Sulfur Dioxide in Acid Environment Facilitates Corn Steeping

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ABSTRACT

An effective steeping procedure for corn is proposed, using sulfur dioxide under acidic conditions. Corn samples were steeped at 50°C in pH 3.0–5.0 citric acid and sodium citrate buffer solutions containing 2,200 ppm SO_2 and 2% sodium chloride. The steeping solutions were refreshed every 5 hr. The highest level of solubilization of corn insoluble proteins was achieved in the steepwater at pH 3.0 and 3.5, with protein degradation ceasing after 20–25 hr. The partition of SO_2 between the corn kernel and the steepwater increased markedly with decreasing the pH of the latter. Apparent partition coefficients of SO_2 (mean ± standard deviation) of 2.23 ± 0.09, 1.64 ± 0.05, 1.16 ± 0.12, and 0.78 ± 0.06 were obtained for steeping solutions at pH 3.0, 3.5, 4.0, and 5.0, respectively. A mechanism is proposed to explain the effective cleavage of the disulfide bonds of the insoluble glutelin matrix, with the subsequent release of the starch granules by the SO_2 under acidic conditions.

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.

Conventional corn steeping occurs over a period of 24–40 hr and consists of a lactic fermentation phase followed by a sulfur dioxide treatment phase. Inherent in the process is an interrelationship between SO_2 levels and development of the lactic fermentation and total titratable acidity. When high levels (>2,000 ppm) of SO_2 are introduced into the process water, fermentation and acidity develop rather slowly and at the later stages of the process. Characteristic of the conventional countercurrent process is an intermediate period of ≈15–20 hr, during which the rate of lactic fermentation slows due to slowly increasing SO_2 levels. Because lactic fermentation cannot occur before the SO_2 concentration drops to low levels, and because the concentration of SO_2 decreases only by oxidation and diffusion into the kernel, little can be done to control and to shorten this intermediate, rather ineffective period.

It is generally accepted that lactic acid is beneficial for corn steeping, due to its softening action on cell walls (Watson 1984) and possibly due to induction of some proteolytic activity which enhances protein degradation (Watson et al 1955, Roushdi et al 1981). The major role of sulfur dioxide in steeping is to cleave disulfide linkages, thereby loosening the protein matrix that encapsulates the starch granules (Watson 1984). The introduction of SO_2 into the process is accompanied by a rapid increase of the soluble proteins as a result of an accelerated degradation of the corn insoluble proteins (Biss and Cogan 1988). In conventional steeping however, the SO_2 action exerted on the corn kernels following the lactic fermentation step may not be optimal.

The objectives of this study were to evaluate the action of sulfur dioxide during steeping at low pH on the degradation of the corn insoluble protein matrix, and to quantify the pH dependence of the distribution of sulfur dioxide between the kernel and the steeping solution. A mechanism is proposed to explain the mode of action of SO_2 under acidic conditions.

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MATERIALS AND METHODS

Materials

The corn used in the study was a mixed hybrid yellow dent No. 2 from the United States. Undamaged kernels were selected for the laboratory-scale studies. A solution of 6% analytical grade sulfurous acid was used as the sulfur dioxide source for the laboratory steeping experiments. The sulfur dioxide used in the industrial steeping system was of commercial grade, obtained as a 30–32% solution of sodium bisulfite (pH 3.8–4.2) (Fertilizers and Chemicals Co. Ltd., Haifa).

Industrial Steeping System

This was a typical countercurrent system consisting of a battery of 18 tanks, with the bisulfite solution being fed into the last steep.

Laboratory Steeping Procedure

Corn samples (1,000 g) were steeped in 1,500 ml of 0.05*M* citric acid and trisodium citrate buffer solutions with pH levels of 3.0, 3.5, 4.0, and 5.0 at 50 ± 1.0°C. The steeping solutions contained 2,200 ppm SO_2 and 2% sodium chloride. The steeping vessels were covered to minimize SO_2 losses, and the solutions were replaced with fresh ones every 5 hr. The salt was added to simulate the salting-in effect on the corn proteins that prevails in regular steeping systems, because the periodical refreshment of the steeping solutions might have resulted in an excessive dilution of the salts leached from the corn. Samples were drawn for analysis at the end of the 5-hr intervals.

