).

Ultrasonics has been widely used in various biological and chemical applications. Zhang et al. (2005) reported the use of ultrasonic treatment to enhance protein-starch separation for use in the wet-milling industry. Ultrasonics has also been employed to assist in the extraction of resveratrol from grapes (Cho et al., 2005). Li et al. (2004) utilized ultrasound treatment to enhance oil extraction from soybeans. Wood et al. (1997), studied ultrasonics to enhance ethanol yield from simultaneous saccharification and fermentation of mixed office paper. They achieved a 20% increase in ethanol yield from their sonicated samples.

Khanal et al. (2007) applied ultrasound to break down the particle size of milled commodity corn for subsequent improvement in sugar released in corn dry-milling. The authors reported a 3-fold increase in sugar production rate from the sonicated corn slurry. Motivated by the preceding success, this study examined the potential of exposing sugary corn slurry to high-power ultrasonics to enhance sugar production. Because sugary corn has a lower crystallinity and a lower gelatinization temperature than normal corn (Singh et al., 2006), it was postulated that the starch in sugary corn would be easier to break down compared to normal maize under the same conditions of ultrasonication. The objectives of the present study were to determine the efficacy of ultrasound treatment in releasing fermentable sugar and to determine its effects on sugary corn starch.

## Materials and Methods

### *Sample Preparation and Ultrasonic Treatment*

Corn slurry samples were prepared with a composition of 3 g of dry milled sugary corn (B90), 25 ml 0.1 M pH 4.5 acetate buffer, and 7 ml de-ionized water (DI). The enzyme used was STARGEN™ 001 (456 granular starch hydrolyzing units(GSHU)/g) from Genencor International (Palo Alto, CA, USA), which contained *Aspergillus kawachi* α-amylase expressed in *Trichoderma reesei* and glucoamylase from *Aspergillus niger* that hydrolyzes starch dextrins into glucose. The composition of sugary corn was obtained using a Near-Infrared (NIR) Infratec™ 1241 Grain Analyzer (FOSS Tecator, Eden Prairie, MN, USA).

**Table 1 Average ultrasonic power dissipated at different amplitudes**

| **Parameters** | **Power levels** | | |
|---|---|---|---|
| | **Low** | **Medium** | **High** |
| Average power dissipated (J/s) | 164 - 174 | 205 - 237 | 248 - 330 |
| Amplitude ($\mu m_{pp}$) | 192 | 256 | 320 |

Corn slurry samples were sonicated using a Branson 2000 Series (Branson Ultrasonics, Danbury, Connecticut, USA) bench-scale ultrasonic unit for 5, 10, 15, 20 and 40 s. The system operates at a maximum power output of 2.2 kW and a frequency of 20 kHz. The ultrasonic treatments were carried out in 50-ml polypropylene centrifuge tubes using three different amplitudes (power): low, medium and high (Table 1). The horn was a standard 20-kHz half-wavelength catenoidal titanium with a flat 13-mm diameter face (gain = 1:8). STARGEN™ 001 enzymes (18 µl) were added after sonication. The samples were then incubated (liquefied and saccharified) for 3 hours in a rotary shaker at 150 rpm and 32°C. All experiments and analytical procedures were conducted in duplicate and triplicate, respectively.

### *Analytical Methods*

After liquefaction and saccharification, 2 ml of 4M HCl-Tris buffer (pH 7) were added to the samples to stop the enzymatic reaction at particular times. The slurry was then centrifuged at 10,000 rpm (12,096 x g) using a Beckman Coulter Avanti J-20xPI with Rotor JA25.5 (Fullerton, CA, USA) for 10 min to separate the unsaccharified corn. The supernatant was then analyzed for reducing sugar using the modified dinitrosalicylic acid (DNS) method (Miller, 1954; Khanal, et al., 2007). A sample size of 100 µl was removed from the batch ~35 ml, then mixed thoroughly with 1ml of DNS reagent. The DNS reagent consisted of 0.25g of 3,5 dinitrosalicylic acid; 75g sodium potassium tartrate; 50ml 2M NaOH; and distilled water up to 250ml. The solution was heated to 100°C for 10min, cooled in an ice bath, then measured for irradiance absorbance at 570 nm in spectrophotometer (ThermoSpectronic Genesys 2 – model W1APP11(Thermo Fisher Scientific, IL, USA)). Glucose concentrations were calculated from calibration graphs obtained using absorbance data for standard solutions of D-glucose reacted with DNS reagent as above.

