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Modification of Wheat Gluten with Citric Acid to Produce Superabsorbent Materials

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ABSTRACT: Wheat gluten was reacted with citric acid to produce natural superabsorbent materials able to absorb up to 78 times its weight in water. The properties of the modified gluten samples were characterized using Fourier Transform Infra-red (FTIR) spectroscopy, thermogravimetric analysis, and water uptake. The reaction between gluten and citric acid was examined for gluten : citric acid ratios of 0.38 : 1 to 0.75 : 1 at temperatures from 100 to 130°C. More citric acid reacted for samples containing higher citric acid concentrations and at higher temperatures. FTIR analyses indicated the presence of carboxylate groups on the modified gluten samples, which resulted in modified samples having higher water uptake values than neat gluten. The sample with a gluten:citric acid ratio of 0.5 : 1 and reaction temperature of 120°C had the largest water uptake value. Also, all modified gluten samples had lower thermal stability than neat gluten. © 2013 Wiley Periodicals, Inc.† J. Appl. Polym. Sci. 129: 3192–3197, 2013

KEYWORDS: biomaterials; proteins; thermal properties

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INTRODUCTION

Most commercial superabsorbent materials are derived from synthetic monomers, such as acrylic acid and acrylamide. For instance, one of the most successful superabsorbent materials is poly(acrylic acid), sodium salt, which is a completely synthetic polymer. These polymers can absorb hundreds of times their original weight in water and are widely used in the hygiene industry. However, the use of petroleum-based polymers has several drawbacks. One is that the polymers are produced from a nonrenewable resource. Another drawback is that these synthetic polymers are not biodegradable, which leads to persistence and accumulation in the environment.

Some researchers have tried to incorporate natural polymers into superabsorbent materials. Various researchers had modified natural polymers, including polysaccharides, such as starch,[1,2] cellulose,[3–5] chitosan,[6–8] carrageenan,[9] guar gum,[10] pectin,[11] and alginate,[12] as well as proteins, such as collagen,[13] soy protein isolate,[14,15] and fish protein.[16] Modification of these natural polymers usually involved grafting poly(acrylic acid), poly(acrylamide), poly(acrylonitrile), or their copolymers onto the polymer backbone. However, these samples still contained a substantial amount of synthetic polymers. There had been few superabsorbent polymers developed primarily from natural polymers. These usually involved just crosslinking the natural polymers.[3–5,8,11]

Wheat gluten has been used to produce completely natural superabsorbent materials.[17–20] This involved reacting gluten with sulfuric[17,18,20] or phosphoric acid.[19] Sulfuric acid reacts with hydroxyl groups in amino acids of gluten, such as serine and tyrosine, to produce sulfuric acid esters. Phosphoric acid also reacts with hydroxyl groups in gluten to form phosphorylated gluten. These modified gluten samples were able to absorb up to hundreds of times their weight in water.

Citric acid has also been used to modify polysaccharides and proteins to form citrates or to crosslink the samples. The sample is first coated with citric acid and then heated to temperatures above 100°C. Two carboxylic acid groups on citric acid then forms an anhydride through loss of a water molecule. This anhydride then reacts with hydroxyl groups on the polysaccharide or protein to form an ester linkage. Crosslinking occurs when an unreacted carboxylic acid group on the attached citric acid forms another ester bond with a different polysaccharide or protein molecule. Citrates of starch,[21] corn fiber,[22] corn gluten meal,[23,24] distillers' dried grain,[23,24] and soy protein isolate[23] had been examined as possible metal chelation agents. Also, starch[25] and xylan citrate foams crosslinked with chitosan[26,27]