Laboratory Wet-Milling Procedure

The procedure described by Biss and Cogan (1988) was employed. A steeped corn sample (100 g) was blended with 100 g of distilled water for 3 min at the highest speed in a household blender. The ground corn was filtered (Whatman No. 1 filter paper) and the residue was transferred to a 40-mesh sieve and washed four times (each with 50 ml of distilled water) to separate the starch and protein from the fiber. 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.

Analytical Methods

The procedures for the determination of total, soluble, and insoluble proteins and the dry solid content of the steeped corn were previously described (Biss and Cogan 1988). Nitrogen

determination (N × 6.25) employed the Kjeldahl method (AOAC 1990). Free sulfur dioxide was determined by titration with 0.1*N* I_2 using starch as an indicator (AOAC 1990). Measurements of the free SO_2 inside the kernel were made on the milled corn filtrate, the results were expressed on the kernel dry weight basis for each steeped corn sample. Apparent partition coefficients of SO_2 (i.e., ratio of kernel to steepwater SO_2) were thus derived. Acidity was determined by titration of 10 ml of the steeping liquor with a 0.1*N* NaOH solution to pH 8.3.

Statistical Analysis

Experiments were done in duplicate and averaged. Analytical determinations of individual samples were done in triplicate. The significance of the results was analyzed by the analysis of variance (ANOVA) test procedure (Student-Newman-Keuls method) included in SigmaStat software (Jandel Scientific, San Rafael, CA). Differences were considered significant at $P < 0.05$.

RESULTS

The interrelationship between the sulfur dioxide levels and the development of lactic fermentation during conventional industrial steeping conditions is presented in Figure 1. This pattern suggests that the benefit of steeping per unit time is likely to be low when lactic fermentation proceeds at a low rate and the SO_2 concentrations are rather low.

The solubilization of the kernel insoluble protein by 2,200 ppm SO_2 during steeping of corn in buffered solutions of citric acid in the range of pH 3–5 is shown in Figure 2. It appears that the protein solubilization increased as the pH of the steeping solution was decreased. After 20 hr of steeping, the levels of insoluble protein were 6.61 and 6.66%, respectively, for corn samples steeped in solutions at pH 3.0 and 3.5, which were significantly lower than the insoluble protein levels of 6.92 and 7.14%, respectively, for corn steeped in solutions at pH 4.0 and 5.0 ($P < 0.05$, 0.13% average standard deviation for insoluble protein determination). Furthermore, it was observed that at the low pH, the protein degradation ceased after ≈25 hr, when solubilization of ≈23% insoluble protein was reached. The corresponding concentration of soluble proteins inside the kernel is shown in Figure 3. Clearly, the highest levels of soluble proteins were achieved under conditions of the reduced pH. After 20 hr of steeping, the levels of soluble protein inside the kernel were 1.93 and 1.87%, respectively, for corn samples steeped in solutions at pH 3.0 and 3.5, which were significantly higher than the soluble protein levels of 1.78 and 1.63%, respectively, for corn steeped in solutions

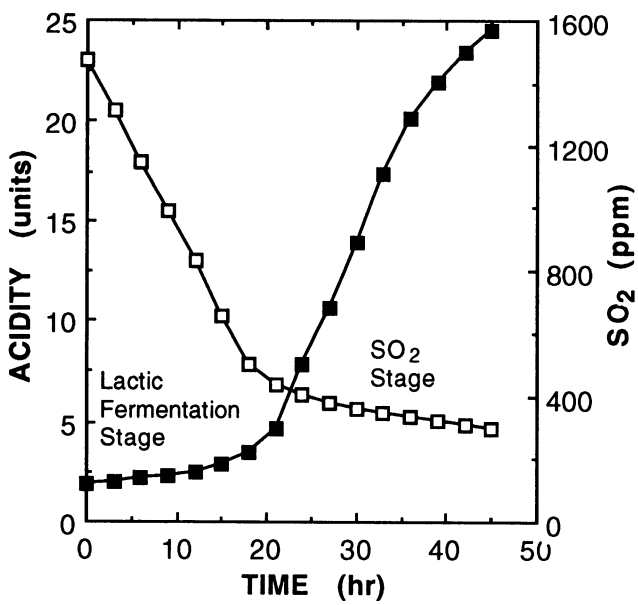

Fig. 1. Pattern of SO_2 levels (■) and the development of lactic acid fermentation (□) in steepwater during a conventional industrial steeping of corn. Acidity units are milliliters of 0.1*N* NaOH required to raise 10 ml of steeping medium to pH 8.3. Data obtained were from a routine operation of a wet-milling plant employing a countercurrent battery of 18 tanks.

