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Wet Cross-Linking Gliadin Fibers with Citric Acid and a Quantitative Relationship between Cross-Linking Conditions and Mechanical Properties

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This paper reports the wet cross-linking of gliadin fibers using citric acid without using phosphorus-containing catalysts or high temperatures. Carboxylic acids such as citric acid are inexpensive and nontoxic chemicals preferred for cross-linking proteins and cellulose. However, previous studies have shown that carboxylic acid cross-linked materials experience substantial strength loss and/or yellowing when cross-linked using phosphorus-containing catalysts after drying and curing at high temperatures. In this research, citric acid has been used to cross-link gliadin fibers and the effects of various cross-linking conditions on the breaking tenacity and breaking elongation have been studied. A mathematical relationship that can predict the breaking tenacity of the fibers at various cross-linking conditions has also been developed. This research shows that citric acid in aqueous solutions can cross-link gliadin fibers at low temperatures using alkali as catalyst. The method of cross-linking developed in this research could be useful to cross-link plant proteins for food, fiber, and other applications.

KEYWORDS: Citric acid; cross-linking; catalysts; protein fibers; gliadin; breaking tenacity

INTRODUCTION

This paper reports the development of a new method of cross-linking plant proteins using aqueous cross-linking solutions without the need for high temperatures or phosphorus-containing catalysts. Specifically, gliadin fibers have been cross-linked in the wet state using aqueous citric acid at low temperatures in the presence of alkali as a catalyst. Protein fibers are desirable over natural cellulose and synthetic fibers due to their comfort, elegance, and luxurious appearance. However, protein fibers are relatively expensive and have limited availability compared to common fibers such as cotton and polyester. For example, protein fibers account for only about 3.5% of the current annual fiber market, and the most common protein fiber, wool, sells at about $3−5 per pound compared to about $0.65 per pound of cotton and polyester (*1*). Therefore, efforts have been made to develop regenerated protein fibers from cereal proteins such as zein, soy protein, and wheat gluten and gliadin (*2*−*6*). Similar efforts are also being made to develop alternative cellulose fibers using lignocellulosic agricultural byproducts (*7*−*9*). However, the regenerated protein fibers developed so far do not have the mechanical properties and/or water stability required for use in textiles and other applications. Fibers intended for textile applications need to have breaking tenacity of at least 1 g per denier (approximately 145 MPa) and be stable in water at high temperatures and various pH conditions that will be experienced during the processing of the fibers. Such high-strength, water-stable 100% regenerated protein fibers are currently not available on the market.

Cross-linking is one of the most common approaches used to improve the properties of regenerated protein fibers and make them useful for various applications. In addition to cross-linking, blending two or more proteins and/or synthetic polymers has also been considered to improve the properties of regenerated protein fibers (*10*−*12*). Fibers developed from soy proteins have been cross-linked with glutaraldehyde, and zein fibers have been cross-linked using carboxylic acids such as citric acid and butanetetracarboxylic acid (BTCA) (*4, 5*). The carboxylic acid cross-linking of zein was carried out using sodium hypophosphite as the catalyst and curing at 170 °C for the cross-linking reaction to occur (*5*). The cross-linked soy protein and zein fibers have been reported to have breaking tenacities suitable for textile applications. Attempts have also been made to cross-link zein in the melt state using citric acid and BTCA (*13*). However, it was reported that the carboxylic acids did not cross-link zein under the conditions studied (*13*). Citric acid was also found to improve the metal-binding properties of zein and also make it stable in acid (*14*). We have recently reported the cross-linking of gliadin fibers using glutaraldehyde, and the cross-linked fibers have properties similar to those of wool (*15*). In that paper, we

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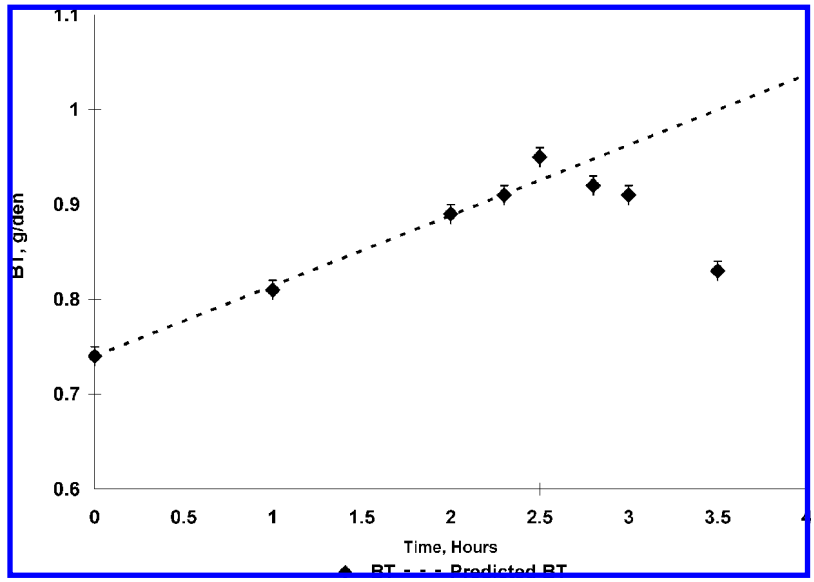


Figure 1. Effect of cross-linking time on the breaking tenacity (BT, shown as data points) of gliadin fibers studied using a citric acid activity 0.67 M, pH 6.8 solution at 50 °C. The predicted tenacity values (broken lines) were obtained from the equations developed.

have also shown that the cross-linking reaction between gliadin proteins and glutaraldehyde is a pseudo 0.6-order reaction and that alkali acts as a catalyst to the reaction.