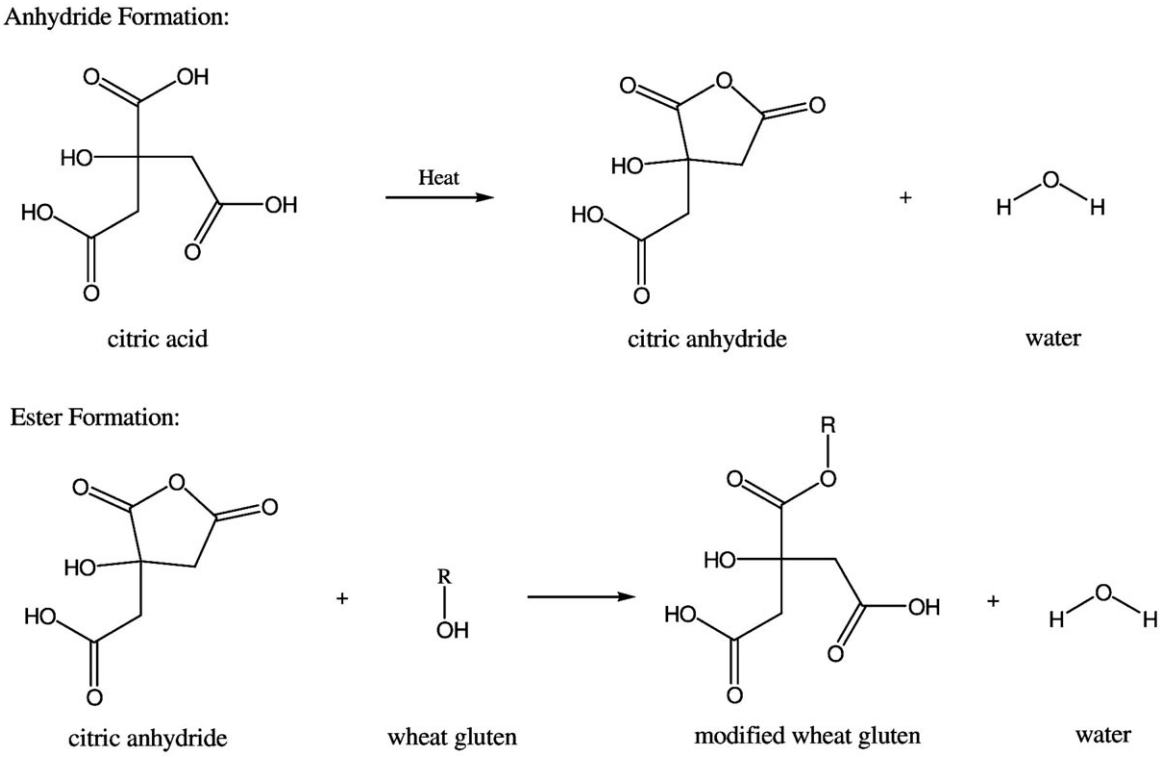

Figure 1. Reaction mechanism of wheat gluten with citric acid.

had shown high water absorbance values. In addition, citric acid had been used to cross-link cellulose,[5,28] gliadin,[29,30] soy protein,[30] and zein.[30]

In this study, we reacted wheat gluten with citric acid at various gluten to citric acid ratios and temperatures to produce completely natural superabsorbent materials. We examined chemical changes of the modified gluten using FTIR spectroscopy and characterized its thermal stability using thermogravimetric analysis. We also measured final water uptake values.

