

# Kinetics of Sulfite Reaction in Corn Grain

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The factors affecting the rate of SO<sub>2</sub> loss in yellow dent corn were determined over the temperature range -16 to +32 °C, moisture contents 15-38%, and initial SO<sub>2</sub> levels from 100 to 4900 ppm. The loss of SO<sub>2</sub> followed first-order kinetics, with rate constants in the range  $2 \times 10^{-9}$  to  $2 \times 10^{-6}$  s<sup>-1</sup>. The Arrhenius activation energy of SO<sub>2</sub> on whole-kernel corn was determined to be 54.9 kJ mol<sup>-1</sup>, while for ground corn it was 17.0 kJ mol<sup>-1</sup>. Moisture content and pH affected the rate. After 2 months of storage at 22 °C, 63% of the total sulfur in SO<sub>2</sub>-treated corn was accounted for by volatile sulfur compounds that did not react with iodine or lead acetate paper. During the same period, 75% of indigenous sulfur that initially was not water soluble was solubilized.

## INTRODUCTION

Sulfur dioxide (SO<sub>2</sub>) is used widely on food products as a preservative, an antioxidant, and an inhibitor of enzymatic and nonenzymatic browning (Joslyn and Braverman, 1954). Its ability to perform these functions depends not only upon the initial level of SO<sub>2</sub> applied but also upon the amount of active SO<sub>2</sub> remaining in the food product during storage (Burroughs, 1981). The ability to predict active SO<sub>2</sub> levels during storage of food products would decrease storage losses, as well as minimize sulfite residue, thiamine degradation, and unwanted bleaching effects. This study on the factors affecting the kinetics of SO<sub>2</sub> loss in yellow dent corn is part of an ongoing project that is developing a process using SO<sub>2</sub> to control microbial growth during ambient-air drying of high-moisture corn (Eckhoff et al., 1980, 1983, 1985, VanCawenberge et al. 1982). During the drying process, several applications of sulfur dioxide are required to maintain sufficient SO<sub>2</sub> levels for microbial control. Treatment applications of 0.05-0.1% w.b. have been used, although since the SO<sub>2</sub> is applied in the bin, higher levels are encountered near the SO<sub>2</sub> inlet. Knowledge of the rate of SO<sub>2</sub> loss, coupled with adsorption and diffusion information, will aid in development of appropriate treatment dosages, application procedures, and application sequencing schemes.

## REVIEW

Of the three forms of absorbed SO<sub>2</sub> (H<sub>2</sub>SO<sub>3</sub>, HSO<sub>3</sub><sup>-</sup>, SO<sub>3</sub><sup>2-</sup>), sulfurous acid is the most active in controlling microbial growth. It has inhibitory capabilities 100-1000 times greater than the bisulfite ion (Rehm and Wittman, 1962). Sulfurous acid's activity results from penetration of the microbial cell wall and binding with cellular ATP (Schimz and Holzer, 1979; Schimz, 1980) or from blocking essential enzyme activity through the reduction of cystine disulfide linkages (Chichester and Tanner, 1972). This blocking of enzyme activity is responsible for the effectiveness of sulfur dioxide in reducing enzymatic browning, although the bisulfite ion appears more active in inhibiting nonenzymatic browning (Burton, et al. 1963).

The amount of SO<sub>2</sub> in each species present in an aqueous solution depends upon the pH and temperature. The first dissociation constant of sulfurous acid is  $2.78 \times 10^{-2}$  and the second is  $1.00 \times 10^{-7}$  at 5 °C. At 25 °C the first and second dissociation constants are  $1.74 \times 10^{-2}$  and  $6.24 \times 10^{-8}$ , respectively (Beilke and Gravenhorst, 1978).

Loss of SO<sub>2</sub> in food products occurs due to either reaction with food constituents including thiamine, proteins, lipids, various sugars, aldehydes, and pectin or oxidation of SO<sub>2</sub> to form sulfate, thiosulfate, pyrosulfate, dithionate, thiourea, tetrathionate, or other polythionates. The rate and extent of these reactions in any particular commodity depends upon water activity, storage temperature, pH, the food product composition, catalytic effects, and inhibition. The chemistry of these reactions has been reviewed in detail by Schroeter (1966) and Gehman and Osman (1956). Other studies or general reviews of the reaction of SO<sub>2</sub> on food products include the following: Burton et al. 1963; Wedzicha and McWeeny, 1974, 1975; Ingles, 1962; Mangan and Doak, 1949; Gilbert and McWeeny, 1976; Sorber, 1944; Thewlis and Wade, 1974; McWeeny et al., 1974; McWeeny, 1981.

Considering the complexity of the SO<sub>2</sub>-food system, it is surprising that earlier researchers found that the overall loss of SO<sub>2</sub> could be approximated by first-order kinetics. Legault et al. (1949) investigated factors affecting SO<sub>2</sub> loss in dehydrated carrots, white potatoes, and cabbage sealed in air or nitrogen-packed jars, Nichols et al. (1939) and Stadtman et al. (1946) studied the loss investigated the loss of SO<sub>2</sub> in dehydrated apples. Data from all four studies showed that the loss could be approximated by first-order kinetics. The first-order rate of reaction also agrees with the observations of researchers studying atmospheric and aqueous SO<sub>2</sub> oxidation (Linek and Vacek, 1981; Hegg and Hobbs, 1978).