Although glutaraldehyde and carboxylic acids have been used to cross-link plant proteins and improve their properties, both of these chemicals have several limitations. Glutaraldehyde is a relatively toxic chemical compared to carboxylic acids and is an irritant to the eyes and throat, and high concentrations (25%) are required to obtain the desired level of improvement in fiber properties (*4*). Although carboxylic acids such as citric acid and BTCA are safe to use and inexpensive chemicals, carboxylic acid cross-linking in the dry state needs phosphorus-containing catalysts and high-temperature curing (150−185 °C) for the cross-linking reaction to occur. Phosphorus-containing catalysts are toxic to use, and cross-linking of proteins with carboxylic acids at high temperatures is reported to cause substantial strength loss and/or yellowing of cross-linked materials (*16*). The cross-linking of molecules leads to lower flexibility and also contributes to the strength loss of the cross-linked materials (*17*). The yellowing of the fabrics is said to be due to the dehydration of the carboxylic acids at high temperature that forms an unsaturated acid that imparts color to the cross-linked materials (*18*).

The objective of this research was to study the possibility of cross-linking gliadin fibers in aqueous citric acid solutions without using high temperature or phosphorus-containing catalysts. The effect of various cross-linking conditions on the breaking tenacity of the fibers has been studied in detail. In addition, a mathematical analysis that describes the reaction kinetics and provides a quantitative relationship between reaction conditions and the breaking tenacity of the fibers has been developed.

MATERIALS AND METHODS

Materials. Wheat gluten (Whetpro 80) used in this research was donated by Archer Daniels Midland Co., Decatur, IL. Gliadin was extracted from the wheat gluten using aqueous ethanol (70%w/w) in a 4:1 (ethanol/gluten) ratio at room temperature overnight. The supernatant formed was centrifuged to collect the gliadin. Citric acid and other reagent grade chemicals were purchased from VWR International, Bristol, CT.

Producing Gliadin Fibers. A 30% (w/w) gliadin solution was prepared by dissolving gliadin in 8 M aqueous urea solution with 1% (w/w) sodium sulfite as the reducing agent. The gliadin solution was allowed to age at room temperature for about 24 h to form a viscous dope suitable for spinning. The spinning dope was extruded as fibers into a coagulation bath consisting of 10% sodium sulfate using a syringe and needle. Gliadin fibers precipitated in the coagulation bath and were washed in warm water three times and dried under ambient conditions. Further details on the production of the gliadin fibers have been reported in our previous publications (*3*).

Cross-Linking. The citric acid solutions had pH of about 1.5−2.5 depending on the concentration of the citric acid. The pH of the cross-linking solution was increased to the desired extent by adding powdered sodium hydroxide flakes. About 0.5 g of the gliadin fibers cut to lengths of about 5−7 cm were dipped in the cross-linking solution of a particular concentration (0.1−1 M) and pH (4−12) for the required amount of time (0.5−48 h) at a certain temperature (21−60 °C). After cross-linking, fibers were taken out of the cross-linking solution, rinsed in distilled water three times, and then drawn by hand. The fibers were later dried at 85 °C for about 90 min and then annealed at 125 °C for about 90 min in a hot air oven. Annealing was done to further improve the mechanical properties of the fibers.

Tensile Properties. All samples were conditioned in a standard testing atmosphere of 21 °C and 65% relative humidity for at least 24 h before testing. The breaking tenacity of the fibers was determined according to ASTM standard D3822 on an Instron tensile testing machine. A gauge length of 1 in. and a cross-head speed of 18 mm/min were used for testing. At least 20 fibers were tested for each condition, and the average ± one standard deviation is reported.

SDS Electrophoresis. About 1 mg of cross-linked and non-cross-linked gliadin fibers was powdered and mixed with 1000 μL of SDS-PAGE 1× sample buffer (4.1 mM Tris-HCl, 2% SDS, 2% β-mercaptoethanol, 10% glycerol, double-distilled water) and left standing at room temperature for 2 h. Ten microliters of the clear top layer of each sample was loaded into each slot in the gel. After electrophoresis, the gel was stained with Coomassie Brilliant Blue G-250. After standing for one night, the gel was flushed with deionized water and put in a destained liquid until a clear background was formed. The molecular weights of the protein standard mixture ranged from 10 to 250 kDa and were obtained from Bio-Rad Chemical Co.

RESULTS AND DISCUSSIONS

Effect of Cross-Linking Conditions on the Mechanical Properties of Gliadin Fibers. *Effect of Time.* The effect of increasing cross-linking time on the breaking tenacity of citric acid cross-linked gliadin fibers is shown in **Figure 1**. The breaking tenacity of the fibers increases linearly with increasing

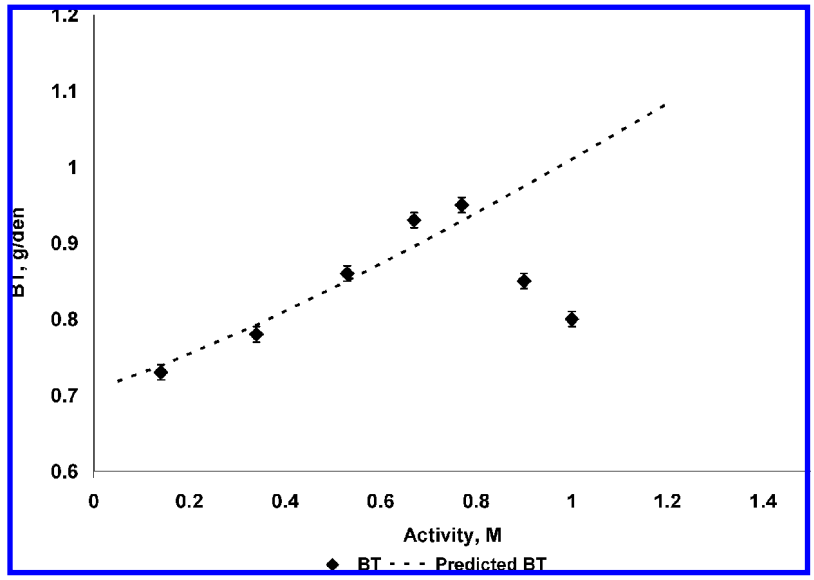


Figure 2. Effect of citric acid concentration (activity) on the breaking tenacity (BT, shown as data points) of gliadin fibers studied using a pH 6.8 solution at 50 °C for 2.5 h. The predicted tenacity values (broken lines) were obtained from the equations developed.

