The Significance of Insoluble Protein Solubilization in Corn Steeping

RACHEL BISS[1] and URI COGAN[2]

ABSTRACT

Adequate determination of the end point of the corn steeping process is important for ensuring the yield and quality of the starch produced. In this study, changes in the soluble and insoluble corn proteins during steeping were investigated. A correlation was found between the protein content of the starch and the corn insoluble protein. The extent of solubilization of the corn insoluble proteins during steeping can serve as a sensitive indicator of the steeping end point.

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The major objectives of corn steeping are to induce chemical and physical changes in the kernel that will result in leaching of soluble components, in effective separation of the endosperm from the germ and hulls, and in quantitative separation of starch and protein during the wet milling and fractionation steps (Watson 1984). The separation of starch and protein is the most difficult step to achieve and constitutes the bottleneck of the entire process.

The steeping process is costly because of its long duration and high energy requirements. Consequently, suitable criteria for the determination of the proper end point of this process are of crucial importance. Nonetheless, tests presently employed by the industry, namely, the extent of water absorption and the leaching of soluble solids, are far from satisfactory, and the need for the development of objective tests is often cited (Grindel 1965, Kempher 1977, Watson et al 1951).

Water-insoluble proteins, namely zein and glutelin, are the major proteins of corn. Starch granules are known to be entrapped within the insoluble protein matrix (Knight 1969). It was therefore of interest to examine the relationship between the loosening up of the glutelin matrix and the ease of separation of starch from protein, hence the starch quality.

[1]Galam Ltd., Menashe Post, Israel.
[2]Department of Food Engineering and Biotechnology, Technion, Haifa 32000, Israel.

MATERIALS AND METHODS

Corn was yellow dent no. 2. Commercial grade sulfur dioxide was obtained from Fertilizers and Chemicals Co., Ltd., Haifa, as a 30–32% solution of sodium bisulfite (pH 3.8–4.2). A solution of 6%

analytical grade sulfurous acid was used in the laboratory experiments.

Industrial Steeping System

A typical countercurrent steeping system consisting of 18 tanks was employed, with the bisulfite solution being fed into the last steep. For analysis of the unsteeped corn (zero time), a 75-g sample of corn mixed with 150 g of distilled water was held at room temperature for 24 hr and subsequently ground.

Analytical Procedures

Distilled water (100 g) was added to a sample of 100 g of steeped corn, and the mixture was blended for 3 min at the highest speed in a household blender. A sample of 6–8 g of the ground corn was dried at 70°C in an oven and subsequently in a vacuum oven at 105°C for 2 hr. A 4–6 g sample of the ground corn was assayed by the Kjeldahl procedure (AOAC 1980). The ground corn was filtered on a filter paper (Whatman No. 1), and 6–8 g of the filtrate was assayed for dry substance as already described. The filtrate of the ground corn was assayed by the Kjeldahl method to give the amount of soluble protein. The insoluble protein content was obtained by subtracting the soluble from the total protein.

Laboratory Wet Milling Procedure

The residue of the filtered corn was transferred to a 40-mesh screen and washed four times, each with 50 ml of distilled water, to separate the protein and starch from the fiber. Following each wash the fiber was squeezed. Fine fibers were removed from the pooled filtrates by sieving through a 100-mesh screen. Separation of starch from protein was accomplished by three consecutive centrifugations (2,800 *g* for 5 min). Following each spin, the upper protein layer was removed, and the starch layer was mixed with a small amount of distilled water before the next spin. The resulting starch fraction was assayed for dry matter and protein content as already described.

RESULTS

All the analytical results were expressed in terms of the dry solid matter of the unsteeped corn; necessary corrections for loss of solids during steeping were made by iterations.

Changes in the total solids and in the soluble solids of corn during typical industrial steeping are shown in Figure 1. As documented in the literature (Watson 1984, Nielsen et al 1970), the rapid absorption of water during the initial stages of steeping subsides, and this parameter bears little sensitivity after 20 hr of steeping. Similarly, a rapid initial drop in the soluble solids content of the steeped kernel is followed by a rather gradual change in this parameter. The changes in total protein during a typical industrial steeping process are presented in Figure 2.