The degree of swelling test was conducted by determining the water absorbed by the corn sample. This test was modified from the swelling power and solubility method by Leach, et al. (1959). Because 68% (dry weight) of the corn kernel is starch, the amount of water absorbed by the sample was correlated to swelling (water absorption) of the starch granules. In this analysis, 3 g of sugary corn was weighed into a centrifuge tube and 35 ml of distilled water was added. For comparison reasons, two batches of samples were prepared. The first batch was treated with ultrasonics at high power for 5, 10, 15, 20 and 40 seconds. At the end of each treatment, the temperature of the samples were recorded, which ranged from 25 to 70°C. In

order to simulate the effect of this temperature, a water bath was used to heat the untreated sample with a similar temperature history. The second batch of samples was added into the 35 ml distilled water and mixed for 15, 120 and 240 seconds. The tubes were immediately cooled in an ice bath to stop the reaction, and then centrifuged at 2,500 rpm for 15 minutes. The supernatant was transferred into a separate container then dried at 40°C until a constant weight was achieved. The precipitate is also weighed after the supernatant is removed. The degree of swelling was estimated using Eq. 1.

$$DS = [(W_p + W_s - W_c) / W_c] \cdot 100 \quad \text{[Eq. 1]}$$

where:

DS – degree of swelling (%, g/g)

$W_p$ – weight of precipitate (g)

$W_s$ – weight of dried supernatant (g)

$W_c$ – weight of corn (g)

In order to confirm that ultrasonic energy introduced mechanical mechanisms that affect gelatinization, these results were compared to samples that were heated only to similar temperatures at a constant value. For example, at the shortest ultrasonic treatment time, 5 s, the temperature was 25-27°C and at the end of the longest treatment time, 40 s, the temperature was 68-70°C. Control samples were adjusted to the same temperatures using electrical heating to separate any gelatinization caused by heating.

### *Relative Energy Gain Calculation*

The dissipated ultrasonic energy is defined as the amount of electrical energy supplied per unit volume of corn slurry in W/ml as detailed in Eq. 2.

$$P_{avg} = \left( \frac{P_{initial} + P_{final}}{2} \right) - P_{air}$$

$$Q_{avg} = \frac{P_{avg}}{V}$$

$$E\_Density_{avg} = Q_{avg} \cdot t$$

*Where*:

$P = \text{Power}$ [Eq 2]

$Q = \text{Average power density (W/ml)}$

$V = \text{Volume of sample (ml)}$

$t = \text{Sonication time (s)}$

$E\_density = \text{Energy density (J/ml)}$

The total energy dissipated ($E_{in}$) into each sample was calculated based on the average power and sonication time:

$E_{in} = \int_{t_0}^{t_f} P dt$ ~$E_{in} = P_{avg}$ t), where $t_0$ and $t_f$ are the initial and final times during sonication.

The total energy delivered from sonication (energy out, $E_{out}$) was calculated based on the chemical energy of the additional glucose produced compared to the control group. In more detail, the change of glucose mass yield compared to the control group was calculated and the energy of the glucose was estimated by assuming an energy density of 15,992 kJ/Kg for glucose if fully oxidized. The overall relative energy gain (*Eff*) of sonication was calculated based on the ratio of the energy balance as detailed below;

$$Eff = \frac{E_{out} - E_{in}}{E_{in}} x100\%$$

### *Microscopy*

Selected samples were viewed at 40x magnification with a cross-polarized filters optical microscope (Nikon Labophot HFX-II, Japan). Scanning electron microscope pictures were taken with Hitachi S-2460N VP-SEM (Hitachi, Ibaraki, Japan).

## Results and Discussion

### *Glucose Yield*

Figure 1 shows the relative sugar release of ultrasonically treated corn slurry at varying sonication times and powers. The relative sugar release is computed as the ratio of the sugar release of ultrasonic treated corn slurry to the sugar release of untreated corn slurry. As shown, all sugar release results of the treated slurries are greater than the results of the untreated ones (control). The relative increase in reducing sugar release ranges from 1.18 at low power to 6.16 at high power. It is seen that the amount of sugar released is generally proportional to sonication time. It is interesting to note that the relative sugar releases at medium and high power are similar while the relative sugar release for the low power is greatly lower compared to the medium and high powers in the ranges of 5 to 20 seconds. However, at the extended sonication time (40 s.), the results are similar. This suggests an optimum amount of sonication energy that is required to promote the release of fermentable sugars. During treatment, it was observed that the viscosity increased, suggesting the corn slurry has gelatinized, thus decreasing the mixing of the corn slurry.