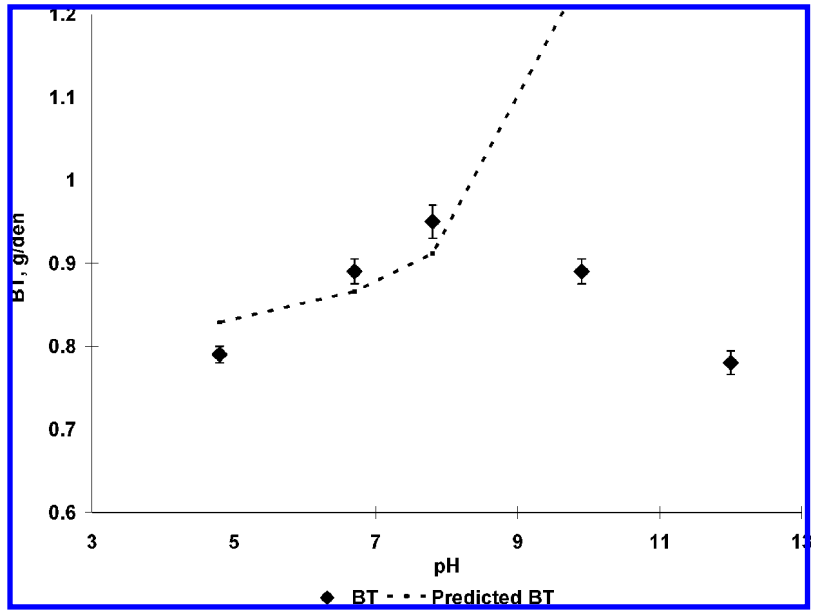


Figure 3. Effect of pH of the cross-linking solution on the breaking tenacity (BT, shown as data points) of gliadin fibers studied using a citric acid activity of 0.67 M, at 50 °C for 2 h. The predicted tenacity values (broken line) were obtained from the equations developed.

time up to 2.5 h but starts to decrease when the cross-linking time is increased beyond 2.5 h. The highest tenacity of 0.95 g per denier was obtained when the fibers are cross-linked for 2.5 h at the particular cross-linking conditions. However, increasing the concentration of citric acid or temperature of cross-linking decreases the cross-linking time necessary to achieve a breaking tenacity of 0.95 g per denier or higher as will be shown later. Cross-linking for more than 2.5 h under the cross-linking conditions of 0.67 M, pH 6.8, and 50 °C causes over-cross-linking and a decrease in the breaking tenacity of the fibers. Cross-linking interconnects polymer chains and therefore improves the strength of the fibers. However, excessive cross-linking or over-cross-linking decreases the mobility of the polymer chains and restricts the movement of the molecules, leading to reduced strength and elongation (*17*, *19*). Over-cross-linking restricts the movement of polymer chains during tensile testing. This reduces the load that can be shared by neighboring molecules, leading to reduction in the breaking tenacity of the fibers. Such decrease in the strength of the cross-linked fibers was also observed in cotton fabrics cross-linked with carboxylic acids. (*17*, *19*, *20*)

Effect of Citric Acid Activity. Increasing the activity of citric acid increases the breaking tenacity of the fibers up to a concentration of 0.8 M, and further increase in the activity decreases the breaking tenacity of the fibers as seen from **Figure 2**. The cross-linked fibers have a breaking tenacity of 1 g per denier when cross-linked using 0.8 M citric acid in pH 6.8 solution at 50 °C for 2.5 h. Further increase in the concentration of citric acid decreases the breaking tenacity of the fibers at the particular time, temperature, and pH used for cross-linking. However, lower concentrations could also increase the breaking tenacity of the gliadin fibers to about 1 g per denier if higher temperature or longer time was used compared to using 0.8 M citric acid at 50 °C for 2.5 h. At concentrations above 0.8 M, the gliadin fibers are over-cross-linked and the polymers become rigid and less flexible, leading to lower breaking strength for

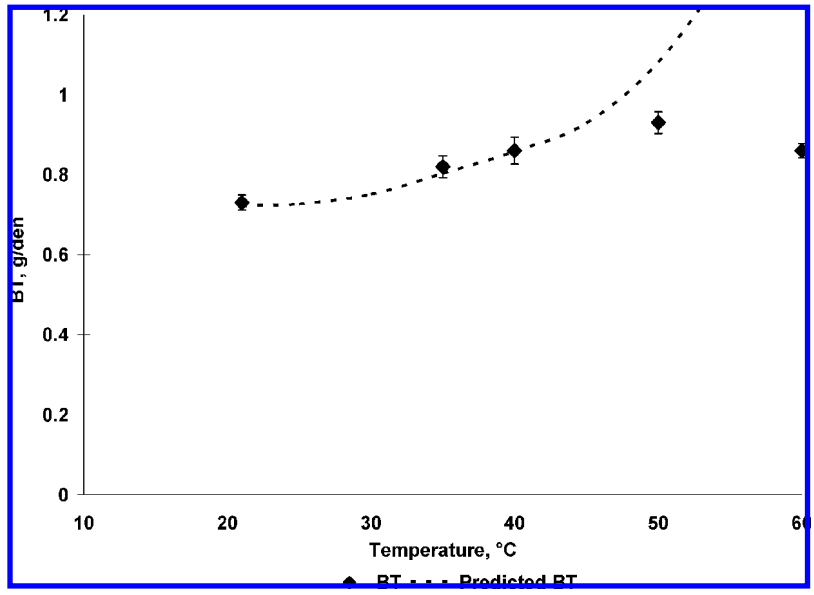


Figure 4. Effect of temperature on the breaking tenacity (BT, shown as data points) of gliadin fibers studied using a citric acid activity 0.67 M in a pH 6.5 solution for 2.5 h. The predicted tenacity values (broken lines) were obtained from the equations developed.