EXPERIMENTAL

Reaction of Wheat Gluten with Citric Acid

A Hobart mixer (model N50A, Troy, OH) was initially used to blend wheat gluten (Giusto, San Francisco, CA) with deionized water in a 1 : 2 weight ratio for five minutes. A 6.93*M* citric acid (Sigma–Aldrich, St. Louis, MO) solution was then slowly added during the blending process for another 30 min. Citric acid solution was added to produce samples containing wheat gluten to citric acid weight ratios of 0.38 : 1, 0.50 : 1, and 0.75 : 1. After mixing, the sample was placed in a 55°C oven for up to 5 days until it became fully dried. During the drying process, the sample was broken into smaller pieces each day to improve drying efficiency. A mill with a one mm size screen was then used to grind the dried sample. The sample was heated *in vacuo* for 30 min to react citric acid with gluten. The oven temperatures were set at 100, 110, 120, and 130°C during reaction. A schematic of the reaction of citric acid with gluten is shown in Figure 1. When citric acid was heated, it dehydrated to form an anhydride. The anhydride then reacted with hydroxyl groups on gluten amino acids, such as serine and tyrosine, to form gluten citrate and water. After reaction, the sample (10g) was mixed with 200 ml deionized water. This mixture was neutralized to a pH (Hach Sension1 pH meter, Loveland, CO) of 7.0 using a 0.4*M* NaOH (Sigma–Aldrich) solution. The sample was then rinsed with 500 mL deionized water and filtered using Whatman (Piscataway, NJ) no. 4 filter paper. After this, the sample was rinsed with 400 mL acetone (Fisher Scientific, Pittsburgh, PA) and allowed to settle before the acetone was decanted. This acetone rinse was repeated three times.

Titration to Determine Amount of Citric Acid Reacted

Titration was performed on the modified gluten samples after the gluten had been reacted with citric acid in the oven. After reaction, the modified sample was mixed with 200 mL deionized water using a stir bar. The mixing was stopped after the pH reading became stable. The gluten was then allowed to settle and 25 mL of supernatant was decanted to a separate container. The supernatant was finally neutralized to a pH of 7.0 with a 0.4*M* NaOH solution. The amount of NaOH solution required for neutralization indicated the amount of unreacted citric acid.

Fourier Transform Infrared Spectroscopy

A Perkin Elmer 2000 FTIR spectrometer (Waltham, MA) was used to characterize the chemical composition of the gluten samples. The samples were ground into powder with a ball mill and placed in a DuraSamplIR attenuated total reflectance attachment (ASI SensIR Technology, Danbury, CT). Each IR spectrum contained an average of 50 scans over a ten minute period with a resolution of 4 cm^{-1}.

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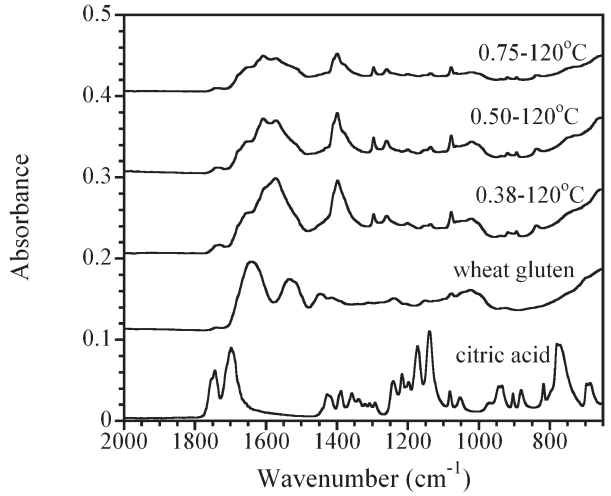

Figure 2. FTIR spectra of citric acid, wheat gluten, and modified wheat gluten samples with gluten:citric acid ratio of 0.5 : 1 at different reaction temperatures. Each spectrum has been shifted up 0.1 absorbance units from the one below it.

Thermogravimetric Analysis

A TA Instruments TGA 2950 (New Castle, DE) was used to characterize the thermal stability of the gluten samples. Each 11 mg sample was heated from 30 to 800°C at a rate of 10°C min^{-1}. The sample was maintained in a nitrogen atmosphere by using a nitrogen gas flow rate of 40 cm^3 min^{-1}. Each sample was conditioned in a 50% relative humidity chamber for at least 48 h before the test. The humidity was maintained in the chamber by using a saturated solution of calcium nitrate tetrahydrate ($Ca(NO_3)$*$4H_2O$; Fisher Scientific) in deionized water.