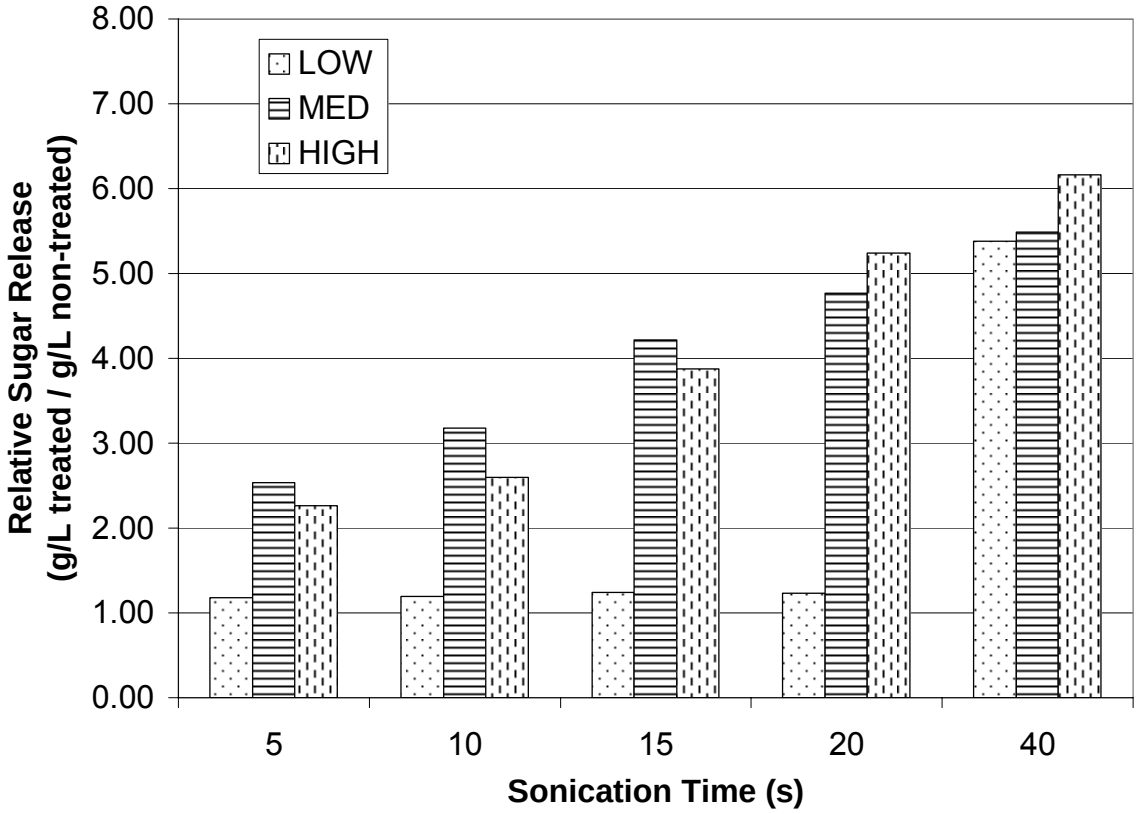


**Figure 1 Relative Sugar Release at Varying Ultrasonics Conditions**

### *Scanning Electron Microscopy*

Scanning electron microscopy (SEM) images of corn slurries with and without ultrasonic treatment are shown in Figure 2. The images in the left column are magnified 300x, whereas the images on the right are magnified 1000x. In the control samples (Figure 2 (A) and (B)), the starch granules are intact. In contrast, as seen in a 20-s ultrasonic treated sample (Figure 2(C)), the starch granules are partially ruptured. At the higher magnification (Figure 4(D)), it can be seen that the granules are coated with what is believed to be gelatinized starch. Additionally, as the corn slurry was treated for 40s (Figure 2(E) and Figure 2(F)), the starch granules are indistinguishable; similar to what was found for ultrasonic treated commodity corn in Khanal et al. (2007). Thus, the samples treated at 40 s appeared to be fully gelatinized.