## PROCEDURES

Yellow dent corn (30% M.C.) was hand harvested, hand shelled, and fractions thereof conditioned to moisture contents of 15%, 20%, 25%, and 38% (w.b.). Breakage susceptibility, as determined by the 4-min Stein breakage test (Eman et al., 1981), was 16.1% for the corn initially at 30% moisture. The corn was held at -16 °C until used in the storage test.

Twenty-eight combinations of storage temperature, moisture content, and SO<sub>2</sub> level were investigated. Twenty-one of the conditions used were in a central composite design consisting of a basic three-level factorial, combined with five levels on each of the central axes. Each condition was run in triplicate, resulting in a total of 63 runs. Storage temperatures were -16, 2, 12, 22, and 32 °C, maintained by using standard laboratory incubators during the 3-month storage test. Sulfur dioxide levels of 0.01%, 0.033%, 0.10%, 0.33%, and 1.0% (w.b.) were used. These levels were chosen to cover the range of SO<sub>2</sub> levels generally found in the process. The seven other conditions were for 30% corn stored at 22 °C, with four SO<sub>2</sub> treatment levels of 0.056%, 0.169%, 0.508%, and 1.523%, and for ground corn treated with 0.35% SO<sub>2</sub> and held at 2, 12, and 22 °C.

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The ground corn storage test lasted 21 days.

The SO<sub>2</sub> gas was applied to the corn samples in polyethylene bags and held at 2 °C for 18 h. The corn then was aerated briefly and transferred to 150 mm × 25 mm screw-top culture tubes with Teflon-lined caps. These caps prevented desorption of SO<sub>2</sub> and eliminated any reaction with the cap material. A total of 10 tubes were used in the first replicate of each run, and six tubes were used in the other two replicates. The tubes were stored at the appropriate temperatures and, at specified times over a 3-month period, tubes were removed from storage and analyzed for total SO<sub>2</sub> and water-extractable SO<sub>2</sub> (WE SO<sub>2</sub>).

Total SO<sub>2</sub> was determined using the distillation method of the Manufacturing Confectioners Alliance and the FMF, as described by Pearson (1977). WE SO<sub>2</sub> was determined by the procedure developed by Eckhoff and Okos (1983). Values of the corn pH was determined following the AACC Official Method 02-52.

A 2-month storage study also was performed at 22 °C with 30% M.C. corn untreated and treated with 0.1% SO<sub>2</sub>. This study had two objectives. The first was to show that the culture tubes with Teflon-lined caps did not allow the SO<sub>2</sub> to desorb from the corn. The second objective was to investigate the reacted forms of the added SO<sub>2</sub> after 2 months of storage by determining the sulfur content of various liquid, gaseous, and solid fractions removed from the corn samples. Total sulfur was determined by use of a combustion-type sulfur analyzer with an infrared detector. The analysis were performed by the LECO Corp. St. Joseph, MI. Accuracy of the instrument is ±1% of the amount of sulfur in the sample. Sensitivity is 0.001% of sample weight.

A total of 23 sulfur analyses were performed. Three procedures were used in preparation of the samples for total sulfur analysis. The first was to analyze the whole kernel for total sulfur. The second was to separate the sulfur into various fractions using a hot-extraction procedure. The third procedure was a cold-extraction procedure.

The hot-extraction procedure involved grinding the stored corn, running a WE SO<sub>2</sub> analysis on 10 g of the approximately 40 g held by the culture tube, and running a total SO<sub>2</sub> analysis on 20 g of the remaining sample. The liquid and solid material remaining in the boiling flask after the total SO<sub>2</sub> analysis was completed and was carefully washed from the flask with hot, recently boiled distilled water. This material was vacuum filtered through weighed No. 1 Whatman ashless filter paper, washing with hot distilled water in sufficient quantity to remove any remaining solubles from the solid residue. The total quantity of filtrate was measured and a sample sent for analysis. The solid residue plus the filter paper was weighed, dried at 103 °C for 24 h, and reweighed. The dried residue sample was then sent for total sulfur analysis.

The cold-extraction procedure involved grinding the sample, placing 20 g in 100 mL of 25 °C distilled water, and then waiting 30 min before vacuum filtering the sample and washing with room-temperature distilled water. The filtrate and the solid residue were handled as previously described for the hot-extraction procedure.

## RESULTS AND DISCUSSION

**Regression of Data.** Average first-order rate constants (*k*) and intercepts (*C*<sub>0</sub>) for each treatment condition is shown in Table I. Each value is an average of three runs. Complete data on each run can be found in Eckhoff's (1983) thesis. A total of 44 of the 48 runs with initial SO<sub>2</sub> levels above 300 ppm had *r*<sup>2</sup> values above 0.80, with 39