Initially there was a net influx of protein resulting in a net increase of the total protein content of the kernel. This is due to the countercurrent flow inherent to the process and results in a high protein concentration in the liquor encountering the fresh corn. These findings also suggest that no substantial leaching of the soluble albumins and globulins took place before these proteins were hydrated. After 3 hr of steeping, there was a continuous decrease in the total protein content, the rate of which was somewhat reduced during the second half of the process (Fig. 2).

The soluble protein content also increased during the first 3 hr of steeping and then began to decrease (Fig. 3). However, as distinct from the total protein pattern (Fig. 2), the soluble protein content underwent a second temporary rise during the 18–24 hr period of the process, and thereafter decreased to a final value comparable to the soluble protein level of the raw corn. Thus, considering the patterns of the total and of the soluble proteins, it is clear that solubilization of initially insoluble protein takes place during steeping. The rise in soluble proteins during the 18–24 hr of steeping was most likely due to solubilization of structural proteins

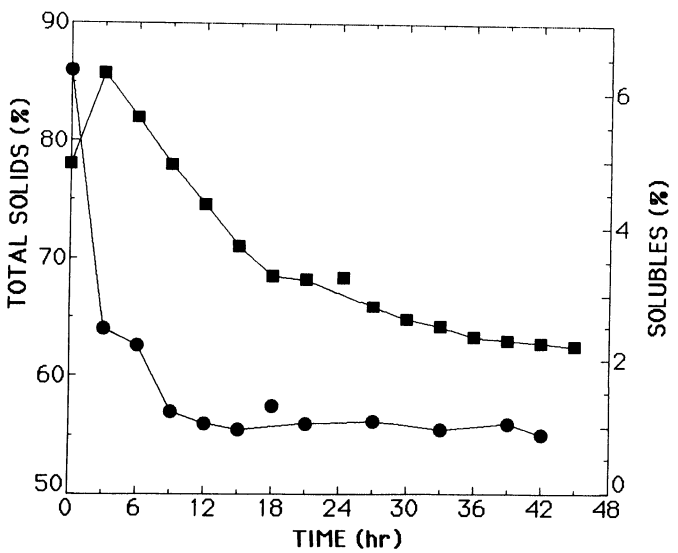

Fig. 1. Changes in the total solids (•) and in the soluble matter (■) of corn during a typical industrial steeping. Data points represent the average of three determinations.

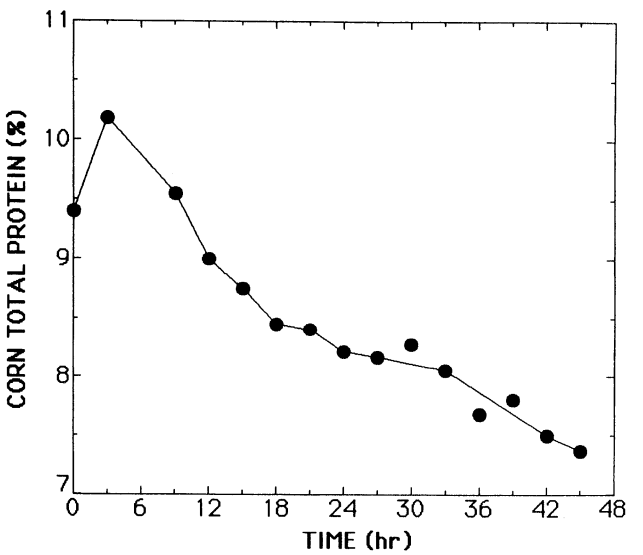

Fig. 2. The total protein pattern of corn during a typical industrial steeping. Data points represent the average of three determinations.