Scanning Electron Microscopy

A Hitachi (Pleasanton, CA) S-4700 Scanning Electron Microscope (SEM) was used to observe the samples under ×1.00k and ×5.00k magnifications. The voltage setting was 15.0 kV and the current setting was 10 μA. The wheat gluten samples were affixed to stubs with carbon adhesives. A Polaron (Hatfield, PA) E5100 Sputter Coater was then used to apply a gold coating at a voltage of 1.5 kV and a discharge current of 20 mA for 90 s. The vacuum chamber in the sputter coater was set to a pressure of 10 Pa.

Water Uptake

Each gluten sample (0.5g) was mixed with 50 mL of deionized water. After 2 h, the sample was vacuum filtered until no water dripped through the filter. The sample was then placed in a Denver Instrument IR-200 moisture analyzer (Bohemia, NY) and heated to 115°C until the sample weight remained constant. Water uptake was calculated as:

$$W = \frac{m_H - m_D}{m_D}$$

where W is water uptake (g of water/g dry gluten), m_H is initial mass of the hydrated sample, and m_D is mass of the dry sample.

RESULTS AND DISCUSSION

FTIR Spectroscopy

The acid modified samples showed the presence of carboxylate ([sbond]CO_2^-) groups, which were not present in neat gluten. This is shown in Figure 2, where we plot the FTIR spectra of citric acid, neat gluten, and modified gluten with different gluten to citric acid ratios. The neat gluten sample contained the amide I (1639 cm^{-1}) and amide II (1533 cm^{-1}) peaks. After reacting with citric acid, all samples showed asymmetric [sbond]CO_2^- stretching (1608 cm^{-1}) and symmetric [sbond]CO_2^- stretching (1399 cm^{-1}) peaks. Sessa et al.[23] found similar peaks after reacting soy protein isolate, distiller's dried grain, and corn gluten meal with citric acid. Also, modified gluten samples contained saturated aliphatic ester (C[dbond]O) stretching vibrations at 1730 cm^{-1}. This indicated citric acid had reacted with hydroxyl groups on gluten to form ester linkages (see Figure 1). Previous studies on distillers' dried grain,[23] corn gluten meal,[23] hydroxy propyl methyl cellulose,[28] and carboxy methyl cellulose/hydroxy ethyl cellulose blends[31] showed similar peaks after reaction with citric acid.

All samples reacted at different temperatures also contained carboxylate group stretching vibrations at 1608 and 1399 cm^{-1} as well as ester group stretching vibrations at 1730 cm^{-1}. This is shown in Figure 3, where we plot the FTIR spectra of 0.50 : 1 samples reacted between 100 and 130°C. The presence of carboxylate and ester groups indicated citric acid reacted with hydroxyl groups on gluten within the experimental temperature range.

Titration Results

More citric acid reacted with gluten at higher reaction temperatures. This is shown in Table I. Previous studies had shown similar results. Wing[21] found that an increase in reaction temperature of citric acid with starch from 110 to 130°C resulted in attachment of more carboxylic acids groups to starch. At 140°C, crosslinking occurred, resulting in lower carboxyl content. Sessa et al.[23,24] also found that an increase in reaction temperature of

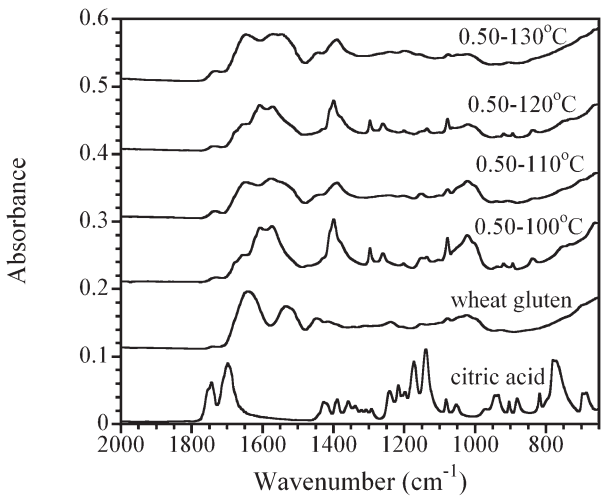

Figure 3. FTIR spectra of citric acid, wheat gluten, and modified wheat gluten samples with different gluten:citric acid ratios at 120°C. Each spectrum has been shifted up 0.1 absorbance units from the one below it.