**Table I. Intercepts (*C*<sub>0</sub>) and First-Order Rate Constants (*k*) for Loss of Water-Extractable Sulfur Dioxide at Various Storage Temperatures, Moisture Contents, and Initial SO<sub>2</sub> Levels**

moisture cont, % w.b.	temp, °C	SO <sub>2</sub> level, % w.b.	<i>C</i> <sub>0</sub> , ppm	<i>k</i> , ×10 <sup>7</sup> s <sup>-1</sup>
15	12	0.100	595	-1.13
20	2	0.032	125	-4.63
20	2	0.320	2359	-1.18
20	12	0.100	649	-1.80
20	22	0.032	123	-6.44
20	22	0.320	2456	-2.83
25	-16	0.100	763	-0.112
25	2	0.100	815	-1.39
25	12	0.010	init levels below 20 ppm	
25	12	0.030	98	-9.68
25	12	0.100	750	-1.87
25	12	0.320	2345	-1.51
25	12	1.000	4430	-3.45
25	22	0.100	914	-4.69
25	32	0.100	750	-11.60
30	2	0.032	320	-15.50
30	2	0.320	2695	-1.09
30	12	0.100	863	-3.60
30	22	0.032	molded after 7 days	
30	22	0.320	2791	-4.07
38	12	0.100	952	-2.94

above 0.90. Three of the four runs below 0.80 were for the -16 °C condition, which showed little loss of SO<sub>2</sub> during the 3 months of storage and were most sensitive to sampling and analytical random errors.

Rate constants for corn are approximately 1 order of magnitude larger than those determined on other food products (Bolin and Boyle, 1982; Nichols et al., 1939; Stadtman et al., 1946; Legault et al., 1949; Mangan and Doak, 1949). Preprocessing may account for some of this difference, as the fruit and vegetable products studied had been previously dried or dehydrated to low-moisture levels. In general, the rate constants for food products are several orders of magnitude less than sulfite oxidation in aqueous solutions. General reviews by Linek and Vacek (1981) and Hegg and Hobbs (1978) showed that uncatalyzed oxidation rates were generally on the order of (3-5) × 10<sup>-3</sup> s<sup>-1</sup>, while catalyzed oxidation (Fe<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup> catalysts) was generally 1 order of magnitude larger.

While there are significant mineral catalysts in food products to enhance the oxidation rate, inhibition, as observed by Fuller and Crist (1941), Sapotanitskii and Glushchenko (1962), Dev and Jain (1962), and Schroeter (1963a,b), accounts for a significant decrease in oxidation rates. Mannitol was shown to reduce the first-order rate constant by a factor of 1000 at a concentration of 3 × 10<sup>-2</sup> mol/L (Fuller and Crist, 1941). Alcohols, phenolic compounds, sugars, and other aromatic compounds also have been shown to inhibit sulfite oxidation. Corn and other cereal products contain a wide variety of volatile compounds (Maga, 1978; Hougen et al., 1971), many of which act as sulfite oxidation inhibitors.

**Comparison of Total SO<sub>2</sub> to WE SO<sub>2</sub>.** The difference observed between total SO<sub>2</sub> values and WE SO<sub>2</sub> values represents the amount of SO<sub>2</sub> bound by food constituents such as sugars and aldehydes (Eckhoff and Okos, 1983). This difference also can include oxidation products such as thiosulfate, tetrathionate, dithionate, and thiourea, which yield SO<sub>2</sub> upon acidification and boiling (Mangan and Doak, 1949). In corn, the amount of SO<sub>2</sub> expected to be bound up by the sugars and aldehydes is insignificant, due to the small amount of each contained within the kernel. Only 1-3% of the kernel is free sugar, primarily sucrose (Inglet, 1970), and according to Ingram and Vas

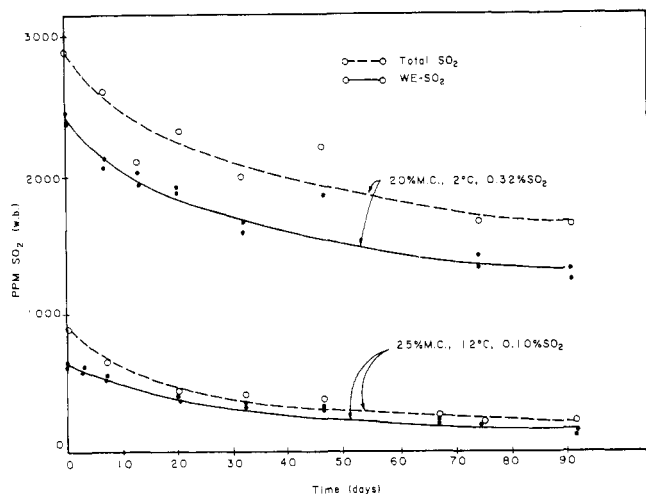


Figure 1. Representative curves showing WE  $\text{SO}_2$  and total  $\text{SO}_2$  values with time.

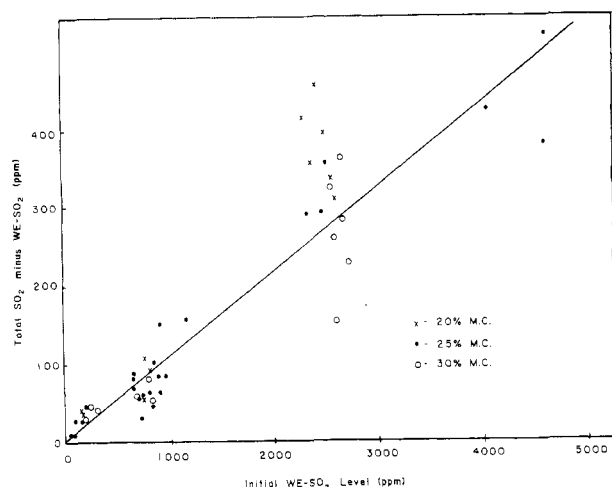


Figure 2. Average difference between WE  $\text{SO}_2$  and total  $\text{SO}_2$  values vs. initial WE  $\text{SO}_2$  level.