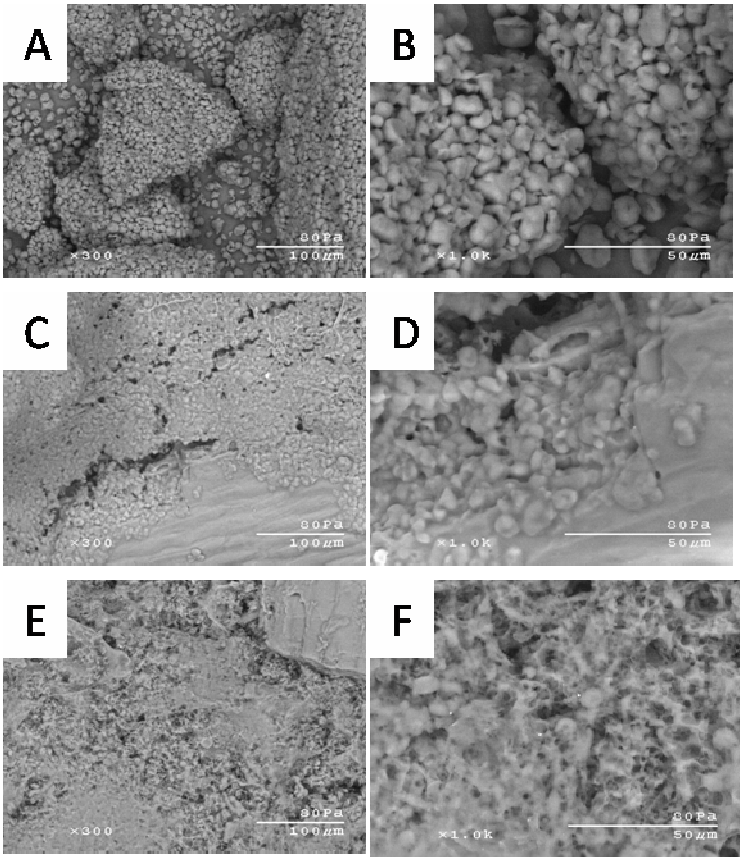


**Figure 2 SEM Images of Control (A & B) and Sonicated Samples [for 20s (C&D) and 20s (E&F)]**

### *Polarized Light Microscopy*

Gelatinization is a critical step in converting the starch into sugar, as it releases the water soluble fractions of the starch granule. After gelatinization the intra-molecular bonds (secondary bonds) of starch are broken in the presence of water. Gelatinization can be achieved by chemical and heat treatment. In ultrasonics, the sample temperature increases due to cavitation implosion. Since the experiments were conducted without controlling the temperature in the reaction chamber increased with ultrasonic power settings and the treatment time. However, it is theorized that the ultrasonic energy and heating were synergetic and partially gelatinized, the starch, allowing more effective enzymatic reactions. In order to confirm this hypothesis, a polarized optical microscopy examination was conducted to characterize sonicated and unsonicated (control) samples that both had been subjected to similar heating profiles.

