

Research Article

Effects of Different Comsteep Liquids on Structure of Starch and Protein Binding in Corn Endosperm

Rong Yan, Xinhua Li and Xiaojun Qi

College of Food Science, ShenYang Agriculture University, Shenyang 110161, China

Abstract: The changes in the structures of corn endosperms immersed in different reagents were studied, in order to preliminarily determine the mode of binding of starch and protein in the endosperm. The corn endosperm was immersed in Sulfurous acid, NaOH, ethanol, L-cysteine and morel berry, respectively. The dissociated starch content was analyzed using water as the reference. The results of the treatment were analyzed by the dissociated starch content, optical microscopy and scanning electron microscopy. The test results showed that by immersing in NaOH solution, the protein matrix was effectively decomposed, the protein in the endosperm was dissolved and the highest amount of starch granules were released. Sulfurous acid and L-cysteine solution could make the protein matrix into pieces by destroying intermolecular disulfide bridge and the starch granules would be released. There were some holes on the surface of starch granules after L-cysteine steeping. Immersed in the morel berry, there were holes on the protein matrix surface and the water absorption of corn endosperm was improved. In case of the sample immersed in ethanol, the zein was dissolved, whereas the matrix protein was still intact with the starch grains that still bound to it. By analysis of the microscopic structure and morphological change of starch and protein in corn endosperms in different comsteep liquor, it is demonstrated that the main reason which the starch grains were tightly wrapped by Glutenin is the intermolecular disulfide bonds in glutelin. In addition, a part of the protein combined with starch granules directly. The study provided a reference for exploring the structure of binding of starch and protein and their interaction in corn.

Keywords: Corn endosperm, microscopic examination, protein, starch, structure

INTRODUCTION

Corn is not only an important food and feed resources for human beings but also an important raw material for producing starch. The starch content in corn is approximately 70% and it is present in the form of starch grains in corn endosperm (Ji *et al.*, 2003). The scope of applications of corn starch is very broad. At present, about one third of the starch material is used as food, in medicines and beverages. Another one thirds is used in paper-making, packaging, in textiles and also for petroleum products across the world (Jinlan, 2005). Corn starch plays an important role in the deeping processing of whole corn grains. Therefore, extraction of starch with high purity has always been a topic of hot research both locally and also abroad. Currently global research on corn starch extraction is focused on the immersion technique of wet extraction, which includes extensive research on the immersion reagents, time required, processes and effects (Perez *et al.*, 2001; Singh and Johnston, 2002; Shadera and Jacson, 1996) and also the structure of corn endosperm. Initial results showed the diameter of starch granules that exist in the powery endosperm and cut in endosperm to be in the range of 7~25 μm and the shape to be irregular (Wang

et al., 1993). Cui *et al.* (2012) studied the characteristics of grain size distribution of starch grains of corn with different endosperm types and related size distribution of corn starch to a three-peak curve. The corn starch grains, whose grain diameter was more than 15 μm , occupied a larger volume and the corn starch grains, whose grain diameter was less than 2 μm , occupied a smaller volume. The ordinary, super sweet and waxy corn starch grains were spherical-shaped. There were a few sunken starch grains in the ordinary corn and the size of the waxy corn starch grains was uniform with a large number of sunken starch grains.

So far, the previous research was important for better understanding and to know the structure of endosperm and immersion process. However, the changes in the microstructure of the corn endosperm at the immersion stage were not clearly understood and also the effects on microscopic structure of the corn endosperm by different reagents have not been reported. In this study, the cross-sections of the corn endosperms which were treated by immersing in different reagents were studied using scanning electron microscopy techniques in order to understand the changes in the structures of starch and protein in the endosperm in presence of different reagents, which is

helpful in exploring the mechanism between the starch and protein in corn.

MATERIALS AND METHODS

Materials: Ordinary dent corn was obtained from Liaoning of china.

Morel berry (*Lactobacillus paracasei* subsp. *Paracasei* L1).

All chemicals used were of analytical grade.

Instrument: uv-vis spectrophotometer (TU-1810); Electronic balance (EL-204); High speed freezing centrifuge (CR-21G); Digital Biological Microscope (ME21, OLYMPUS); S-4800FESEM (HI-BON)

Methods:

The corn steeping process: The broken segment corn grains was respectively steeped by mixing 5 g of corns (dry matter) with 50 mL of 0.2% sulfurous acid, 0.3% NaOH, 75% alcohol, 1.0% L-cysteine, morelberry and water for 12, 36 and 60 h, respectively at 50°C. After removed the seed coat and germ, the corns were grinded with isometric water. The suspension was then centrifuged (4000 r/min, 5 min) and the supernatant was discarded. Fifty milliliter water was added to residue and then shaken well to measuring dissociated starch content in solution. The experiment was repeated three times.