(1950), sucrose does not bind with sulfur dioxide. Even assuming that the  $\text{SO}_2$  binds with the reducing end of starch molecules, complete binding would account only for approximately 9 ppm of  $\text{SO}_2$ .

Throughout the storage test, total  $\text{SO}_2$  values were larger than WE  $\text{SO}_2$  values, and the difference between them,  $d$ , remained fairly constant during the course of each run. Figure 1 shows two runs representative of the experimental data. Mangan and Doak (1949) found in their studies that there was a measurable difference between the values determined by the alkali-extraction procedure they used and the Monier-Williams distillation procedure. Their study on the storage of dehydrated cabbage, with an initial  $\text{SO}_2$  level of 34 000 ppm, showed that  $d$  increased to a maximum value of 5200 ppm after 60 days of storage then decreased rapidly back to the initial  $d$  value of 1350 ppm. They concluded that the change in  $d$  was due to the development of intermediate reaction products that were detectable by distillation but not by alkali extraction. In the present tests, the levels of initial  $\text{SO}_2$  were sufficiently lower than levels in Mangan and Doak's work to result in an analytical error in determination of total  $\text{SO}_2$  and WE  $\text{SO}_2$  levels that masked any subtle changes in the value of  $d$ . Gross changes in  $d$ , as observed by Mangan and Doak, were not observed in any of the runs.

The magnitude of  $d$  appears to be due to some type of equilibrium phenomenon as can be seen in Figure 2, where the average  $d$  value for each experimental run is plotted

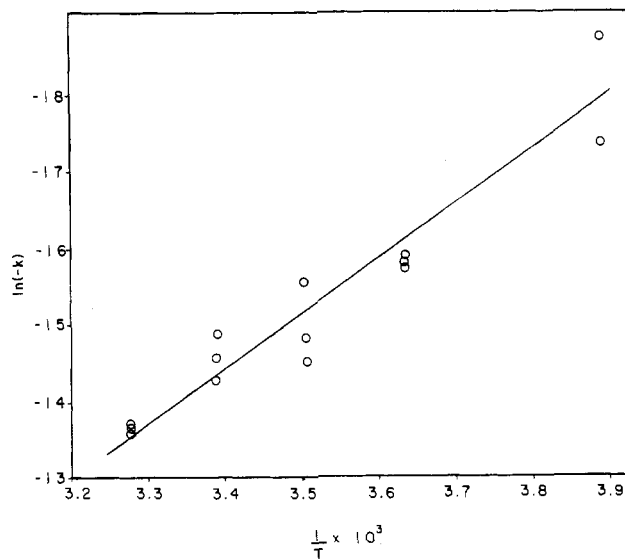


Figure 3. Arrhenius plot of first-order rate constants vs. temperature.

as a function of the initial WE  $\text{SO}_2$  level. Although there is considerable variability, a general linear trend can be observed. Most of the variability is at the 2500 ppm level, where it is evident that the moisture content of the sample affects the  $d$  value. Temperature did not correlate well with  $d$  even within a given moisture content.

**Temperature Effect.** Figure 3 shows that the first-order rate constants follow an Arrhenius-type temperature dependence with an activation energy of 54.9  $\text{kJ mol}^{-1}$ . This is similar to the value of 48.2  $\text{kJ mol}^{-1}$ , determined from the data of Nichols et al. (1939) on dried apricots over the temperature range of  $-17.8$  to  $+37.8$   $^{\circ}\text{C}$ , but is considerably lower than the value of 85.6  $\text{kJ mol}^{-1}$  determined by Bolin and Boyle (1972) for dehydrated apples between 10 and 32.2  $^{\circ}\text{C}$ . Legault et al. (1949) also found the higher values of 138–180  $\text{kJ mol}^{-1}$  in their study of dehydrated carrots, potatoes, and cabbage over the temperature range 24–49  $^{\circ}\text{C}$ .

The present study also found that the apparent activation energy for ground corn was only 17.0  $\text{kJ mol}^{-1}$  over the temperature range 2–22  $^{\circ}\text{C}$ . This difference between whole kernels and ground corn could be due to a change in the reaction mechanisms, or an increase in the number of lower energy reaction sites caused by grinding. Grinding disrupts the natural structure of the corn and exposes more surface area. This lack of agreement on the activation energy occurs in the literature on aqueous oxidation of sulfite, as well. In general, experimentally determined values for  $E_0$  ranged from 7.1 to 142.0  $\text{kJ mol}^{-1}$  (Hegg and Hobbs, 1978; Penkett et al., 1979a,b; Linek and Vacek, 1981; Larson et al., 1978; Beilke and Gravenhorst, 1978). Since aqueous solutions are homogeneous, it would seem that a change in the reaction mechanism is the most plausible explanation.