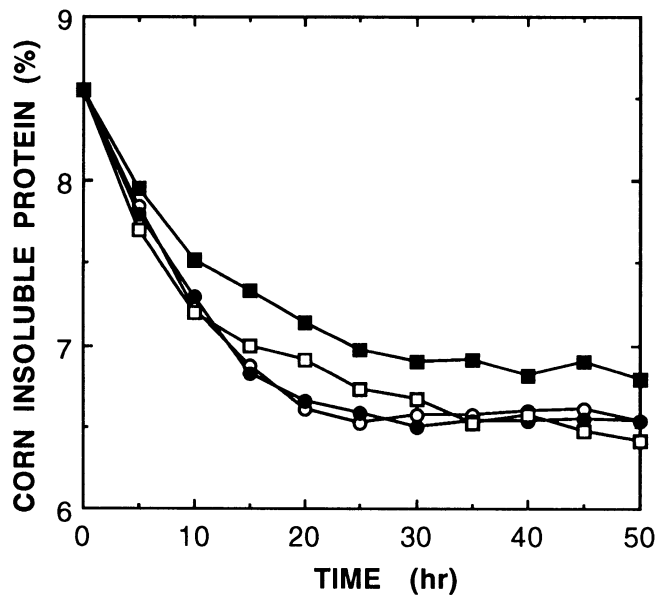

Fig. 2. Decrease in the level of the insoluble protein during steeping of corn in model solutions in the pH 3–5 range at 50°C. Solutions contained 2,200 ppm of SO_2 and 2% sodium chloride and were buffered with 0.05*M* citric acid and sodium citrate. Solutions were refreshed every 5 hr. Results are expressed on a dry weight basis. An average standard deviation of 0.13% was observed for the insoluble protein determination. Initial pH values of the steeping solutions were: pH 3.0 (○); pH 3.5 (●); pH 4.0 (□); pH 5.0 (■).

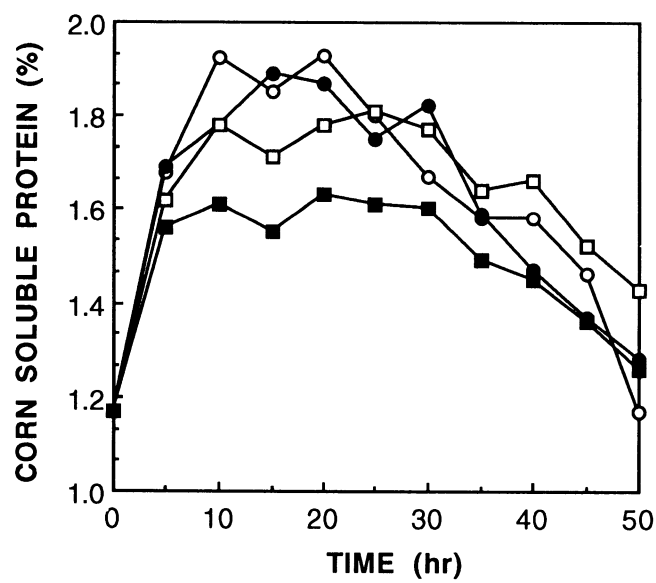

Fig. 3. Soluble protein pattern of corn during steeping in model solutions in the pH 3–5 range at 50°C. Solutions contained 2,200 ppm of SO_2 and 2% sodium chloride and were buffered with 0.05*M* citric acid and sodium citrate. Solutions were refreshed every 5 hr. Results are expressed on a dry weight basis. An average standard deviation of 0.045% was observed for the soluble protein determination. Initial pH values of the steeping solutions were: pH 3.0 (○); pH 3.5 (●); pH 4.0 (□); pH 5.0 (■).

at pH 4.0 and 5.0 ($P < 0.05$, 0.045% average standard deviation for the soluble protein determination). However, with the steeping solutions at pH 3.0–3.5, the level of the soluble protein inside the kernel was low at the end of steeping. This can be explained by the finding that degradation of insoluble proteins in the low pH range stopped after 20–25 hr of steeping (Fig. 2). Consequently, in the second half of the steeping period, soluble proteins leached out from the kernel without the generation of new soluble proteins.