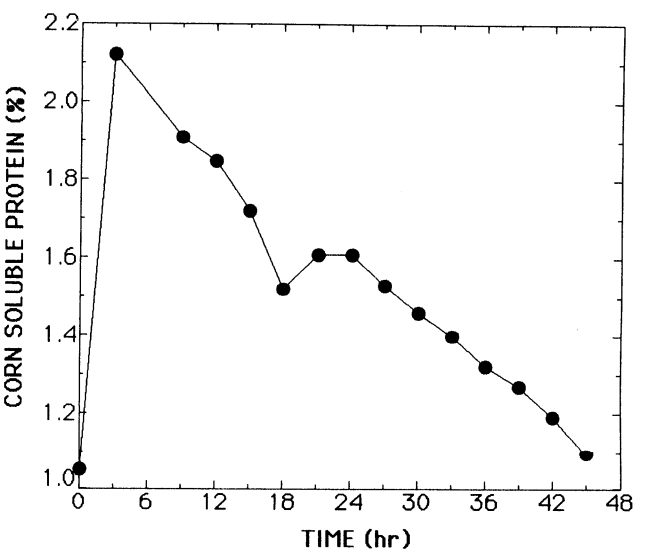

Fig. 3. The soluble protein pattern of corn during a typical industrial steeping. Data points represent the average of three determinations.

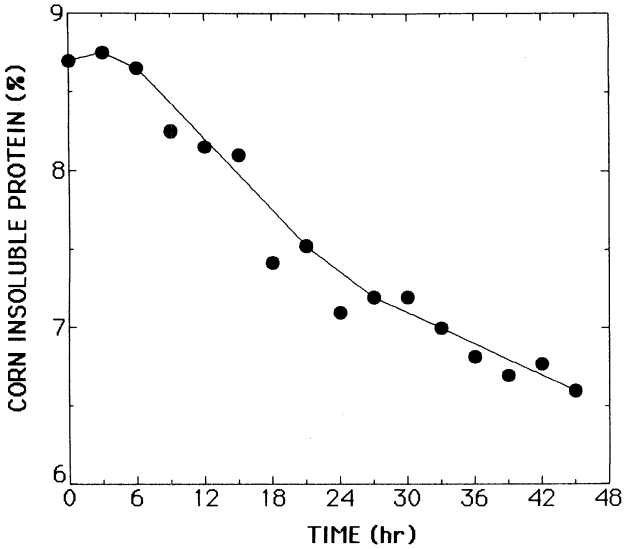

Fig. 4. The insoluble protein pattern of corn during a typical industrial steeping. Data points represent the average of three determinations.

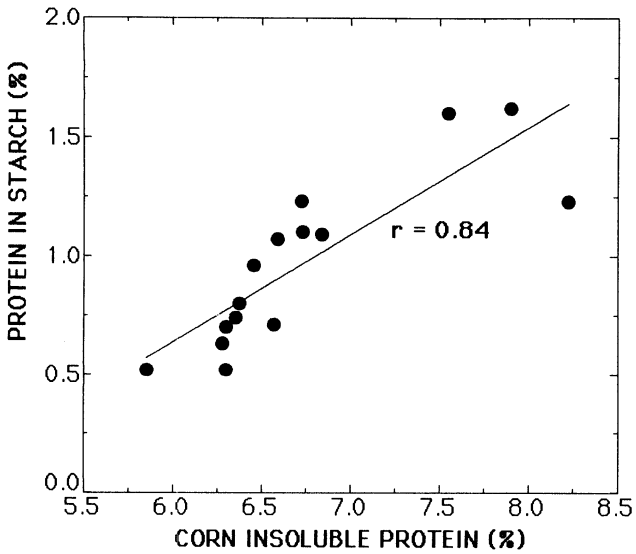

Fig. 5. The relationship between the protein content of crude starch obtained by laboratory wet milling and separation and the insoluble protein content of the steeped corn. Data points represent the average of three determinations; r is the correlation coefficient obtained by linear regression analysis.

upon the introduction of SO_2 to the process.