**Effect of Concentration and pH.** Figure 4 shows a strong dependence of the first-order rate constant on the initial WE  $\text{SO}_2$  level. The 25% M.C. 12  $^{\circ}\text{C}$  set of data shows a horseshoe-shaped relationship for the WE  $\text{SO}_2$  level with a minimum around 2200 ppm. The 30% M.C. 22  $^{\circ}\text{C}$  set of data shows a similar trend.

Schroeter (1963a, 1966) discusses the theoretical work of Able (1951) on the oxidation of sulfite, in which it was determined that the rate of loss of sulfur dioxide species can be expressed by the relationship

$$-d[\text{SO}_2]_T/dt = g[\text{HSO}_3^-]/[\text{H}^+]^{1/2} \quad (1)$$

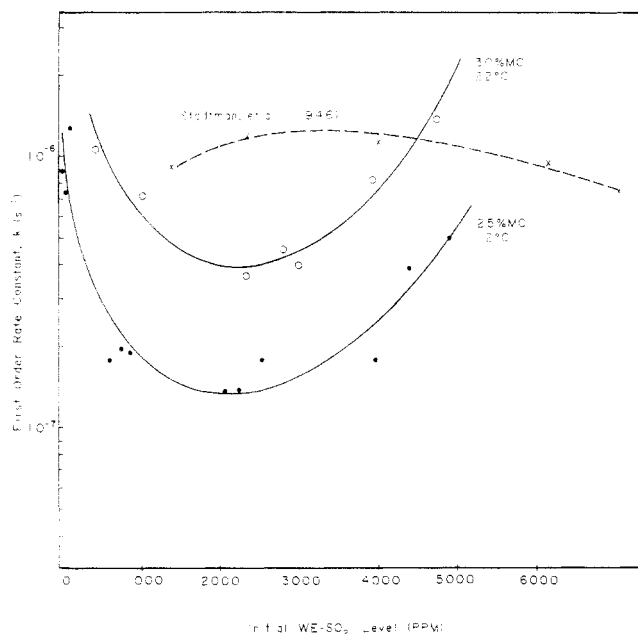


Figure 4. First-order rate constants vs. initial WE  $\text{SO}_2$  level.

where  $[\text{SO}_2]_T$  = the total concentration of sulfur dioxide species and  $g$  = catalytic constant. Schroeter used this equation to explain the observation that in solutions where bisulfite is the dominant species, the rate of oxidation is substantially decreased. He cites a 10-fold decrease due to the decrease in pH from 8, where sulfite ions dominate, to pH 4, where bisulfite ions are the major  $\text{SO}_2$  species. However, eq 1 was derived by Able (1951) considering only sulfite and bisulfite oxidation and thus is only valid for conditions with pH above 4.0. Below a pH 4.0 the rate should increase again. Sapotanitskii and Glushchenko (1962) demonstrated this phenomenon in their work on the air oxidation of ammonium sulfite, magnesium sulfite, and sodium sulfite solutions as functions of pH. The extent of oxidation was minimum at pH 4.0–4.5. Less than 1% of the sulfite was oxidized at pH 4.0–4.5, as compared with 40% oxidized during the same period at pH 3.0 and 6.0.

The relationship between pH and the initial  $\text{SO}_2$  level on corn was determined by putting 20 g of ground un-sulfited corn in 200 mL of distilled water and then injecting 5 mL of  $\text{SO}_2$  into the stirred slurry. The pH was recorded following each addition of 5 mL of  $\text{SO}_2$  (representing 677 ppm  $\text{SO}_2$ ). The relationship between pH and  $\text{SO}_2$  level is shown in Figure 5. The curve was the same for corn samples ranging from 15% to 38% moisture. Five samples of whole corn (30% M.C.) treated with various levels of  $\text{SO}_2$  were analyzed for initial WE  $\text{SO}_2$  levels and pH. The values for whole kernels coincided well with the values determined by injecting  $\text{SO}_2$  into the corn slurry. It also was found that, during storage, the pH of the corn remained constant although the amount of  $\text{SO}_2$  decreased substantially. This indicates that the products formed from sulfite reactions have an acidity of similar strength to sulfurous acid.

The pH decreased asymptotically to a value of 1.83 at an  $\text{SO}_2$  level of 34 822 ppm (255 mL of  $\text{SO}_2$ ). This is due to the buffering nature of the  $\text{HSO}_3^-$  ions and the sulfurous acid. By use of the Henderson-Hasselbalch equation (Martin et al. 1969)

$$\text{pH} = \text{p}K_a - \log \left[ \frac{[\text{HSO}_3^-]}{[\text{H}_2\text{SO}_3]} \right]$$

the pH of this buffered system should approach the  $\text{p}K_a$  value of 1.81. Since the added  $\text{SO}_2$  partitions itself between