The pH of the steeping medium and the corresponding pH of the corn kernel (the pH of the milled corn filtrate) are given in Figure 4A and B. The steeping medium in the range of pH 3–4 kept drifting upward, from the initial pH value of the specific buffer solution used, due to a buffering effect exerted by the continuous leaching of soluble components from the kernel into the medium. The values shown in Figure 4A are those monitored at the end of each 5-hr interval, before the solution was replaced by a fresh one. Thus, steeping solutions with an initial pH of 3.0, 3.5, and 4.0 had, respectively, approximate pH of 3.5, 3.8, and 4.2 at the end of the 5-hr periods. The pH 5.0 steeping solution was essentially unchanged at the end of the 5-hr interval. As expected, the pH inside the kernel was high initially and dropped to levels quite close to those of the medium after 15–20 hr of steeping (Fig. 4B). There was certainly a pH gradient inside the corn kernel, particularly in the initial stages of steeping, and the values observed experimentally were only overall average figures.

The SO_2 levels in the steeping media at the different pH values, and the corresponding SO_2 levels inside the kernel (measurements made on the milled corn filtrate) are shown in Figure 5A and B. Obviously, the rate of penetration of the SO_2 into the kernel increased as the pH of the steeping medium was decreased, and higher steady-state average levels of SO_2 were obtained inside the corn at the low pH range (pH 3–3.5). In this pH range, the concentration of SO_2 inside the kernel appears to have reached nearly maximum levels after 15 hr of steeping, whereas with steepwater in the range of pH 4–5, the level of SO_2 inside the kernel increased with time up to 40 hr of steeping. The apparent constant levels of SO_2 inside the kernel at the low pH range (3–3.5) beyond 15 hr of steeping, along with only a concomitant small rise in the SO_2 levels in the respective steeping solutions, most likely reflect losses of SO_2 due to evaporation which are more pronounced as the pH is lowered.

Apparent partition coefficients of free SO_2 between the kernel interior and the respective steeping solution were calculated from the data of Figure 5 for steeping periods of 20–50 hr, yielding coefficients (mean ± SD) of 2.23 ± 0.09, 1.64 ± 0.05, 1.16 ± 0.12, and 0.78 ± 0.06 for steeping solutions at pH 3.0, 3.5, 4.0, and 5.0, respectively. The differences between the four derived partition coefficients were significant ($P < 0.05$). Thus, there was an

