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Mechanochemical effects of micronization on enzymatic hydrolysis of corn flour

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Abstract

Corn flour samples with different particle size were prepared by ball milling, and then liquefaction and saccharification of the corn flour samples were carried out by using commercially available α-amylase and glucoamylase, respectively. Mechanochemical effects of micronization on the enzymatic hydrolysis were studied for developing a new technology of low-temperature enzymatic hydrolysis of corn flour.

The commercial corn flour of 273.6 μm could be micronized to 17.5, 15.4, 14.6, 13.3 and 9.8 μm in median diameter by wet-milling for 20 min, 1, 2, 3 and 5 h, respectively. Microscopic observation and X-ray diffractometry revealed the starch crystal structure of corn flour could be destroyed by wet-milling for more than 3 h. All the wet-milled corn flours could be liquefied at 30 °C. The liquefaction rate of corn flour increased with increasing wet-milling time. The glucose yield was 46.8% for the 20 min milled corn flour. It was increased to 83.7% by wet-milling for more than 3 h. The increase of glucose yield corresponded with the destruction of starch crystal structure and the decrease of gelatinization temperature.

Our experimental results indicated that wet-milling had mechanochemical effects, which resulted in a remarkable increase of glucose yield in low-temperature enzymatic hydrolysis of corn flour.

Keywords: Corn; Enzymatic hydrolysis; Micronization; Mechanochemical effect; Biorefine

1. Introduction

Biomass is considered as one of the key renewable resources that possess significant economic potentials and have various social and environmental benefits (Demirbas, 2001; Hall, 1997). Research efforts have been focused on the development of a cost effective technology for commercial production of liquid fuel and chemicals such as ethanol, lactic acid and succinic acid from renewable resources. Corn is a main feedstock in the bio-chemical industry. It is estimated that the energy content of bio-ethanol derived from corn grain is higher than the energy content of ethanol (Shapouri, Duffield, & Wang, 2002).

Hydrolysis is an essential process in the bio-chemical industry using corn flour as raw material. The role of hydrolysis is to convert the starch in corn flour to fermentable sugars. A two-step enzymatic hydrolysis of corn flour includes a liquefaction step and a saccharification step. Corn flour, the ground endosperm, usually contains 75–87% starch and 6–8% protein. In native corn flour, starch granules are surrounded by a continuous phase of protein (Chanvrier, Colonna, Valle, & Lourdin, 2005), which protects the starch granules from the attack of enzymes. Therefore, in order to promoting the hydrolysis of corn starch, it is necessary to destroy the protein network by cooking at a high temperature. The cooking process, however, requires 30–40% of the total energy consumption in the bio-ethanol production, and produces non-fermentable impurities. A number of studies have been carried out to select thermostable and effective enzymes for hydrolysis at a relatively low temperature. However, reports on the development

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of hydrolysis process are rare (Ma, Shi, & Zhang, 2005; Mojovic, Nikolic, Rakin, & Vukasinovic, 2006).

In a previous work, the authors compared the liquefaction rate, the glucose yield in a two-step enzymatic hydrolysis of commercially available and micronized corn flour samples. We found that the activation energy and reaction rate in the liquefaction was decreased, and the glucose yield was increased by micronizing of corn flour (Miao, Wu, Jiang, & Yang, 2007). However, the mechanism of the enzymatic hydrolysis of micronized corn flour was not clarified. The objectives of the present work were, (1) to investigate mechanochemical effects of micronization on the enzymatic hydrolysis of corn flour, and (2) to determine the optimum conditions for micronizing of corn flour.

2. Materials and methods

2.1. Corn flour samples

Commercially available corn flour (Anhui Yanzhifang Food Co., China) was obtained from a supermarket. It had a median diameter of 273.6 μm, and contained 10.5% water, 77.8% starch, 5.2% protein, 4.1% fat and 0.7% ash.