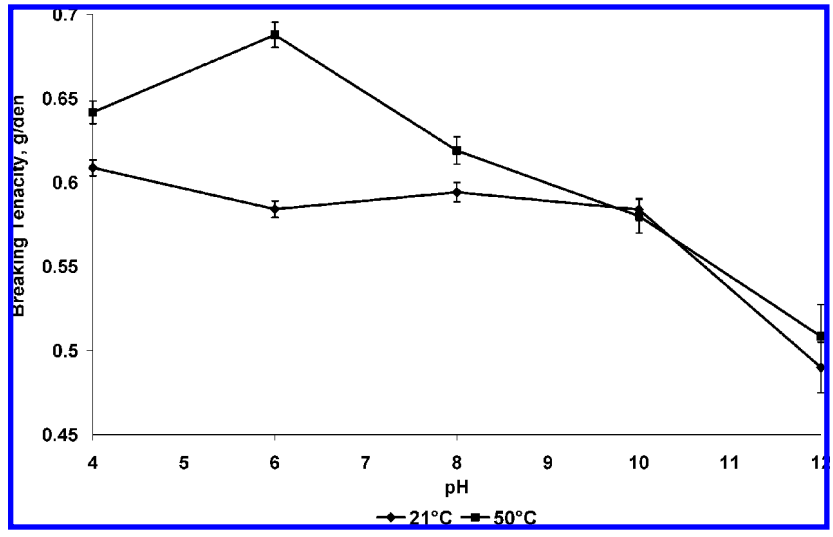


Figure 5. Effect of pH and temperature on the breaking tenacity of the non-cross-linked gliadin fibers. The fibers were treated at 21 °C for 48 h and at 50 °C for 2.5 h. The untreated fibers had a breaking tenacity of 0.72 ± 0.06 g per denier.

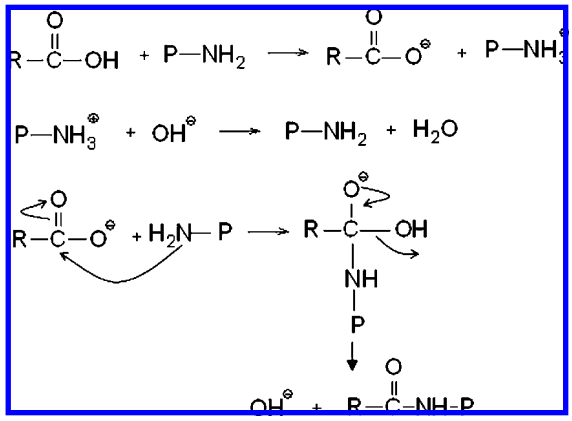
the fibers. Because a citric acid concentration of 0.67 M gave the highest strength, this concentration was chosen to study the effect of pH, time, and temperature on the breaking tenacity of the fibers.

Effect of pH. The effect of the pH of the cross-linking solution on the breaking tenacity of the fibers is shown in **Figure 3**. As seen from the figure, the pH of the cross-linking solution has relatively less effect on the increase in breaking tenacity of the fibers compared to the effects of time and activity discussed earlier. At strong acidic conditions, there is not enough alkali (catalyst) in the solution to catalyze the reaction and improve the strength of the fibers. Weak acidic and alkaline conditions provide the fibers with higher tenacity (about 1 g per denier) compared to the tenacity of the fibers at strong acidic or alkaline conditions. At pH 12, the breaking tenacity of the cross-linked fibers is similar to that of the non-cross-linked fibers with breaking tenacity of 0.7 ± 0.1 g per denier. The lower tenacity of the fibers at high-alkaline conditions should be due to the hydrolysis and/or over-cross-linking of proteins. Because weak acidic conditions provide better strength to the fiber than strong acidic or alkaline conditions, a pH of 6.8 was chosen to study the effect of cross-linking time and temperature and citric acid concentration on the breaking tenacity of the fibers.

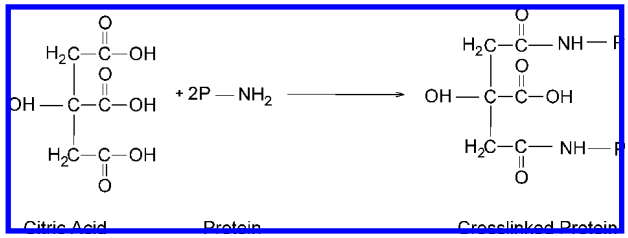
Effect of Temperature. Increasing the temperature up to 50 °C increases the breaking tenacity of the fibers as seen from **Figure 4**. At 50 °C, the cross-linked fibers have a breaking tenacity of about 0.93 g per denier, whereas the tenacity decreases to about 0.85 g per denier when cross-linked at 60 °C under similar cross-linking conditions. The hydrolysis and/or over-cross-linking of proteins at higher temperatures could be the reason for the decrease in breaking tenacity of the fibers when cross-linked at 60 °C. However, lower temperatures do not provide sufficient energy for the cross-linking reaction to occur and improve the breaking tenacity when cross-linked for 2.5 h, but may increase the breaking tenacity to about 0.95 g per denier or higher if longer cross-linking times are used.

Figure 5 shows the effect of treating the gliadin fibers at various pH values at two temperatures without the cross-linking agent. The non-cross-linked fibers lost strength after treatment at both temperature and all pH values studied compared to their

Scheme 1. Possible Mechanism for the Alkali-Catalyzed Reaction between Citric Acid and an Amine Group in a Protein (P)



Scheme 2. Schematic Representation of the Cross-Linking between Citric Acid and Protein (NH_2-P)



strength before the treatment. At 50 °C, the fibers had a relatively lower decrease in tenacity when treated in weak acid conditions (pH 4 and 6) but experienced substantial strength loss when treated at high-alkaline conditions. The fibers had similar strength losses at pH 6, 8, and 10 but experienced a high strength loss of about 32% when treated in pH 12 water at 21 °C for 48 h. The strength loss of the fibers in high-pH (alkaline) solutions should mainly be due to the hydrolysis of the proteins. However, gliadin fibers exhibited an increase in strength after treatment in various pH conditions and temperatures in the presence of citric acid as shown in **Figures 1** and **4**. This confirms that the increase in the breaking tenacity of the fibers should be due to cross-linking and not due to pH or temperature.