The changes in the insoluble protein upon steeping obtained by a simple material balance of the total and of the soluble proteins is shown in Figure 4. The rate of degradation of the insoluble protein during the steeping process appears to consist of three phases (Fig. 4): a lag period with little or no change (0–6 hr), followed by rapid (6–24 hr) and gradual (24–45 hr) solubilization phases. Because the starch granules are entrapped within the glutelin network, it was of interest to establish the relationship between the degradation of insoluble protein during steeping and the removal of protein from starch during the subsequent milling steps. A laboratory wet-milling procedure was used to assess the suitability of corn for milling during the various stages of the steeping process. The relationship depicted in Figure 5 shows the correlation ($r = 0.84$) between the protein content of the crude starch obtained by the laboratory wet-milling method and the insoluble protein content of the steeped corn.

Examination of the relationship between the protein content of the crude starch and the insoluble protein content of the corn along the steeping process (Fig. 6) indicated that at the later stages of the

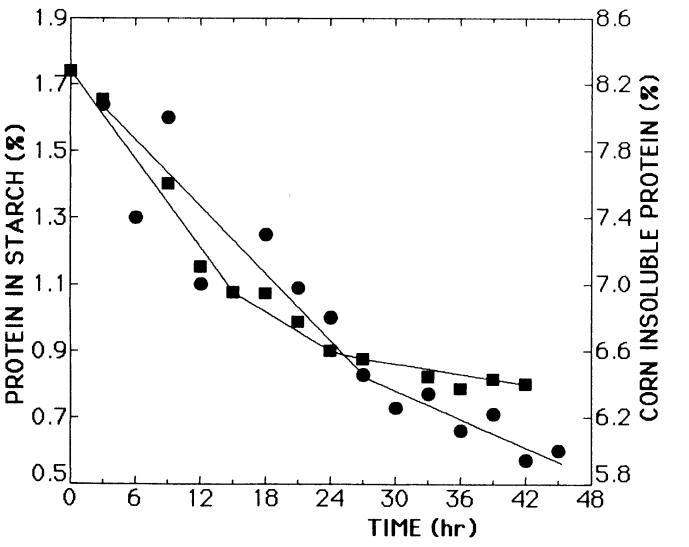

Fig. 6. The insoluble protein pattern of corn (■) and the respective pattern of the protein content of the crude starch (●) as a function of steeping time. The crude starch was derived from the steeped corn by a laboratory wet milling and separation procedure. Data points represent the average of three determinations.

process a small decline in the corn insoluble protein resulted in a substantial decrease in the protein content of the starch.

DISCUSSION

Milling improperly steeped corn will result in starch of poor quality or will necessitate the sacrificing of yield or of production capacity for quality (MacMasters et al 1954). A low protein content of the starch is one of the major quality requirements. Approximately 17% of the corn proteins consist of albumins and globulins (Paulis and Wall 1969), which readily undergo dissolution and diffusion during the steeping of the kernel. The commonly accepted figure that approximately 20% of the corn proteins leach out during the steeping process (Miwa 1980) may lead one to conclude that very little, if any, of the matrix proteins undergo solubilization during steeping. However, examination of the total and soluble protein content of the kernel throughout the course of steeping (Figs. 2 and 3) revealed that there was a continuous breakdown of insoluble protein, thereby generating additional soluble proteins. These compensate for the soluble proteins that diffuse outward from the kernel. Overall, 20–25% of the initially insoluble protein (depending on the SO_2 concentration) was solubilized during the steeping process.

The correlation between the protein level of the crude starch and the insoluble protein content of the steeped kernel (Fig. 5) supports the notion that some decomposition of the glutelin matrix during steeping is essential for the quantitative recovery of high quality starch. The extent of solubilization of the insoluble kernel proteins appears to be an important steeping parameter. Monitoring this parameter by means of a laboratory wet milling procedure can ensure proper assessment of the state of the steeped corn and of its suitability for wet milling.

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