The commercial corn flour was dry-milled for 5 h and wet-milled for 20 min, 1, 2, 3 and 5 h respectively, by a planet-type ball mill (XQM-4 L, Nanjing Kexi Institute of Experimental Instruments, China) with a rotate speed of 500 rpm. In the wet-milling, the weight ratio of corn flour to water was 1:3. The particle size distribution of corn flour samples was measured with a particle size analyzer (2000, Malvern, UK). The 5 h dry-milled corn flour had a median diameter of 28.9 μm, and the 20 min, 1, 2, 3 and 5 h wet-milled corn flours had a median diameter of 17.5, 15.4, 14.6, 13.3, 9.8 μm, respectively. Fig. 1 shows the particle size distribution of commercial, 5 h dry-milled and 3 h wet-milled corn flours.

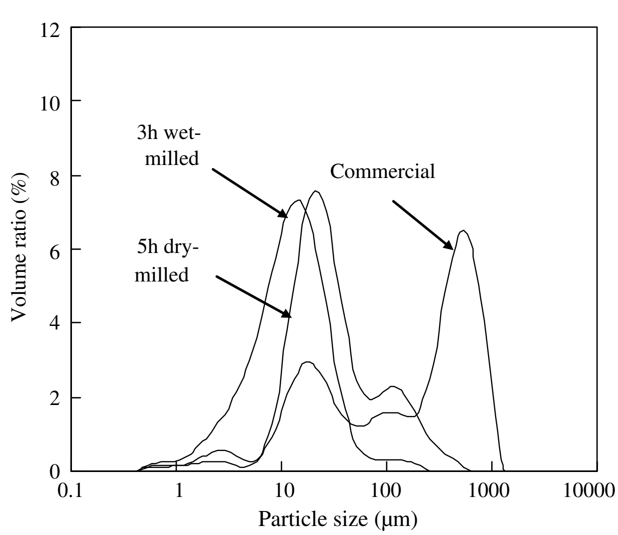

Fig. 1. Particle size distribution of commercial, 5 h dry-milled and 3 h wet-milled corn flours.

2.2. Measurement of gelatinization temperature

A microscope equipped with a heating table (XP-203, Nanjing Donglilai Optics Co., China) was used to observe the granule shape and Maltan cross of the corn flours, under general and polarized light condition, respectively. Suspension of each corn flour was prepared with deionized water. A drop of the suspension was sandwiched by two flat glass slides, and heated by the heating table at a rate of 2.6 °C/min. The clearance between the two flat glass slides was sealed up with edible salad oil to prevent the suspension from evaporation. The temperature at which 2% of the particles with Maltan cross disappeared was defined as initial gelatinization temperature, and the temperature at which 98% disappeared was defined as final gelatinization temperature (Liu, 2003; Xiang, Tian, & Li, 2006). Images of granule shape and Maltan cross were recorded by a color CCD camera (TK-C921EC, Victor Company of Japan Limited) and a personal computer (M4600, Lenovo).

2.3. X-ray diffractometry

The X-ray diffraction pattern of a corn flour sample also reveals the presence and characteristics of the crystal structure of its starch granules (Wang, Yu, Gao, et al., 2007). X-ray diffractometry of the commercial, 5 h dry-milled and 3 h wet-milled corn flours were performed by an X-ray diffractometer (ARL X'TRA, Thermo Electron Corporation, USA). The 3 h wet-milled corn flour was air-dried under the room condition before the analysis. Each corn flour sample was packed tightly in a square plastic cell (20 × 20 mm). The samples were exposed to the X-ray beam from an X-ray generator running at 45 kV and 35 mA. The scanning regions of the diffraction angle 2θ were 5–80°.

2.4. Enzymatic hydrolysis

A two-step enzymatic hydrolysis method including liquefaction and saccharification was applied to the wet-milled corn flours.