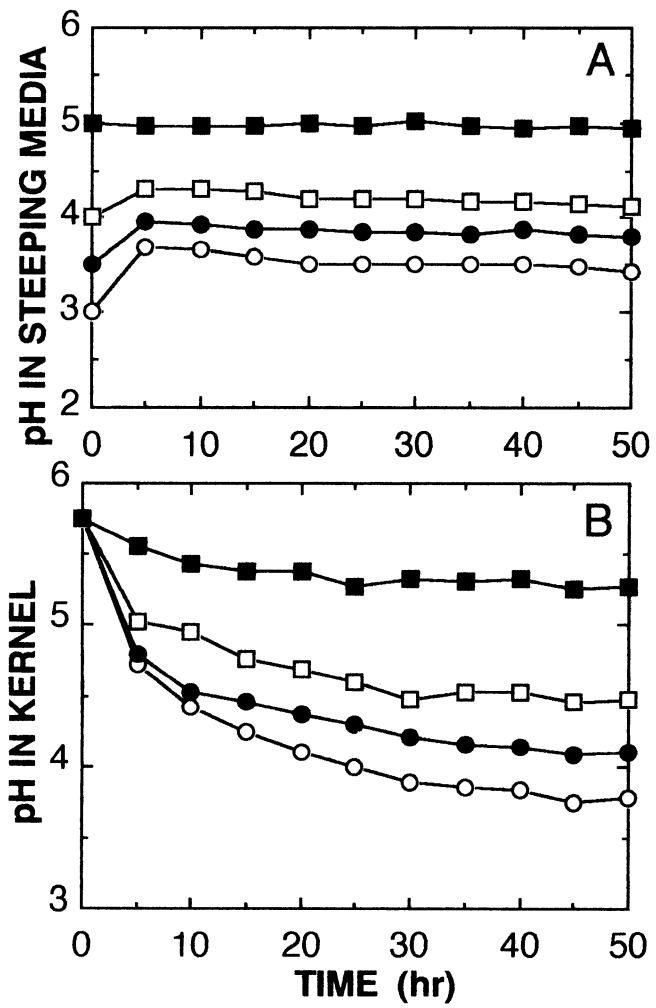

Fig. 4. Profile of pH with time of the steeping media (**A**) and of the corn kernel (**B**) (measurements made on the milled corn filtrate) for corn steeped in model solutions in the pH 3–5 range at 50°C. Solutions contained 2,200 ppm of SO_2 and 2% sodium chloride and were buffered with 0.05*M* citric acid and sodium citrate. Solutions were refreshed every 5 hr. Results shown were those monitored at the end of each 5-hr period, before replacing the solution with a fresh one, and reflect the buffering effect of the corn on the steeping medium. Initial pH values of the steeping solutions were: pH 3.0 (○); pH 3.5 (●); pH 4.0 (□); pH 5.0 (■).

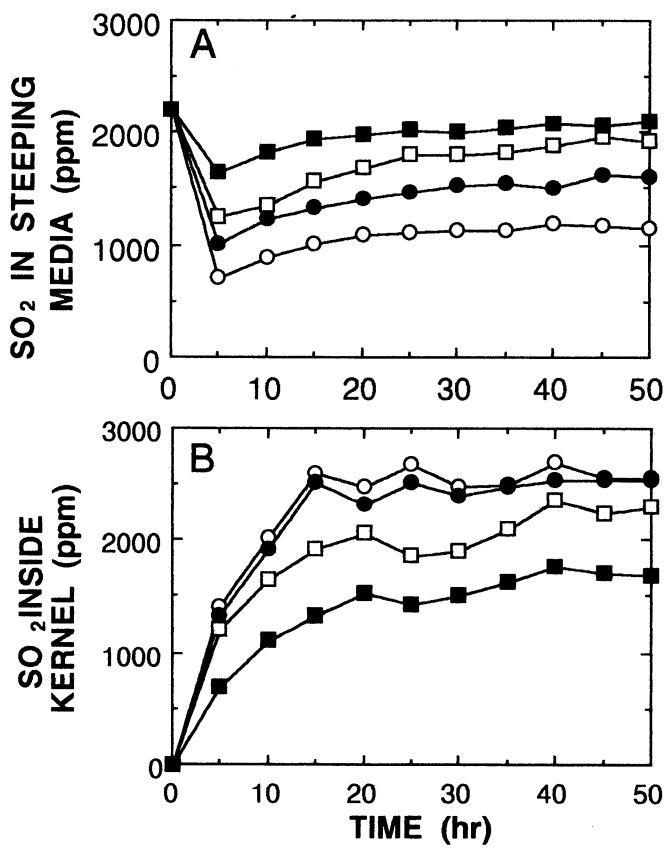

Fig. 5. Distribution of SO_2 between the steeping media (**A**) and the corn kernel (**B**) for corn steeped in model solutions in the pH 3–5 range at 50°C. Solutions contained 2,200 ppm of SO_2 and 2% sodium chloride and were buffered with 0.05*M* citric acid and sodium citrate. Solutions were refreshed every 5 hr. Measurements of the free SO_2 levels inside the kernel were made on the milled corn filtrate. Values are expressed on a dry weight basis for each steeped corn sample. Initial pH values of the steeping solutions were: pH 3.0 (○); pH 3.5 (●); pH 4.0 (□); pH 5.0 (■).

inverse relationship between the apparent SO_2 partition coefficients and the medium pH range (3–5) that could be best described by a second-order polynomial fit (Fig. 6). A partition coefficient of 1.0 appeared to correspond to a steeping solution at about pH 4.3 under the experimental conditions used.