Proposed Mechanism of Wet Cross-Linking of Gliadin with Citric Acid. On the basis of the results discussed above, we propose a new mechanism of wet cross-linking of plant proteins using citric acid at low temperatures and alkali as a catalyst. As described earlier, weak alkaline conditions improve the breaking tenacity of the fibers to the greatest extent, because strong alkaline conditions can hydrolyze the proteins. The relatively higher amounts of amine groups in gliadin proteins (6.2 g per 100 g of protein) facilitate the cross-linking of the proteins with citric acid. A possible reaction between citric acid and the amine groups in the protein is proposed in **Scheme 1**. This reaction occurs by nucleophilic substitution as shown in **Scheme 1**. Without the addition of any alkali, it will be difficult for the positively charged amine in the protein to react with the partially positively charged carbonyl carbon in citric acid. In the presence of alkali, the amine group is less likely to carry a positive charge as shown in **Scheme 1**, and it therefore can attack the carbonyl carbon and more readily form an amide linkage. A schematic of the cross-linking reaction between citric acid and proteins is shown in **Scheme 2**.

SDS-PAGE. The SDS-PAGE depicted in **Figure 6** shows that the proteins in gliadin powder (lane 2) and gliadin fibers (lane 3) have similar molecular weight bands. This indicates

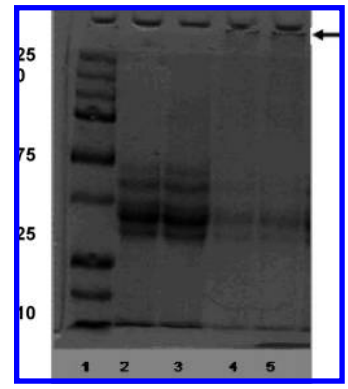


Figure 6. SDS-PAGE of molecular weight standards (lane 1), gliadin powder (lane 2), non-cross-linked gliadin fiber (lane 3), gliadin fibers cross-linked with 0.3 M citric acid at 50 °C for 2.5 h (lane 4), and fibers cross-linked with 0.6 M citric acid at 21 °C for 48 h (lane 5).

that the proteins are not damaged during dissolution or fiber production. Most of the strong bands between 25 and 75 kDa seen in lanes 2 and 3 for the gliadin powder and non-cross-linked fibers, respectively, have disappeared in the cross-linked fibers (lanes 4 and 5). The removal of low molecular weight proteins and/or the lower weight proteins being cross-linked and becoming higher molecular weight proteins results in weak bands in the 25−75 kDa region (*21*, *22*). Although the same amount of proteins was used for each lane, the cross-linked proteins (lanes 4 and 5) have fewer and less intense bands compared to lanes 2 and 3. Cross-linking results in higher molecular weight proteins, and most of the higher molecular weight proteins cannot be dissolved in the SDS-PAGE solution. Most of the higher molecular proteins are unable to penetrate the gel, resulting in less intense and fewer bands in the cross-linked proteins in lanes 4 and 5 (*21*, *22*). However, a narrow low-intensity band can be observed (indicated by the arrow) at the top of the gel in both lanes 4 and 5, indicating that some high molecular weight proteins were able to penetrate the gel.

Developing the Quantitative Relationship between Reaction Conditions and Breaking Tenacity of the Fibers. *Determining the Kinetics of the Reaction.* The cross-linking reaction between citric acid and proteins can be shown as eq 1.

$$a\ \text{protein} + b\ \text{citric acid} \leftrightarrow c\ \text{cross-linked protein} + d\ H_2O \quad (1)$$

where $a-d$ are the molar constants at equilibrium.

The reaction rate, r, could be expressed as

$$r = \frac{dC_{XLP}}{dt} = k_1 {a_G}^m {C_p}^n \quad (2)$$

where k_1 is the reaction rate constant, C_{XLP} and C_P are the concentrations of functional groups in the cross-linked protein and in the non-cross-linked protein, respectively, a_G is the activity of citric acid, and m and n are the powers related to the reaction mechanism.

During the cross-linking reaction, only a small portion of the total functional groups available in the proteins are cross-linked; therefore, C_p could be considered as constant, and eq 2 could be rewritten as

$$r = \frac{dC_{XLP}}{dt} = k_2 {a_G}^m \quad (3)$$

where $k_2 = k_1 C_p n$, k_2 is the pseudo-reaction rate constant, and m is the pseudo-rate order.

Because the breaking tenacity (BT) is a function of the concentration of the cross-linked protein, based on eq 2, we have

$$\mathrm{BT}(t) = f[C_{\mathrm{XLP}}(t)] = f[rt] \tag{4}$$

where BT(t) and $C_{\mathrm{XLP}}(t)$ are the breaking tenacity of the fibers and the concentration of the cross-linked protein at time t, respectively. Assuming BT has a linear relationship (shown previously) with C_{XLP} before over-cross-linking, eq 4 could be simplified to

$$\mathrm{BT}(t) = k_3[rt] + k_4 \tag{5}$$

where k_3 and k_4 are the slope and intercept of the linear relationship, respectively. The constants k_3 and k_4 do not change with the cross-linking conditions.

From eq 5, we have

$$\mathrm{d}[\mathrm{BT}(t)] = k_3(t\,\mathrm{d}r + r\,\mathrm{d}t) \tag{6}$$

In the cross-linking of gliadin fibers, we have a reaction bath much larger in volume than the protein fibers (by a factor of about 5000). Therefore, the activity of citric acid (a_{G}) does not change during the cross-linking reaction. On the basis of eq 3, r is a constant, and therefore eq 6 becomes

$$\mathrm{d}[\mathrm{BT}(t)] = k_3 r\,\mathrm{d}t \tag{7}$$

From eqs 3 and 7, we have

$$\mathrm{d}[\mathrm{BT}(t)] = k_2k_3{a_{\mathrm{G}}}^m\,\mathrm{d}t = k{a_{\mathrm{G}}}^m\,\mathrm{d}t \tag{8}$$

where $k = k_2k_3$. k here is defined as the constant associated with the rate of change of breaking tenacity.