Assay methods for dissociated starch: iodine colorimetry (Changjie *et al.*, 1998): One milliliter determinand was taken out immediately from the shaking well suspension and was transferred to graduated test tube to diluted with 80% Ca (NO₃)₂ to 20 mL. After water bath in boiling water for 10 min, suspension was centrifuged (4000 r/min, 5 min). After that, 100 µL samples from supernatant were volume to 4 mL in 80% Ca (NO₃)₂ and 100 µL 0.01 N I₂-KI were added. The dissociated starch content of the samples was determined by absorption value at 620 nm. The standard curve: $y = 0.0113x - 0.0033$, $R^2 = 0.9999$.

Analysis of optical microscopy of corn cutin endosperm: To make the same cross surface of endosperm, the corn grains with the same size and shape were cut slightly around median area with one side blade and broke along the cuts. The broken corn grains were respectively steeped in 0.2% sulfurous acid, 0.3% NaOH, 75% alcohol, 1.0% L-cysteine and morel berry for 36 h. and then, the cross section of cutin endosperm were dyed by I-KI. After dyeing, the starch turned blue and the protein turned yellow.

Analysis of scanning electron microscopy of corn cutin endosperm: According to the method 3, the cross-section of cutin endosperm with immersed by different comsteep liquids were dehydrated and the structure change of the starch and protein in corn cutin

endosperm were observed by scanning electron microscopy.

RESULTS AND DISCUSSION

Results and analysis:

Results of optical microscopy study of corn cutin endosperm immersed in different liquids: After the treated corn samples were immersed in different reagents for 36 h, slices in the region of the cutin endosperm were prepared and the changes in the structures of starch and protein were observed under an 100X oil mirror optical microscope, in order to obtain the images of cutin region (Fig. 1). Figure 1a shows that in the endosperm cutin region, the protein matrix was fully swollen but was not decomposed after immersion. There were whole, square or polygonal-shaped starch grains in the protein matrix and protein matrix is reticular connective in shape, which wraps the starch grains in layers and the size of starch grains is uniform. After immersing in different reagents, the microstructure in the horny region of the corn endosperm shows different degrees of changes. Figure 1d to f showed that after immersing in solutions of NaOH, L-cysteine or sulfite, all the protein matrices were destroyed. The degree of protein breakdown was found to be the greatest on treatment with alkali. The reticular structure almost disappeared, as most of the proteins were decomposed into pieces and a large number of starch grains were dissociated. Treatment with sulfurous acid and L-cysteine solutions showed clearly that due to fracture of protein network structure, the starch grains were released. Figure 1c shows the

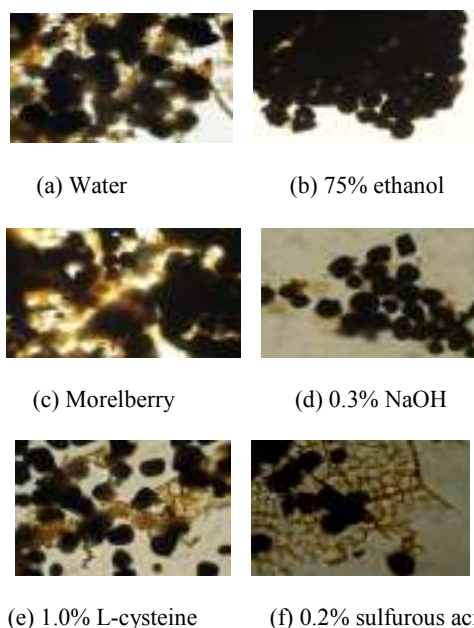


Fig. 1: Microscopic structure of horny endosperm of corn after different comsteep liquor ($\times 100$ oil microscopy)