It was of interest to examine the pH profile and the SO_2 distribution between the corn kernel (measurement made on the milled corn filtrate) and the steeping medium in the conventional industrial steeping process (Fig. 7). Initially, the pH of the corn is substantially higher than that of the steepwater, but within 15–18 hr, it drops to a level similar to that of the medium (Fig. 7A). The partition of SO_2 between the milled corn filtrate and the steeping medium in conventional steeping was lower than one throughout the entire process under the steady state conditions, with values of 0.55–0.65 at the second phase of the process (Fig. 7B). Thus, at the termination of steeping, SO_2 analysis of the contents of the last tank revealed 2,180 ppm in the steeping medium and only 1,260 ppm in the kernel. Furthermore, at the phase where the corn encounters high SO_2 levels (in the later stages of the steeping), the pH inside the kernel is ≈4.2, which is close to that of the steepwater (Fig. 7A).

Starch of high quality should contain minimal quantities of contaminating protein. The relationship between the degradation of the corn insoluble proteins in conventional steeping and the quality of the produced starch was established in an earlier study (Biss and Cogan 1988). We used the laboratory wet-milling procedure to assess the extent to which the starch granules were freed from the protein matrix during the steeping studies. A linear relationship was found between the protein content of the crude starch and the insoluble protein content of the steeped corn under the steeping conditions in which the SO_2 was applied at the reduced range of pH 3.0–3.5 (Fig. 8). The observed correlation coefficient (r = 0.898) indicates, as one would expect, that also under the particular steeping conditions examined in the present study, (exposing the corn to SO_2 at low pH from the initiation of steeping) solubilization of the insoluble proteins of the kernel during the process is crucial for the attainment of high quality starch and can serve as an indicator of the steeping endpoint.

It is important to note that the quality of the starch prepared from corn steeped in the steeping solutions at pH 3–3.5 was essentially identical to that prepared from corn steeped at pH 5.0, as observed by evaluation of the derived starches with the Brabender Amylograph (Table I).

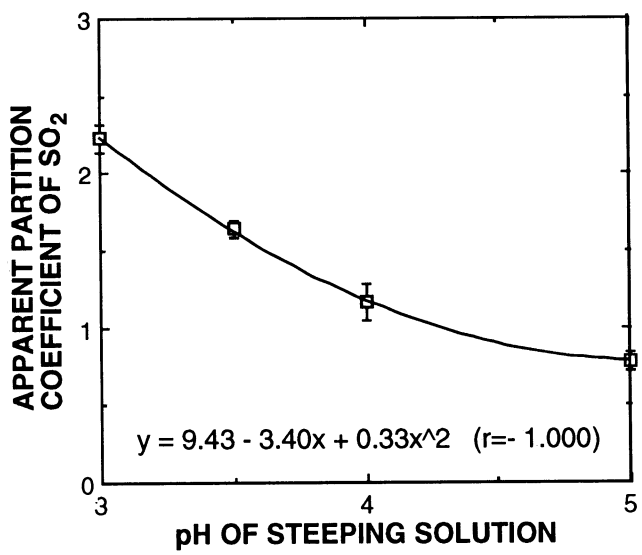

Fig. 6. The pH dependence of the apparent partition coefficients of free SO_2 between the steeped corn kernel (measurements made on the milled corn filtrate) and the steeping solution. Apparent partition coefficients (mean ± standard deviation) were calculated from the data of Fig. 5 for steeping periods of 20–50 hr. The pH values correspond to the initial pH of the steeping solutions. r = correlation coefficient for a second-order polynomial fit

DISCUSSION

Sulfite ion (SO_3^{-2}) and not bisulfite ion (HSO_3^-) was shown to be the prime reactive species in cleaving S-S bonds in a variety of disulfide compounds to form S-sulfonates according to the following equation (Cecil and McPhee 1955, McPhee 1956):

$$\text{Protein-S-S-Protein} + SO_3^{-2} \rightarrow \text{Protein-S}^- + \text{Protein-SSO}_3^-$$

As an aqueous solution of SO_2 forms the following equilibria (Watson 1984):

$$H_2SO_3 \leftrightarrow H^+ + HSO_3^- \quad pK_{a1} = 1.81$$
$$HSO_3^- \leftrightarrow H^+ + SO_3^{-2} \quad pK_{a2} = 6.99$$

where the pH in the vicinity of the protein molecules will be a determinant factor governing the rate of S-S bond cleavage by SO_2.