In the liquefaction step, a wet-milled corn flour sample equivalent to 60 g dry matter was mixed with 540 ml deionized water in a 1000 ml three-meatus flask. The pH of the mixture was adjusted to 6.0–6.5 by 5% H_2SO_4 solution and 1% NaOH solution. A commercially available α-amylase (Wuxi Saide Bio-technology Co.), which had an optimum temperature of 70 °C and pH of 6.0–6.5, was added to the mixture at a ratio of 10 U/g. The liquefaction was performed at 30 °C on a thermostated water bath with an electric motor-driven stirrer. Reducing sugar content of the mixture was analyzed with the DNS method (Miller, 1959; Mojovic et al., 2006; Yu & Zhang, 1991) and expressed by dextrose equivalent:

$$\text{Dextrose equivalent}(\%) = \text{mass of reducing sugar/mass of corn flour} \times 100 \quad (1)$$

After liquefaction, a commercially available glucoamylase (Wuxi Saide Bio-technology Co., China), which had an optimum temperature of 60 °C and pH of 4.0–4.5, was added to the mixture at a ratio of 200 U/g. The saccharification was performed at 60 °C and pH 4.0–4.5. Glucose content of the mixture was analyzed by an auto-analyzer (SBA-50B, Biology Research Institute of Shandong Academy of Sciences, China). Glucose yield was expressed on the basis of the source corn flour.

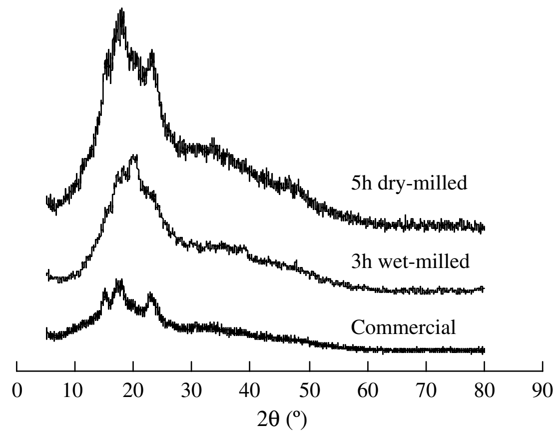

Fig. 3. X-ray diffraction patterns of corn flours.

3. Results and discussion

3.1. Changes in crystal structure

The granule shape and Maltan cross of 5 h dry-milled, 5 h water-soaked, 1 h wet-milled and 3 h wet-milled corn flours are shown in Fig. 2. The 5 h water-soaked corn flour was prepared by soaking the commercial corn flour to water at the room temperature for 5 h. It can be found that all particles in the 5 h dry-milled and 5 h water-soaked corn flours had smooth surfaces and clear Maltan crosses. In the 1 h wet-milled corn flour, some of the particles swelled up and their Maltan crosses disappeared. In the corn flour wet-milled for more than 3 h, almost all of the particles were dissolved and all Maltan crosses disappeared, indicating that starch crystals of the corn flour were destroyed.

The X-ray diffraction patterns of commercial, 3 h wet-milled and 5 h dry-milled corn flours are shown in Fig. 3. The commercial corn flour had the same X-ray diffraction pattern as that of corn starch, in which there were three peaks at $2\theta = 16.7°$, 17.9° and 23.5° (Wu, Li, Su, & Xie, 2004). In the X-ray diffraction pattern of 3 h wet-milled corn flour, there was no obvious peak. In the X-ray diffraction pattern of 5 h dry-milled corn flour, the peak still existed at 17.9° and 23.5°, but there was no peak at 16.7°. The changes in X-ray diffraction pattern corresponded to disappearing of Maltan crosses in the microscopic observation.

Corn starch granules originally have a multi-layer structure of crystals and non-crystals. It was indicated that the crystals could be destroyed by wet-milling for 3 h in Figs. 2 and 3. Compared with wet-milling, dry-milling had less effect on the crystals. The main reason is that the starch granules swell up when they absorb enough water, which results in a decrease of bond strength between the crystal layer and non-crystal layer. Particularly, mechanical force