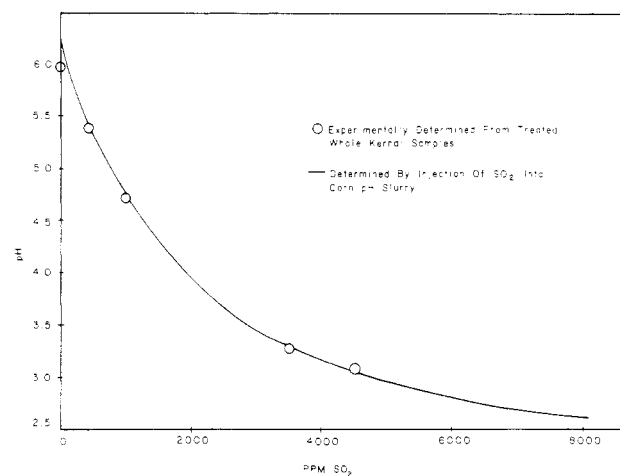


Figure 5. Relationship between pH and initial WE  $\text{SO}_2$  level.

the sulfite ion and sulfurous acid, the system will not behave like a normal buffered system in that its buffering capacity can never be exceeded. This phenomena should occur in all food products, so that pH values below 1.81 will not be encountered unless the pH is decreased by the use of another acid.

On Figure 4, a comparison is made to the work of Stadtman et al. (1946), which shows that with dehydrated apricots there is a rate dependence on the initial  $\text{SO}_2$  level that is opposite to what has been observed on the corn samples. While there is no known correlation of pH to initial  $\text{SO}_2$  levels in apricots, a similar trend would be expected. This difference between Stadtman's data and the relationship found for corn is due to the difference in composition between the two food products. Sapotanitskii and Glushchenko (1962) have shown that while ammonium sulfite, magnesium sulfite, and sodium sulfite solutions show a minimum extent of oxidation at pH approximately 4.0, calcium sulfite showed a maximum extent of oxidation at pH 4.0. They could give no explanation for the phenomenon. The only known variables were the cations in the system. Corn is composed of 0.03% calcium, 0.17% magnesium, and 0.01% sodium (Inglet, 1970), while apricots are composed of 0.017% calcium, 0.001% sodium, and 0.012% magnesium (Salunkhe, 1974). In corn, the magnesium and sodium cations that cause a minimum extent of oxidation at pH 4.0 are in excess by a factor of 6 over the calcium [ $\text{Ca}/(\text{Mg} + \text{Na}) = 0.17$ ]. This is in contrast to apricots, which have a  $\text{Ca}/[\text{Mg} + \text{Na}]$  ratio of 1.31. These trends agree with the work of Sapotanitskii and Glushchenko (1962).

**Effect of Moisture.** The moisture content of the corn samples had a significant effect on the rate of  $\text{SO}_2$  loss, as can be seen in Figure 6. There is approximately a 3-fold increase in reaction rate with an increase in moisture from 15% to 38%. Legault et al. (1949) also found that the moisture content affected the rate of  $\text{SO}_2$  loss on vegetables. Their work with dehydrated carrots, potatoes, and cabbage in the range of 5–9% moisture showed that an increase of 1.5–2.0% moisture doubled the rate of  $\text{SO}_2$  loss.

**Nature of Reactant Products.** The nature of  $\text{SO}_2$  food reactions and the products formed as a result are complex, varied, and have not yet been elucidated fully. As late as 1981, McWeeny stated "The lack of specific information about the nature of the substances into which  $\text{SO}_2$  is converted in foods is remarkable in view of the wide range of products in which it is permitted...". Prior to about 1970, observations on the type of products formed were minimal (McWeeny, 1981), and while some progress has been made in the last decade on the identification of reaction prod-

Table II. Total Sulfur in Various Samples

	sulfur (d.b.), ppm					
	replicate 1		replicate 2		replicate 3	
	0	2 months	0	2 months	0	2 months
I. untreated whole corn	1377					
II. treated whole corn	2598	2896	2655	2669	2385	2740
Hot Extraction						
III. treated						
dried residue	742		626	237	716	186
WE SO <sub>2</sub>	653		667	0	588	0
total SO <sub>2</sub> - (WE SO <sub>2</sub> )	47		134	30	96	33
liquid	1486		2010	1029	1458	848
total	2928		3437	1296	2858	1067
IV. untreated						
dried residue	622					
liquid	1074					
total	1696					
Cold Extraction						
V. treated						
dried residue						1180
liquid						572
total						1752
VI. untreated						
dried residue	1138					
liquid	401					
total	1539					

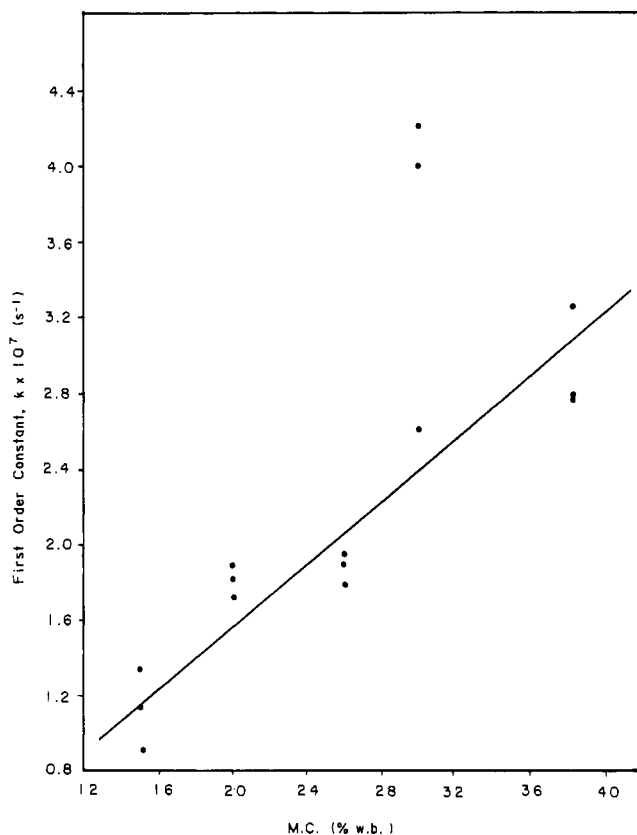


Figure 6. First-order rate constants vs. moisture content.