The present investigation demonstrates that the simultaneous action of SO_2 and acidity (high SO_2 levels at pH 3.0–3.5) enable

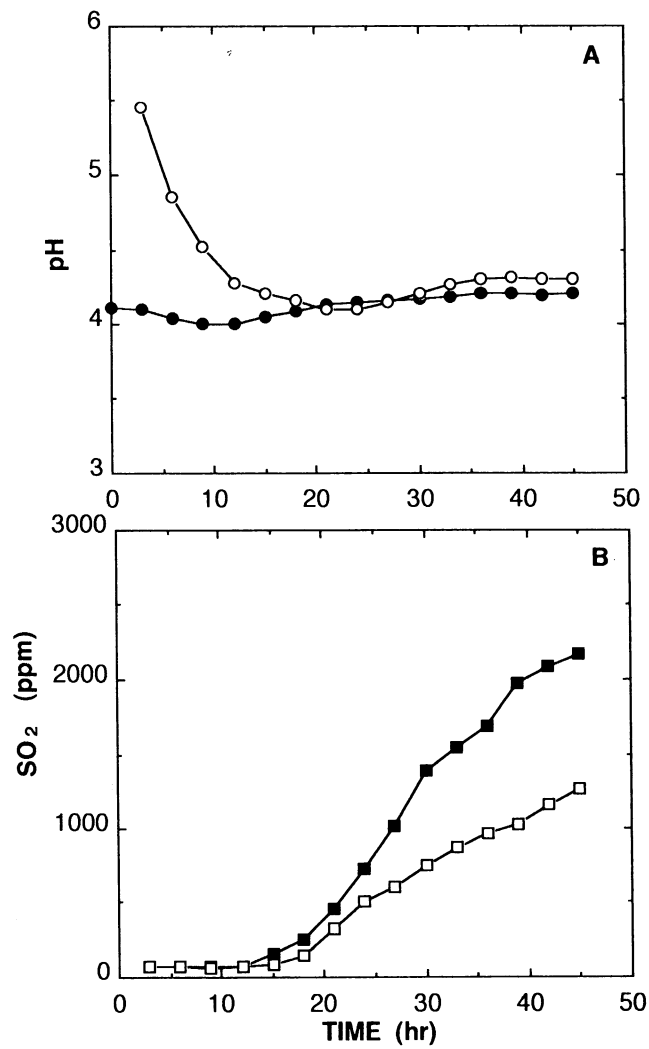

Fig. 7. A, The pH profile of steepwater (●) and corn kernel (○). **B,** The SO_2 levels of steepwater (■) and corn kernel (□) in a conventional industrial steeping. Values for the corn were obtained from measurements made on the milled corn filtrate and expressed on a dry weight basis for each sample of steeped corn.

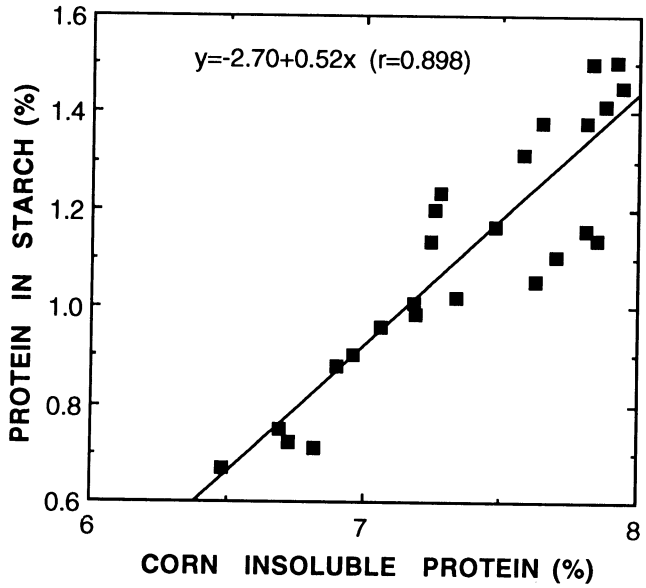

Fig. 8. Relationship between the protein content of the crude starch, obtained by laboratory wet milling and the insoluble protein content of corn steeped in model solutions in the pH 3–3.5 range. Solutions contained 2,200 ppm of SO_2 and 2% sodium chloride and were buffered with 0.05*M* citric acid and sodium citrate. Solutions were refreshed every 5 hr. *r* = linear correlation coefficient.