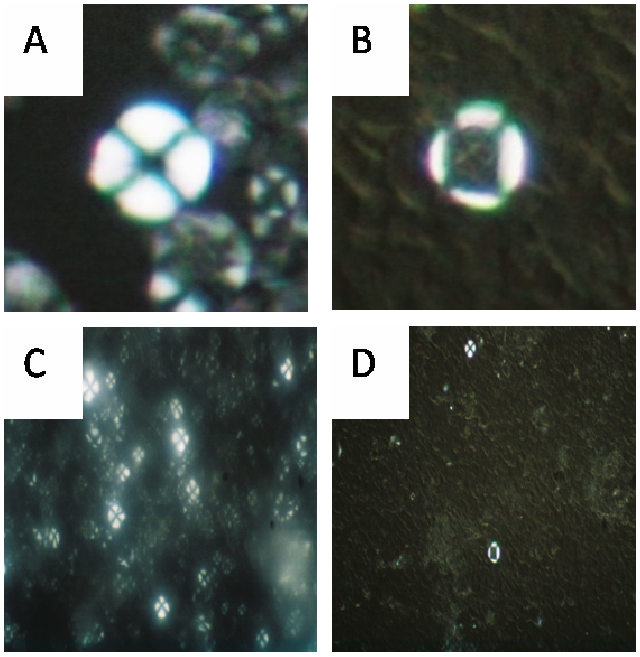


**Figure 3 Polarized Microcopy Images of Control (A&C) and Sonicated (B&D) Samples**

Figure 3 shows the polarized microscope images of sugary starch subjected to sonication (B and D) and without sonication treatment (A and C). Images 3C and 3D were viewed under a polarized microscope at 40x magnification. Images A and B were zoomed in 22.5x from Images C and D to focus only on a single Maltese cross pattern. The control samples demonstrated a clear Maltese cross pattern that is normal for non-gelatinized starch granules (French 1973; Guler et al., 2002; Zhong et al., 2007). These patterns relate to semi-crystalline structures of starch and are often called spherulites. Spherulites and birefringence are more pronounced in the control groups (Figure 3 (A) and (C)) compared to the sonicated groups (Figure 3 (B) and (D)). The amount of birefringence correlates to the degree of crystallinity (Zhong et al., 2007). At the higher magnification (Images (A) and (B)), sonicated starch had no birefringence in the center of the spherulite (Soares et al., 2007) indicating that the sample had a lower degree of crystallinity and therefore was partially gelatinized.

### *Degree of Swelling*

The degree of swelling as a function of time for using conventional heating and ultrasonics is shown in Figure 4. It could be noticed that the swelling in the sonicated sample starts as early as 5s and increases rapidly compared to conventional heating. This is similar to the results found by Isono et al. (1994), where the rate of degradation of sonicated waxy rice starch accelerated at/or above the gelatinization temperature. In addition, the final degree of swelling is substantially higher (350%) for the ultrasonic treatment compared to samples that were heated only (~200%). This increase is believed to be related to a finding of Huang et al. (2007) that ultrasonics affected the starch amorphous regions and enhanced water absorption. In addition, Seguchi et al. (1994) reported that ultrasonics gradually separated starch agglomerate and reduced the starch average molecular weight. This could have solubilized the amylose fraction and released it in the suspension forming a three dimensional gel network (Lehmann, et al., 2004).