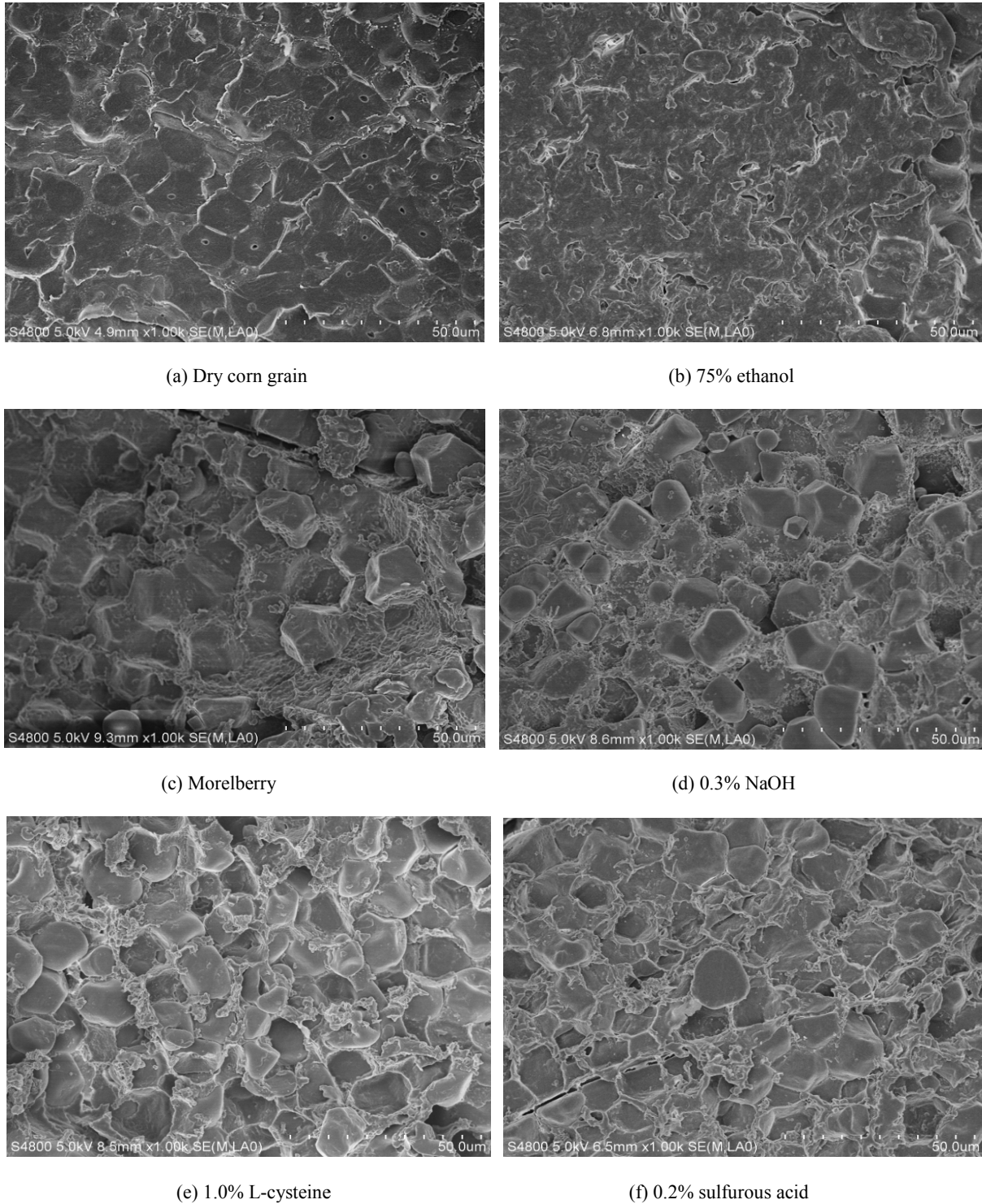


Fig. 2: SEM photograph of horny endosper of corn after different comsteep liquor ($\times 1000$)

micrograph after immersing in morel berry. Compared to the water immersed sample, the matrix protein in this case had higher degree of swelling, dispersion and a more loose structure, which resulted in formation of holes. This was due to the effect on the corn endosperm cell wall by lactic acid, present in morel berry. This

resulted in increase of moisture in the corn grains which promoted the dissociation of the corn protein and starch (Dailey *et al.*, 2000). The sections of cutin regions of the corn endosperms, which were immersed in ethanol (Fig. 1b), showed almost no swelling of the protein matrix and it was found to be in close

combination with starch grains, thereby preventing the release of starch.