TABLE I
Pasting Temperature and Viscosity of Starches[a] Prepared from Corn Steeped with pH 3–3.5 and pH 5 Steeping Solutions

	Starch from Corn Steeped at	
	pH 3–3.5	pH 5
Pasting temperature (°C)	71	71
Peak viscosity at 95°C (BU)	880	900
Viscosity after 1 hr at 95°C (BU)	760	760
Viscosity after cooling to 50°C (BU)	1,500	1,500

[a] Starch slurry prepared from 40 g of starch and 420 g of water.

rapid rates of endosperm insoluble protein solubilization. We hypothesize that this is due to effective penetration of sulfur dioxide into the kernel while the interior is still maintained at a high pH.

In accordance with the foregoing results, we would like to present the following mechanism for the action of SO_2 on the corn proteins under acid pH.

At any pH, the SO_2 is present in its various forms, the relative concentrations of which are dictated by the actual pH. From a kinetic point of view, sulfurous acid in its unionized form is the species that can effectively penetrate the kernel (Lindsay 1985). This is so because the ionized forms are likely to be hindered by the charged surfaces of cellular membranes and cell walls and, consequently, possess poor permeability. As the free acid penetrates into the kernel, the equilibrium conditions dictated by the pH lead to the generation of additional undissociated sulfurous acid molecules in the steeping medium.

Inside the kernel in the initial period of steeping, the pH is relatively high (≈ pH 5.8, Fig. 4B) and this causes the H_2SO_3 penetrating the kernel to dissociate. The ionized forms, which under these circumstances consist of relatively significant proportions of SO_3^{-2}, can then act effectively to cleave the protein disulfide bridges. With time, a pH gradient is formed in the kernel in a radial direction. As the pH inside the kernel drops locally, free sulfite and bisulfite ions in the external layers will tend to reassociate to the free acid. These newly formed H_2SO_3 molecules will then continue to penetrate deeper into the kernel and will further dissociate into the ionized forms when reaching inner layers of the endosperm, which still possess relatively high pH levels. Consequently, the glutelin matrix becomes available to degradation by the active forms of SO_2 in a radial direction and in a continuous manner. Thus, the unique conditions of low pH in the steeping medium and high SO_2 levels from the onset of steeping enable the creation of pH gradient inside the kernel, which acts as a driving force in pushing sulfurous acid deeper into new regions where it dissociates and cleaves the protein, thereby releasing the starch granules. The effective penetration of SO_2 into the kernel at low pH is evident from the high SO_2 partition coefficients between the kernel and the steeping medium, which were 2.23 ± 0.09 for steeping medium of ≈ pH 3 (Fig. 6).

Contrary to the above, the conditions that prevail in conventional steeping with respect to the SO_2 action on the glutelin matrix are quite unfavorable. The range of the steeping medium pH in conventional steeping varies at 4.0–4.2 throughout the process (Watson 1984) (Fig. 7). With a pK_{a1} = 1.81 for the first dissociation reaction of H_2SO_3, this means, that >99% of the SO_2 in the steeping medium is present in the ionized bisulfite form and, therefore, its penetration into the corn is relatively poor. In addition, when the pH inside the kernel is still high (during the initial phase of the process when the protein can be acted on effectively by the SO_3^{-2} form), the levels of SO_2 in the steepwater are very low (Fig. 1). Furthermore, when elevated levels of SO_2 reach the corn in the second phase of the process, the pH inside the kernel is already quite low (after being exposed to lactic fermentation for a long period). Consequently, the cleavage of disulfide linkages will be relatively ineffective as only negligible amounts of sulfite ions will be present within the protein matrix. All this is further manifested by the low SO_2 partition coefficients of 0.55–0.65 between the corn and the steeping medium demonstrated for the conventional industrial steeping (Fig. 7B).

In conclusion, this study showed that proper timing between high SO_2 levels and acid pH in the steeping process enables the achievement of rapid degradation of the glutelin matrix, concomitant with effective release of the starch granules within 20 hr of steeping. The economic advantages of cutting the steeping time are evident in view of the large vats used and the energy expenditure required for pumping and maintaining the steeping media at 50°C.

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