Table I. Citric Acid Reaction with Wheat Gluten

Gluten : citric acid ratio	Mmol citric acid reacted/g wheat gluten			
	100°C	110°C	120°C	130°C
0.38 : 1	-	-	6.7 ± 1.1	-
0.50 : 1	3.8 ± 1	5.0 ± 0.2	4.1 ± 0.6	5.9 ± 1
0.75 : 1	-	3.6 ± 0.9	4.0 ± 0.9	-

distiller's dried grain, corn gluten meal, and soy protein isolate with citric acid from 110 to 120°C resulted in an increase in carboxyl content. However, Wing[22] determined that an increase in reaction temperature for corn fiber and citric acid from 110 to 140°C resulted in lower carboxyl content. The author postulated that crosslinking occurred at higher temperatures, reducing carboxyl content.

The addition of more citric acid (lower gluten:citric acid ratio) also resulted in an increase of citric acid reacted with gluten (Table I). These results applied to reactions at both 110 and 120°C. Previous studies on corn starch[21] and corn fiber[22] had shown similar results. Wing[21,22] found that increasing the mass ratio of citric acid to corn starch and corn fiber up to 1 : 1 had resulted in increases of carboxyl contents.

Thermal Stability

The modified wheat gluten samples at different reaction temperatures were less thermally stable than the neat gluten sample. This is shown in Figure 4(a). All samples exhibited two main decreases in their mass during heating. The first one occurred at 100°C, which indicated loss of water. All the modified gluten samples had greater water loss than the neat sample due to higher equilibrium moisture contents. After this point, the modified gluten samples had rapid decreases in weight at a temperature range of 225–234°C. In comparison, the neat gluten sample began to rapidly decrease in weight at ~250°C due to degradation of gluten. These results might indicate lower thermal stability of carboxylate groups in the modified samples. A previous study[20] found similar results for gluten films modified with sulfuric acid. In that study, the sulfated gluten films showed rapid decreases in weights at ~230°C.

The lower thermal stability of the modified gluten samples can also be seen in the derivatives of their weight loss, which are shown in Figure 4(b). All the modified gluten samples exhibited an increase in their derivative values at lower temperatures than the neat gluten sample. Also, the derivative curves for the modified samples had more jagged peaks than that of the neat sample. This indicated more heterogeneous structures might be present in the modified samples.

The modified gluten samples with various gluten : citric acid ratios were also less thermally stable than the neat gluten sample. This is shown in Figure 5. All the modified gluten samples had higher equilibrium moisture levels than the neat gluten sample. Also, the modified samples with gluten : citric acid ratios of 0.38 : 1 and 0.75 : 1 showed rapid weight loss at a temperature range of 229–235°C, similar to the samples with gluten : citric acid ratio of 0.50 : 1 reacted at different