Integration of eq 8 from time zero to t gives

$$\mathrm{BT}(t) = k{a_{\mathrm{G}}}^m t + \mathrm{BT}(0) \tag{9}$$

where BT(0) is the breaking tenacity of the non-cross-linked gliadin fibers. Equations 8 and 9 provide the quantitative relationship between the breaking tenacity of gliadin fibers and the cross-linking time and cross-linking activity.

Determining the Reaction Order. To obtain the constants k and m in eq 9, the breaking tenacity of the fibers at two different activities of citric acid were plotted against cross-linking time as shown in **Figure 7**. The plot gives the breaking tenacity of the fibers at two different activities (a_{G}) at various cross-linking times (t). Linear regression of the two curves gives

$$\mathrm{BT} = 0.0801(t) + 0.7351\ (R^2 = 0.9866) \text{ for } 0.67\ \mathrm{M} \tag{9a}$$

and

$$\mathrm{BT} = 0.0608(t) + 0.7349\ (R^2 = 0.9915) \text{ for } 0.53\ \mathrm{M} \tag{9b}$$

From eqs 9, 9a, and 9b and the slope of the linear relationships in **Figure 7**, we obtain

$$k{a_{\mathrm{G}}}^m = k0.67^m = 0.0801 \text{ when } a_{\mathrm{G}} = 0.67$$

and

$$\mathrm{BT} = 0.0608(t) + 0.7349\ (R^2 = 0.9915) \text{ for } 0.53\ \mathrm{M}$$

Solving these two equations gives k and m as 0.12 g dm$^{3.6}$ mol$^{-1.2}$ denier^{-1} h^{-1} (9.4×10^4 MPa dm$^{3.6}$ mol$^{-1.2}$ s^{-1}) and 1.2, respectively, at 50 °C and pH 6.5. The constant k is lower and reaction order m is higher for citric acid cross-linking of gliadin fibers compared to cross-linking gliadin fibers with glutaraldehyde (*15*). Glutaraldehyde cross-linking of gliadin proteins had k and m of 0.18 and 0.6, respectively (*15*).

With m = 1.2, eq 3 now becomes

$$r = \frac{\mathrm{d}C_{\mathrm{XLP}}}{\mathrm{d}t} = k_2{a_{\mathrm{G}}}^{1.2} \tag{10}$$

Using the values for k and m obtained above [at 50 °C, pH 6.8, BT(0) = 0.70 g denier^{-1}), eq 9 becomes

$$\mathrm{BT}(t) = 0.12{a_{\mathrm{G}}}^{1.2}t + 0.70 \tag{10a}$$

Or at different temperature and pH, eq 10a will become

$$\mathrm{BT}(t) = k{a_{\mathrm{G}}}^{1.2}t + \mathrm{BT}(0) \tag{11}$$

because the order of the reaction will not change with temperature and pH.

According to the Arrhenius equation, we have

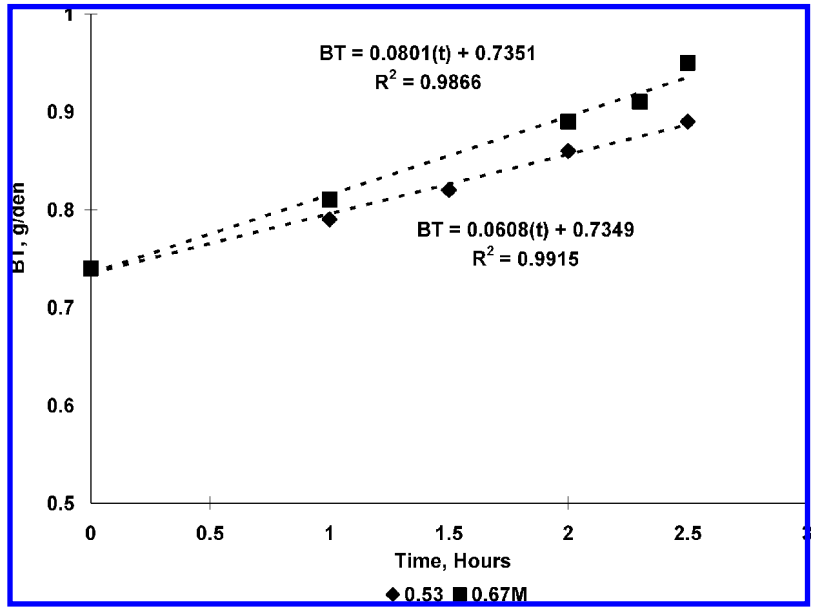


Figure 7. Effect of activity of citric acid on the breaking tenacity of gliadin fibers at two activities of citric acid at 50 °C and pH 6.

$$k_2 = A\, e^{-E_a/RT} \quad (12a)$$

where k_2 is the reaction rate constant and A is the Arrhenius constant. Because $k = k_2k_3$, eq 12a becomes

$$k = A_1\, e^{-E_a/RT} \quad (12b)$$

where $A_1 = Ak_3$ and A_1 is a constant that changes with pH as will be discussed later.

From eqs 11 and 12b, we have

$$BT(t) = A_1\, e^{-E_a/RT} a_G^{1.2} t + BT(0) \quad (13)$$

Equation 13 provides the relationship between the breaking tenacity of the fibers at different cross-linking conditions such as pH, temperature, time, and citric acid activity. However, because the relationships above are developed with an assumption that the breaking tenacity has a linear relationship with the activity of the cross-linked protein, eq 13 is valid for predicting the breaking tenacity only up to 1.0 g denier^{-1}. Above 1.0 g denier^{-1}, the fibers are over-cross-linked and the linear relationship does not hold; therefore, the equations are not valid.