ucts, there has been very little work done on determining the rates and factors influencing the formation of these products in model systems or in the more complex food systems. Reactions observed in the literature can be classified into six broad categories: reactions with sugars or aldehydes, oxidizing agents, thiamine, protein, reducing sugars, and lipids (Eckhoff, 1983). An extensive general review of these reactions can be found in Schroeter (1966).

In the present study, an attempt was made to estimate the nature of the reactant products found in sulfited corn. Table II presents the results of sulfur analysis on samples

of untreated 30% M.C. corn and treated corn stored at 22 °C for 2 months. General observations of the data in Table II indicate that no SO<sub>2</sub> was lost to desorption during storage (line II). Total sulfur increased by approximately 1300 ppm due to SO<sub>2</sub> addition. Sulfur in the fractions, prepared by acidification and boiling, had values higher by approximately 300 ppm than in the whole corn samples. This most likely is due to sample variability and to additive errors in preparation of the extraction fractions.

Untreated whole corn had a sulfur level of 1377 ppm, which agrees with the range of sulfur found in corn (100–1800 ppm, with a mean value of 1200 ppm; Inglet, 1970). Duplicate values agreed within 5% of each other. The initial sulfur in the corn is due to two amino acids, cystine (average 211 ppm) and methionine (average 430 ppm), to thiamine (average 0.5 ppm), and probably to sulfate or sulfate-type products. In forage crops it has been shown that sulfur can accumulate in the plant in excess of that needed for protein synthesis. The excess is in the form of inorganic or sulfate sulfur and acts as a sulfur reserve for the plant in times of stress (Metson, 1973). Since all the corn used in this study was of one variety from one small area of the field, all the corn samples should be fairly uniform in sulfur content. Total SO<sub>2</sub> analysis of untreated corn in this study had near-zero blank values. This indicates that the sulfur in excess of that bound in cystine and methionine is in a form that does not yield SO<sub>2</sub> upon acidification and boiling. This fortifies the position that the excess sulfur exists in a form of inorganic or sulfate sulfur. Since most sulfate products are water soluble, some of the products must have existed in combined form, since cold-water extraction of the untreated sample (line VI) showed the total sulfur in the liquid at 401 ppm. This is half of the estimated 900 ppm of nonprotein sulfur in the corn.

From data of Wall and Paulis (1977), the amount of sulfur expected to be extracted into the liquid as albumin methionine is approximately 17 ppm. Water-soluble cystine sulfur is approximately 25 ppm. This would leave approximately 616 ppm of amino acid sulfur in the solid residue. The remaining sulfur in the solid residue must have been in a form that is not extracted by water at room temperature.

When the untreated corn was acidified and boiled (line IV), the remaining dried residue had 622 ppm sulfur and the liquid had 1074 ppm. Aitken (1930) determined sulfate or inorganic sulfur in grass by boiling a powdered sample in dilute HCl for approximately 80 min. Acid hydrolysis of protein requires boiling in 6 N HCl for 20–70 h (Harper, 1973). The sulfur in the dried residue would appear to be primarily cystine and methionine sulfur. The nonwater-extractable protein as estimated in the previous paragraph is 616 ppm, which is within a reasonable error of the dried residue sulfur values for the initial treated and untreated samples (lines III and IV).

Two months of storage decreased the sulfur in the dried residue by 68%. It is known that aqueous SO<sub>2</sub> at a concentration below 0.2% activates proteolytic enzymes and hydrolyzes proteins at the steeping temperature of 55 °C (Wall and Paulis, 1977). The decrease of sulfur in the solid residue observed during this storage test is most likely due to protein hydrolysis, even though the temperatures were considerably lower.

Line III also shows that the total sulfur recovered from the 2-month samples was considerably less than that recovered from the initial treated samples or the whole corn, 2-month samples. The amount lost was approximately 60% of the sulfur initially in the treated samples. The only possible times for SO<sub>2</sub> loss were during the determination of total SO<sub>2</sub> values, during filtration, or during the drying of the solid residue. It would appear that a major fraction of the added SO<sub>2</sub> was converted to a volatile form that is not reactive with iodine.

Sorber (1944) found that over 50% of the total sulfur in stored, dried apricots could not be recovered as either SO<sub>2</sub> or sulfate sulfur. He concluded that the remaining sulfur was in the form of stable nonvolatile compounds that do not precipitate upon the addition of barium chloride. However, he did not measure the sulfur remaining in the residue following the distillation procedure. On the basis of the present data, it would appear that Sorber's missing sulfur might be due to similar volatile compounds that boil over in the distillation but do not react with iodine. Thewlis and Wade (1974), using radiotracer techniques, determined that sulfur other than sulfur dioxide came over during the distillation of sulfited biscuit dough. They concluded it was sulfate; however, sulfate products such as dithionate and thiosulfate decompose to yield SO<sub>2</sub> figures as described by Mangan and Doak (1949) and would not boil over as a nontitratable sulfur product.