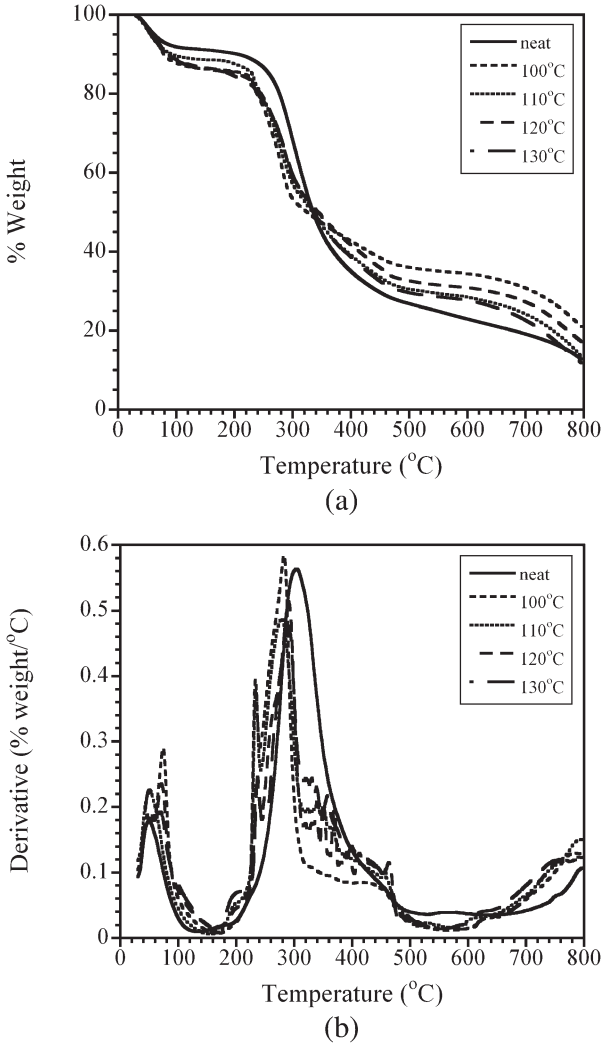

Figure 4. TGA curves of (a) neat and modified wheat gluten samples with a gluten:citric acid ratio of 0.5 : 1 at different temperatures and (b) their derivative of TGA curves.

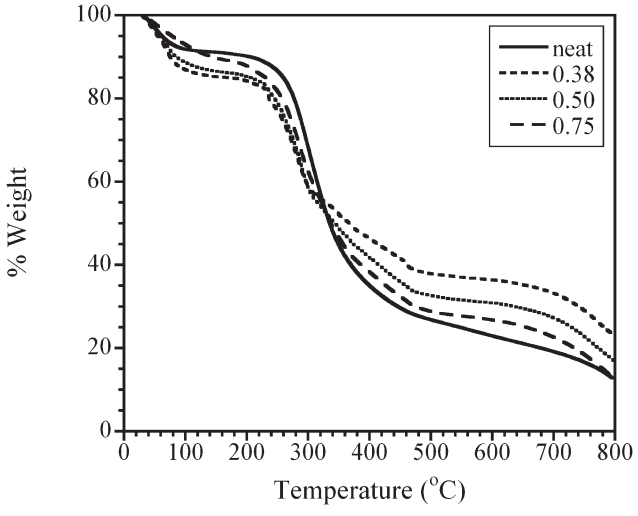

Figure 5. TGA curves of neat and modified wheat gluten samples with different gluten : citric acid ratios at 120°C.