Determining the Relationship between Constant k and pH. To obtain the relationship between the constant (k) and pH, log k calculated using eq 11 was plotted against pH as shown in **Figure 8**. From the plot, it can be seen that log k has a linear relationship with pH from 4.5 to 12 at 50 °C. The linear relationship with $R^2 = 0.9897$ is

$$\log(k) = 0.1215\text{pH} - 1.5099 \quad (14)$$

From eq 14, k is proportional to the hydroxyl ion activity, and the slope of the equation gives the negative power of the activity of the hydrogen ions ($a_{H^+}^{-0.1}$). Equation 14 shows that the higher the pH, the higher the constant k and vice versa. From this, we can infer that hydroxyl ions act as a catalyst to the reaction and change the constant k.

Determining the Average Activation Energy (E_a) and Constant A_1 at Different pH Values. Between pH 4.8 and 12, the natural logarithm of k (ln k) has an inverse linear relationship with temperature. **Figure 9** shows the inverse linear relationship ($\ln k = -10.774 \times 1/T + 31.688$; $R^2 = 0.9937$) between ln k and temperature at pH 6.7. Because $-E_a/R = -10.774$, the average E_a of 90 kJ mol^{-1} (10.774 × 8.314) at pH 6.7 was obtained. The intercept of the linear relationship gives the constant A_1 as 1.09×10^{14} g dm$^{3.6}$ mol$^{-1.2}$ denier^{-1} h^{-1}. The activation energy for cross-linking gliadin proteins with

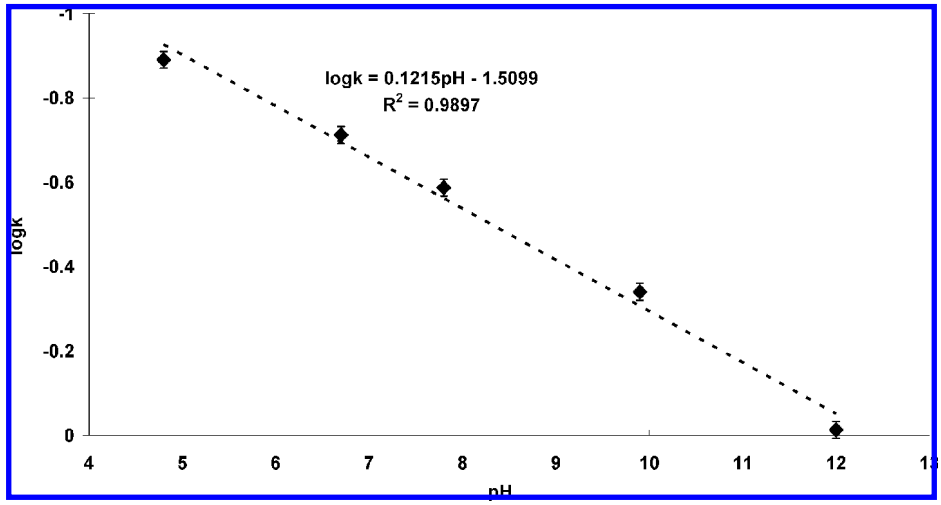


Figure 8. Effect of pH on constant log k studied using a citric acid activity 0.67 M at 50 °C for different reaction times.

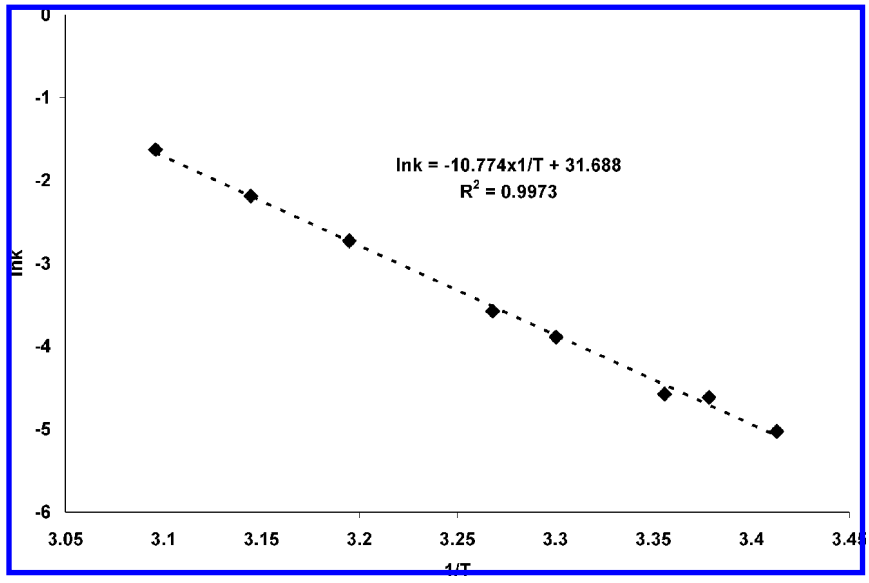


Figure 9. Effect of the inverse of temperature (1/T) on ln k studied using a citric acid activity of 0.67 M at temperatures from 21 to 50 °C for 1.5 h at pH 6.7.

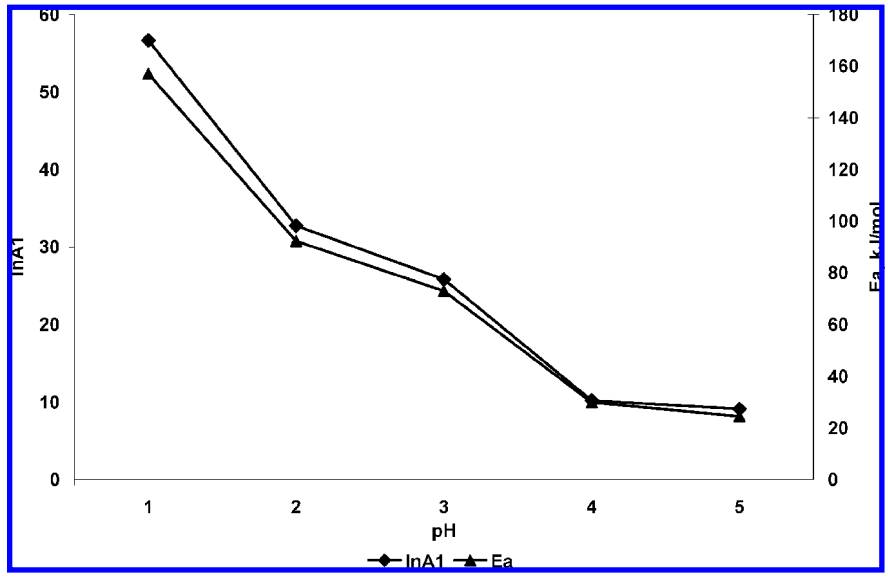


Figure 10. Effect of pH on the activation energy and natural logarithm of A_1 (ln A_1) studied using a citric acid activity of 0.67 M and temperatures from 21 to 60 °C for different cross-linking times.