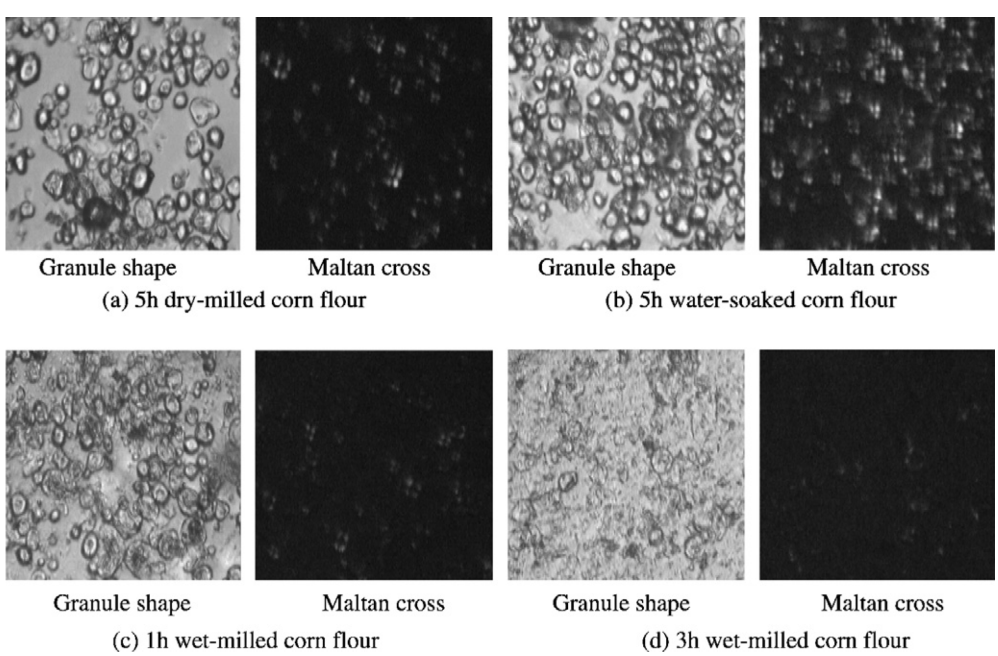

Fig. 2. Microscopic photographs of corn flours.

may facilitate the penetration of water into starch granules during wet-milling.

Corn starch generally consists of amylose, a mostly linear α-D-(1–4)-glucan, and amylopectin, a α-D-(1–4)-glucan with α-D-(1–6) linkage at the branch points. Compared with amylose, amylopetin is easier to be damaged by mechanical force (Wu et al., 2004). In addition, the destruction of starch crystals by micronization depends not only on milling method, but also on the type of starch. For example, broomcorn starch crystal can be seriously damaged, while corn starch crystal is not affected by dry-milling (Hu, 2003).

3.2. Changes in gelatinization temperature

It was indicated in Fig. 2 that the number of particles with Maltan crosses decreased in the wet-milled corn flours. Therefore, in the present work, the particles with Maltan crosses were defined as remaining particles, and gelatinization temperature was, in fact, measured for the remaining particles of corn flours. Table 1 shows the gelatinization temperatures of corn starch and corn flours. The corn starch had an initial and final gelatinization temperature of 60 °C and 69 °C, respectively, which corresponded to 62 °C and 72 °C reported by Gao (2001) with a Maltan cross method, and 66–70 °C by Zhang, Li, Zhao, Li, and Yang (2005) with a differential scanning calorimetric method.

The gelatinization temperature was decreased significantly by wet-milling. The 2 h wet-milled corn flour had an initial and final gelatinization temperature of 31 °C and 59 °C. After 3 h wet-milling, there was no remaining particle, suggesting that the corn flour had been completely gelatinized. On the other hand, however, there was only a decrease of 4–9 °C in gelatinization temperature for the 5 h dry-milled corn flour.

3.3. Liquefaction rate and glucose field

All the wet-milled corn flours could be liquefied at 30 °C, which was much lower than the gelatinization temperature of corn starch. Changes in dextrose equivalent during the liquefaction of wet-milled corn flours are shown in Fig. 4. The liquefaction rate, or change rate of dextrose equivalent, increased with increasing wet-milling time. For each wet-milled corn flour, the dextrose equivalent gradually became constant during the liquefaction. After 6 h liquefaction, the 1–5 h wet-milled corn flours had a dextrose equivalent of 30%, while the 20 min wet-milled corn flour had a dextrose equivalent of 23%. The 5 h dry-milled corn flour and commercial corn flour could not be liquefied at 30 °C.