Results of scanning electron microscopy of the corn cutin endosperm immersed in different liquids: After treatment of corn endosperm samples in different reagents, the microstructures of endosperm cutin regions were studied using the scanning electron microscopy, which was magnified 1000 times in order to obtain the cross-sectional pictures as shown in Fig. 2. Figure 2a shows the untreated corn endosperm cutin region. Irregular shaped starch grains tightly wrapped in thick protein matrices can be seen, which are still a whole and there were lesser number of exposed starch grains. The results were consistent with that of the previous study. Compared with the untreated cutin endosperm, the microstructures of protein matrix in cutin endosperm varied to different degrees under different reagents immersed. Figure 2d to f showed that after immersing in NaOH, L-cysteine and sulfite solutions, all the protein matrices, which wrapped the starch grains, were decomposed into pieces and the effect of treatment by NaOH was most obvious. It could be seen that the matrix protein with large size and irregular structure was dissolved in alkaline solution and a large number of starch grains were exposed. The ball-shaped un-dissolved protein matrices were embedded in the regions combined with starch grains and no obvious changes were seen on the surface of starch grains. A similar phenomenon was also observed on the matrix protein, which was treated with L-cysteine and sulfite solutions. The entire protein portion was decomposed into pieces, the protein binding was destroyed and the starch grains were exposed and released. However, the protein fragments accumulated together in the form of blocks after treatment with L-cysteine solution. Visible holes on the surface of starch grains were seen in the sample treated with L-cysteine solution. Observation of the starch grains showed that L-cysteine played a significant role in the modification of starch. For the corn endosperm immersed in morelberry (Fig. 2c), the protein matrix entirely wrapped the starch grains and there were lesser number of exposed starch grains. However, compared to the untreated ones, the protein matrix was thinner and had many small holes, which may have been caused due to the partial protein matrix structures being destroyed. The protein and the other components were dissolved in the lactic acid and enzymes generated in the process of fermentation of morel berry and the binding modes with the starch were destroyed. Figure 2b shows the results of the treatment with ethanol. It can be obviously seen that the protein matrix was not decomposed and it was intact with less exposed starch grains. Compared to the untreated one, irregular-shaped holes and gaps are present on the thick protein matrix, which may have been caused by liquefaction of the partially alcohol

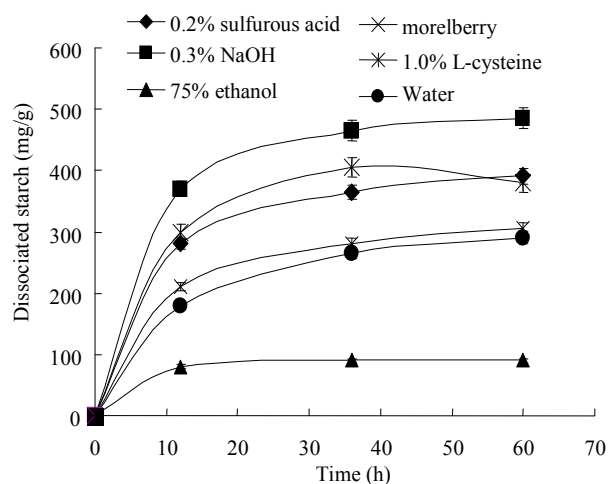


Fig. 3: The amount of dissociated starch of corn after different comsteep liquor

soluble protein. Therefore, the zein, which dissolved in ethanol, was not the protein that was tightly bound to the starch.

Yields of dissociated starch of corn samples immersed in different liquids: Figure 3 shows that there are some differences in dissociated starch contents immersed in different chemical reagents. The dissociated starch content of the sample immersed in 0.3% NaOH solution, 0.2% Sulfurous acid and 1.0% L-cysteine solution is higher than that of reference water. The effect in case of the sample immersed in 0.3% NaOH solution was found to be the best, with highest dissociated starch content. The time required for the saturation of the grain in water was the shortest and it reached maximum in about 20 h. This was due to the fact that the structure of cellulose was destroyed by saponification reaction in alkali solution, which partially dissolves the hemicellulose and lignin in the seed coat. This facilitates the fast entry of water into the grain. The effect of 0.2% sulfurous acid and 1.0% L-cysteine solutions was also obvious. However, the water-absorption saturation time for the corn grains was more than NaOH solution. It reached a maximum in about 36 h. The content of dissociated starch of the sample immersed in 1.0% L-cysteine solution is slightly more than that immersed in 0.2% sulfite acid. With an increase in immersion time, the dissociated starch content reduced. The dissociated starch content of the sample immersed in more lberry was found to be almost the same as that of reference water. However, the speed of water absorption of the grain was higher as compared to that of water immersed sample and it reached the saturation point in about 24 h. The effect in case of immersion in more lberry was found to be the worst. With an increase in immersion time, there was almost no change in the dissociated starch content and was in fact found to be lower than that of water reference. In addition to this, the grain was not softened

or swollen in the process of immersion. The color of liquid became yellow, probably due to dissolution of a certain amount of pigment and alcohol soluble protein. The measured dissociated starch content was consistent with the micrographs of the samples after treatment with different reagents. Therefore, it can be said that the microstructure between the starch and protein in corn endosperm cutin region was affected by different comsteep liquids.