The nature of the volatile products found in this study is not known, however, from the data it appears that they exist in at least two forms. One is loosely bound to the corn constituents and will desorb upon heating. The second is more tightly bound and requires heat and acidification for desorption. This is shown in Table II, line V, where the cold extraction yielded a total sulfur content of 1752 ppm, while line IV shows that the hot extraction yielded only 1067 ppm. Acidification of the sample before heating resulted in 685 ppm more sulfur being lost. If the unknown compound required only heat for desorption, the 24 h at 103 °C used in drying the residue would have been sufficient to drive it off. Distillation of a stored sample of corn was performed with lead acetate paper in the vapor stream. No change in the color of the paper was observed, indicating that the volatile compound was not a sulfide.

In summary, it appears that the major products of the reaction of SO<sub>2</sub> in corn, when heated, or acidified and heated, yield volatile sulfur compound that do not react with iodine. Figure 7 shows an estimate of how the forms

	Initial	2 Month Storage
Sulfur Added As SO <sub>2</sub> (1300 ppm)	Bound and Unbound Sulfate (550 ppm)	Unknown Compound. Volatile S Product upon Heating. (1050 ppm)
	Bound SO <sub>2</sub> (100 ppm)	
	WE - SO <sub>2</sub> (650 ppm)	
Indigenous Sulfur (1535 ppm)	Bound Sulfate and Inorganic Sulfur (525 ppm)	Unknown Compound. Volatile S Product upon Acidification and Heating. (750 ppm)
	Water-Soluble Sulfate and Inorg. Sulfur (350 ppm)	B. Sulfate and ins. S. (200 ppm)
	(45 ppm)	Bound SO <sub>2</sub> (30 ppm) (100 ppm)
	Non-Water Soluble Proteins (615 ppm)	Water-Soluble Proteins (460 ppm)
		Non-Water Soluble Proteins (200 ppm)

Figure 7. Approximate distribution of sulfur in corn initially after treatment and after 2-months of storage.

of sulfur changed during the 2 months of storage. The other major change apparently occurring in the corn during storage is the solubilization of proteins. Approximately 75% of the protein that initially was not water soluble was solubilized by the SO<sub>2</sub> during the 2-month storage period.

**Model.** The central composite design used in the experiment presupposes the use of a quadratic model to fit the data. Two models were tested. The first was a three-factor (M.C., initial SO<sub>2</sub> level, temperature) model with all cross-product terms. The second was similar except instead of using the initial SO<sub>2</sub> level the logarithm of the initial SO<sub>2</sub> level was used. The log values were evenly spaced discrete intervals whereas the unmodified values were not evenly spaced. The data was fed into an SASS program for analysis.

Neither model was satisfactory, with overall regression coefficients of 0.4207 and 0.4133, respectively. More importantly, neither model could fit the trends observed by the plotting of the data. The log model did not show the horseshoe-shaped dependence for *k* vs. initial SO<sub>2</sub> level, and the other model showed a temperature dependence with an activation energy 3 times lower than observed graphically. A cubic or higher order model can be made to fit the data better, but there are insufficient data in this study to statistically support development of such a model.

**Summary and Conclusions.** The rate of SO<sub>2</sub> loss in corn is a function of moisture content, temperature, and initial SO<sub>2</sub> level. The first-order rate constants were generally 1 order of magnitude larger than those found for other food products, but 3 orders of magnitude less than sulfite oxidation in aqueous solutions. Inhibition by solubles in the corn caused this rate decrease.

The data indicate that during storage the SO<sub>2</sub> reacted to form products that, upon heating or acidification and heating, then yielded volatile sulfur compounds that did not react with iodine or lead acetate paper. These unknown products accounted for 63% of the sulfur in corn

after 2 months of storage at 22 °C. The activation energy of the reaction on whole-kernel corn was 54.9 kJ mol<sup>-1</sup>, which was well below the 80–120 kJ mol<sup>-1</sup> determined for sulfite oxidation in aqueous solution. During storage, 75% of the protein not soluble in water was solubilized.

The dependence of the rate constant upon the initial SO<sub>2</sub> level is a pH effect caused by the slower reaction rate for bisulfite ions. The rate decreased with decreasing pH down to 3.8, which is the pH of maximum bisulfite concentration, and then increased again with lower pH values. The magnitude of this pH effect is apparently a function of the food composition, particularly the ratio of calcium to magnesium and sodium. The relation of the corn pH to the SO<sub>2</sub> level showed that the sulfuric acid and bisulfite ion form a buffered system at pH 1.81, as predicted by the Henderson-Hasselbalch equation.

The difference between the SO<sub>2</sub> values determined by the WE SO<sub>2</sub> procedure and standard distillation procedures appeared to be a function of the initial SO<sub>2</sub> level and the moisture content. The relationship may be an equilibrium phenomenon, but the nature of such a relationship was not evident from the data.

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