Table 1
Gelatinization temperatures of corn starch and corn flours

Material	Initial gelatinization temperature (°C)	Final gelatinization temperature (°C)
Corn starch	60	69
5 h dry-milled corn flour	51	65
20 min wet-milled corn flour	47	66
1 h wet-milled corn flour	38	61
2 h wet-milled corn flour	31	59
3 h wet-milled corn flour	–	–
5 h wet-milled corn flour	–	–

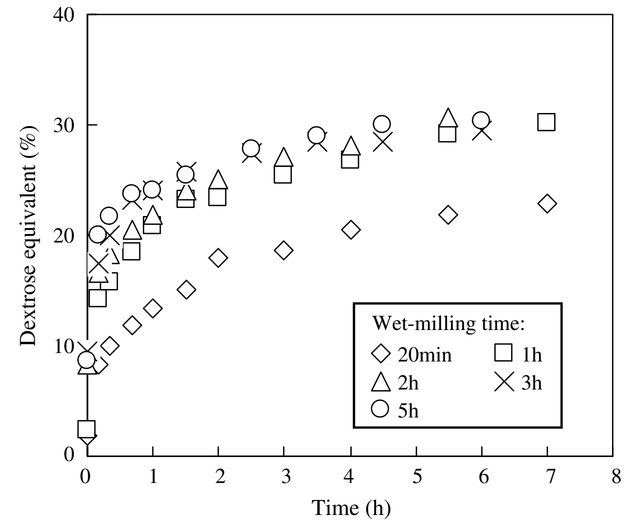

Fig. 4. Changes in dextrose equivalent during the liquefaction of wet-milled corn flours.

Fig. 5 shows the changes in glucose yield during the saccharification of wet-milled corn flours. The glucose yield increased fast in the first 2 h. After saccharification for 6 h, the glucose yield was 46.8% for the 20 min wet-milled corn flour. It was increased to 83.7% by the wet-milling for 3 h. The commercial corn flour had a glucose yield of 79.2% in an enzymatic hydrolysis process of liquefaction at 70 °C followed by saccharification at 60 °C (Miao et al., in press). It is obvious that the glucose yield at a relatively low liquefaction temperature can be improved by wet-milling. The improvement of glucose yield corresponds

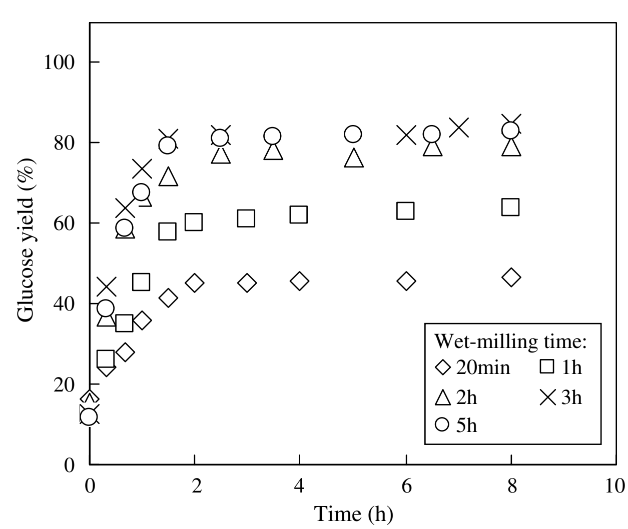

Fig. 5. Change of glucose yield during the saccharification of wet-milled corn flours.

to the decrease in gelatinization temperature, which is a result of the destruction of protein network and starch crystal structure.

4. Conclusions

(1) The commercial corn flour of 273.6 μm was micronized to 13.3 μm in median diameter, and the starch crystal of corn flour was destroyed by wet-milling for 3 h.
(2) The gelatinization temperature of wet-milled corn flour was decreased with increasing wet-milling time.
(3) All the wet-milled corn flours could be liquefied at 30 °C. The liquefaction rate of corn flour increased with increasing wet-milling time.
(4) Glucose yield in the enzymatic hydrolysis of corn flour increased with increasing wet-milling time in the range of 20 min to 3 h. The 3 h wet-milled corn flour had a glucose yield of 83.7%.

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