Discussion: In the corn endosperm, starch grains were wrapped in the protein matrix and starch and protein were closely combined. In order to release starch, the combination of starch grains and the protein matrix must be broken or weakened, so that starch gets dissociated in the process. The mode of action between starch grains and protein and the starch extraction had close relationship. The protein matrix of the corn endosperm was studied using several selected reagents to treat the corn endosperm. Depending on the constitution and characteristics of protein the mode of action between starch and protein matrix in corn endosperms was studied and also the changes in the microstructure of starch grains and matrix protein were noted. The protein in corn endosperm mainly consists of gluten and zein. Zein has specific solubility and it is soluble only in 60-95% alcoholic solution. On the other hand, gluten is not soluble in either water or alcohol, however, it is soluble in dilute sulfuric acid and dilute alkali solutions (Chaohui *et al.*, 2006). The photomicrograph of the endosperm after treatment with NaOH solution and ethanol showed that the matrix protein in the endosperm treated with alkali was adequately broken down and the degree of protein cross-linking and polymerization was reduced. The solubility of the protein was increased due to the destruction of the secondary bonds, such as the disulfide bonds and hydrogen bonds present in the protein molecules in the endosperm of the grain by the action of the alkali solution, such that the starch grains were released. However, in case of immersion in ethanol, although the alcohol soluble protein was dissolved, the structure of matrix protein was still not decomposed as a whole and it was closely integrated with the starch grains. This explains that the protein which surrounds the starch grains and tightly wraps around the starch grains is composed of glutenin and that the alcohol soluble protein was embedded in the gluten network structure.

Sulfurous acid immersion is the commonly used method for the industrial production of corn starch. Haros *et al.* (1995) discovered that the addition of sulfite acid solution could accelerate the absorption of moisture in corn and the yield of starch and glutenin was increased by increasing the sulfite acid concentration. In the process of immersion, sulfite acid could destroy the intermolecular and intra-molecular disulfide bonds in glutenin so that the protein network structure gets

irreversibly damaged resulting in increased permanent solubility of the protein which facilitates washing and separation of starch. As the reducing agent, L-cysteine can destroy the disulfide bonds in the protein molecules, the spatial structure of protein molecules is destroyed by its active sulfhydryl groups (-HS-). However, it is different from the permanent damage caused by sulfurous acid. It is reversible in case of L-cysteine, in which the cleaved disulfide bond and the protein fragment which contains-HS- can be re-oxidized to form a new disulfide bond (Guoheng, 2010). Figure 1e and 2e in the test showed that the protein matrix fragments treated with L-cysteine gathered together, which is consistent with the reversible reactions between the disulfide bonds in protein molecules. The gathered protein fragments were re-wrapped by the starch grains which affected the further separation of starch and protein. In addition, Fig. 1 and 2d to f obviously showed that there were some protein matrix fragments on the surface of the released starch grains treated with the reagents. This explains that the protein matrix and starch grains in the corn endosperm not only had a simple wrapping structure but also combined protein and starch grains. The two parts to be combined, in terms of chemical bonds or interaction is yet to be studied further.

Morelberry is a kind of traditional starch extraction method to extract starch of mung bean and sweet potato. Morelberry could promote the separation of starch and protein from the other impurities in the process of production of starch using microbes which rapidly settle the starch. Moreover, lactic acid and enzymes generated in the process of microbial fermentation can break down the large protein molecules into smaller peptides in the process of starch purification. Dailey *et al.* (2000) reported that the yield of starch was increased and starch was easily separated in this process. Singh *et al.* (1999) also reported that the yield of starch increased and that the starch could be easily separated from the protein by adding lactic acid in the immersion liquid. The protein content in the starch region was reduced by adding lactic acid to the immersion liquid. Teng *et al.* (2008) found after the broken corn grains immersed in Bifida ferment lysate, that the purity of the extracted starch increased, the content of amylose in starch was increased, gelatinization temperature of starch was reduced and the processing properties of corn were significantly improve.

CONCLUSION

Based on the investigation of the endosperm structure changes of corn under different comsteep liquor, the structure of starch-protein-combination in corn endosperm was explored. By analysis of the microscopic structure and morphological change of

starch and protein in corn endosperm in different comsteep liquor, it is demonstrated that the main reason which the starch grains were tightly wrapped by Glutenin is the intermolecular disulfide bonds in glutenin. In addition, a part of the protein combined with starch granules directly. The study provided a reference for exploring the structure of binding of starch and protein and their interaction in corn.

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