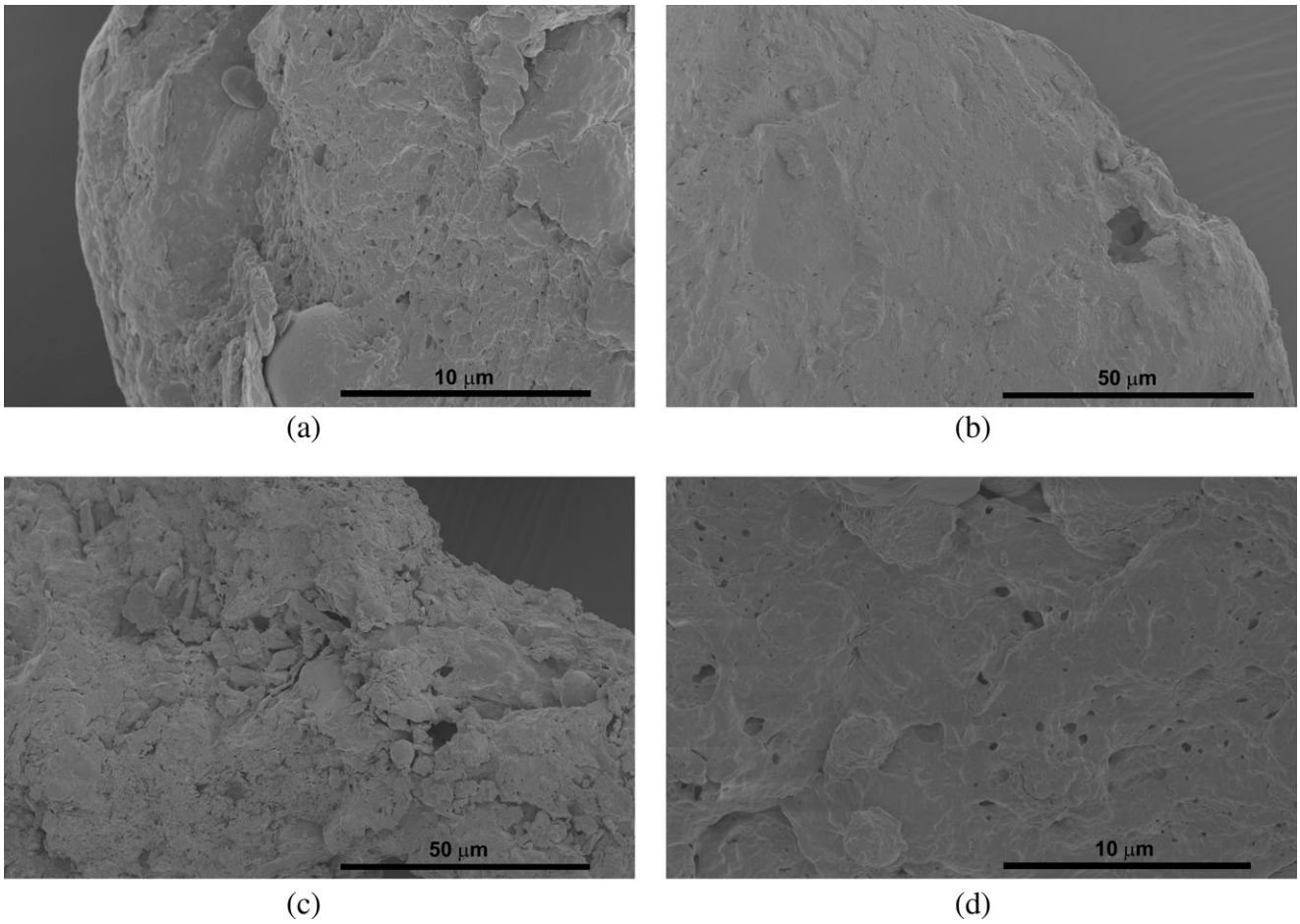

Figure 6. SEM micrograph of (a) neat wheat gluten, (b) 0.75 : 1 wheat gluten to citric acid sample heated at 120°C, (c) 0.50 : 1 wheat gluten to citric acid sample heated at 120°C, and (d) 0.38 : 1 wheat gluten to citric acid sample heated at 120°C.

temperatures [see Figure 4(a)]. In addition, the derivative curves of the samples with different gluten : citric acid ratios also had jagged peaks, indicating heterogeneous structures (data not shown).