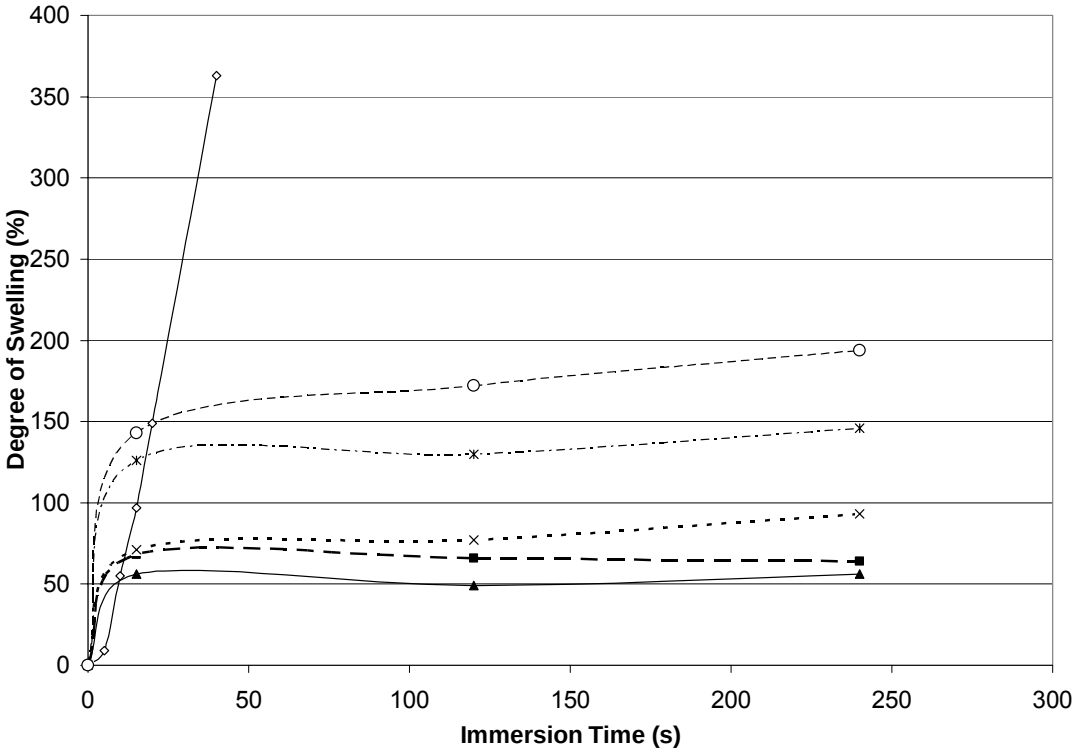


**Figure 4 Comparison of Degree of Swelling between Conventional Heating and Ultrasonics**

### *Relative Net Energy Gain*

Relative net energy gain is a comparison between the additional energy of the reducing sugar produced due to ultrasonics and the ultrasonic energy dissipated. As shown in Figure 5, the relative energy efficiency exceeds 100% for the majority of the experimental design range except at the low power setting and shorter sonication periods (≤20 s). It is believed that this could be due to under-treatment and inadequate gelatinization as well as lack of particle size reduction. The energy gain that is greater than 100% only indicates that the energy produced due to ultrasonics exceeds the ultrasonics energy dissipated. It has been found that for longer sonication times (>25 s), gelatinization increased the viscosity of the slurry and the process became less efficient (>0%). At higher power settings, maximum efficiency occurred with a treatment time of approximately 5 s. The additional energy most likely did not reduce the particle size of starch granules further as described by Khanal et al. (2007), nor promote additional gelatinization. The lower efficiency of the high power condition was attributed to the denaturation/degradation due to over-treatment as well as an increase in viscosity.

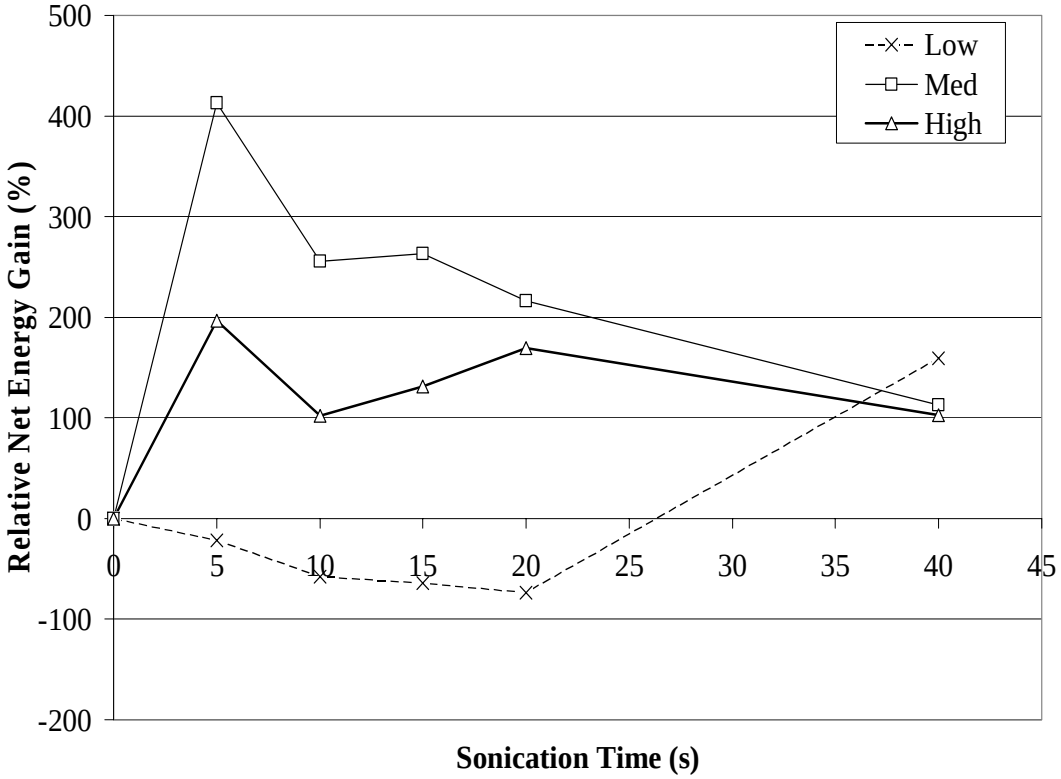


**Figure 5 Relative Net Energy Gain for Different Ultrasonic Conditions**

## Conclusion

This work evaluated the effects of high-power ultrasonics in converting sugary-2 maize to fermentable sugars for ethanol production. The resulting ultrasonic glucose yield was 6-fold that of the unsonicated samples. SEM and polarized light microscope images also confirmed that the ultrasonic treatment gelatinized the starch. Moreover, the ultrasonic energy efficiency exceeded 100%, which indicated that more energy was produced in the form of chemical energy contained in sugar than the amount of ultrasonic energy introduced. This study further investigated the effect of ultrasonics on swelling in comparison with conventional heating. It was found that samples started to swell when subjected to ultrasonics even for as short as 5 seconds. Conventional heating initiated a higher degree of swelling at 30 seconds but increased at a lower rate than ultrasonics. The swelling rate of ultrasonicated samples was 66 times higher than with conventional heating

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