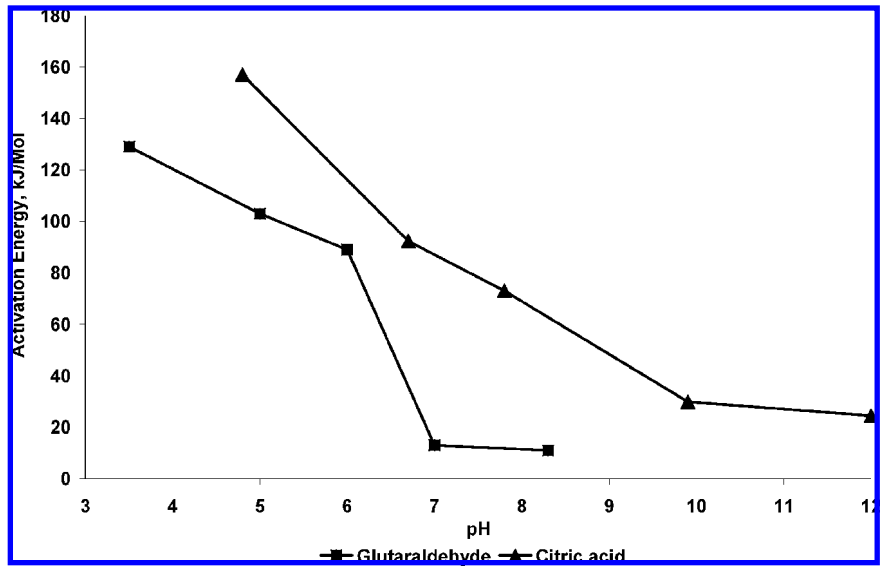


Figure 11. Comparison of the activation energies at various pH conditions for the reactions between gliadin proteins and citric acid and glutaraldehyde.

citric acid is slightly higher than that reported for cross-linking gliadin fibers with glutaraldehyde (85 kJ mol^{-1}) (*15*).

Constants E_a and A_1 were also obtained at pH between 1 and 5. The relationship among E_a, ln A_1, and pH is shown in **Figure 10**. Using eq 13 and the constant for E_a and A_1 from **Figure 10**, it is now possible to predict the breaking tenacity as between 0.7 and 1.0 g $denier^{-1}$ at different cross-linking conditions such as temperature, activity of citric acid, pH, and time.

Comparison of the Predicted and Experimental Data and Verification of the Validity of the Quantitative Relationship. The equations developed above were used to predict the breaking tenacity of the gliadin fibers at various activities of citric acid, time, pH, and temperature of cross-linking. The broken lines in **Figures 1−4** show the predicted breaking tenacity of the fibers at various times, activities, pH values, and temperatures, respectively, in comparison to the actual experimental results. As seen from the figures, the predicted breaking tenacity of the fibers closely follows the experimental results until the fibers are over-cross-linked with a maximum difference between the predicted and experimental values of 4% for time, 5% for activity, 7% for pH, and 7% for temperature. The equations developed can be used to predict the breaking tenacity of the fibers at various other cross-linking conditions up to a breaking tenacity of 1 g per denier.

Comparison of the Reactions between Citric Acid and Gliadin and between Glutaraldehyde and Gliadin. The reaction between citric acid and gliadin proteins is considerably different from the reaction between glutaraldehyde and gliadin. First, the reaction order between citric acid and gliadin (1.2) is twice that of the reaction between glutaraldehyde and gliadin proteins (0.6), but the reactions have similar rate constants of 0.12 and 0.18 g $dm^{3.6}$ $mol^{-1.2}$ $denier^{-1}$ h^{-1}, respectively (*15*). Second, glutaraldehyde cross-linking occurs more easily under neutral and weak alkaline conditions, whereas relatively high alkaline pH values are more suitable for citric acid cross-linking as seen from the activation energies at various pH conditions in **Figure 11**. In both reactions, alkali acts as a catalyst and decreases the activation energy of the reaction. However, the activation energy for citric acid cross-linking is more than twice that

of the reaction between glutaraldehyde and gliadin proteins at the respective highest pH conditions studied. The higher activation energy for citric acid cross-linking indicates that a higher amount of catalyst (alkali) is necessary to obtain similar reaction rate constants compared to glutaraldehyde cross-linking of gliadin.

The systematic study of the effect of citric acid cross-linking conditions on the breaking tenacity of gliadin fibers shows that gliadin fibers can be cross-linked using low concentrations of citric acid at low temperatures and using alkali as a catalyst. Alkali catalyzes the reaction between citric acid and the amine groups in the proteins and allows the cross-linking to occur in aqueous solutions. Cross-linked gliadin fibers have about 60% increases in breaking tenacity compared to the non-cross-linked fibers. The reaction between citric acid and gliadin proteins is a pseudo-1.2-order, and alkali acts as a catalyst to the reaction. The mathematical relationships developed allow us to choose the cross-linking conditions (time, temperature, pH, and concentration) depending on the desired processing conditions and level of improvement in the breaking tenacity of the fibers. The new method of wet cross-linking of plant proteins eliminates the need for phosphorus-containing catalysts or high temperatures and therefore avoids the strength loss and yellowing problems previously experienced during carboxylic acid cross-linking of plant proteins. This research shows that citric acid could be a low-cost, nontoxic, "green" cross-linking agent for cross-linking plant proteins for food, fiber, and other applications.

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