Scanning Electron Microscopy

The citric acid modification did not seem to have much effect on the surface features of wheat gluten samples. This is shown in Figure 6, which presents SEM micrographs of neat and modified gluten samples at different gluten to citric acid ratios. The neat gluten sample had a relatively rough surface and contained pores of various sizes. The modified gluten samples also had similar features, indicating the citric acid treatment did not alter surface morphology to a large extent. These results differed from a previous study involving sulfuric acid modification of wheat gluten films. In that case, sulfuric acid treatment resulted in the appearance of uneven spots.[20]

Water Uptake

The modified gluten samples were able to absorb up to 78 times their weight in deionized water. This is shown in Table II. Neat gluten has a water uptake value of 1.5. This increase in water uptake of modified gluten was due to the attachment of carboxylic acid groups onto gluten from reaction with citric acid. After neutralization with sodium hydroxide, the carboxylic acid groups loose a proton to become carboxylate groups. These carboxylate groups are negatively charged and repel each other within the wheat gluten matrix. When the gluten sample was placed in water, the gluten chains began to swell due to absorbance of water. The repulsion between the carboxylate groups forces the gluten matrix to swell even further, allowing for more incorporation of water. Eventually, swelling stopped due to restrictions in movement of gluten chains from disulfide crosslinks.

One advantage of using gluten to produce superabsorbent materials is the presence of disulfide crosslinks. In previous studies, a crosslinking step was usually required to produce superabsorbent materials from natural polymers. In some studies, the authors first grafted poly(acrylic acid), polyacrylamide, polyacrylonitrile, or their copolymers onto the natural polymer backbones. After the grafting step, these polymers were usually cross-linked to improve their mechanical properties. In other studies, the authors produced superabsorbent materials by directly crosslinking the natural polymers. In comparison, gluten required no further crosslinking after reaction with citric acid since it already contained disulfide crosslinks.

Table II. Water Uptake of Modified Wheat Gluten

Gluten : citric acid ratio	Water uptake[a]			
	100°C	110°C	120°C	130°C
0.38 : 1	-	-	45 ± 14	-
0.50 : 1	32 ± 21	68 ± 19	78 ± 13	50 ± 20
0.75 : 1	-	37 ± 17	27 ± 2	-

[a]Water uptake of neat gluten is 1.5.

There seemed to be an optimal gluten to citric acid ratio and reaction temperature range that resulted in the highest water uptake value. In this study, a gluten to citric acid ratio of 0.50 : 1 and a reaction temperature of 120°C resulted in the highest water uptake value of 78. This is shown in Table II. The 0.50 : 1 sample reacted at 110 and 130°C also had comparable water uptake values. Several studies had found that the reaction between citric acid and distillers' dried grain,[23,24] corn gluten meal,[23,24] and soy protein isolate[23] at 120°C resulted in samples having the highest carboxylic acid content. In another study with corn starch, Wing[21] found the reaction temperature of 130°C led to the highest carboxylic acid content. However, an increase in reaction temperature to 140°C resulted in lower carboxyl content. The author suggested that this decrease might be due to crosslinking of starch molecules with citric acid. Crosslinking occurred when citric acid that had formed one ester bond with a starch molecule formed another ester bond with a different starch molecule. For gluten samples in this study, the 0.50 : 1 sample at 130°C and the 0.38 : 1 sample at 120°C contained the largest amount of reacted citric acid (see Table I). However, this increase in citric acid reaction did not result in the highest water uptake values. This might indicate more crosslinking occurred in these samples, leading to an increase in crosslink density and lower swelling capacity when placed in water. The lower swelling capacity would reduce water uptake, which was consistent with the results in Table II.

CONCLUSIONS

We modified wheat gluten with citric acid and produced natural superabsorbent materials that absorbed up to 78 times their weight in deionized water. FTIR analyses showed that reaction with citric acid and subsequent neutralization with sodium hydroxide resulted in attachment of carboxylate groups on gluten chains. These negatively charged carboxylate groups repelled each other, resulting in increased water uptake. More citric acid reacted with gluten at higher citric acid concentrations and higher temperatures. The sample with a gluten : citric acid ratio of 0.5 : 1 at 120°C had the largest water uptake value. Also, all modified gluten samples had lower thermal stability than neat gluten.

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