Production of composites by using gliadin as a bonding material

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A B S T R A C T

In previous papers, a new technology that produces biopolymer composites by particle-bonding was introduced. During the manufacturing process, micrometer-scale raw material was coated with a corn (Zea mays L.) protein, zein, which was then processed to form a rigid material. The coating of raw-material particles with zein makes use of the unique property of this protein in aqueous ethanol solution. In this paper, it is shown that the behavior of a wheat (Triticum aestivum L.) protein, gliadin, is very similar to zein in aqueous ethanol. Size variation of aggregates in 45–65% aqueous ethanol was investigated with a turbidimeter in conjunction with Size Exclusion Chromatography. Confirming the resemblance of gliadin to zein in terms of aggregation behavior in aqueous alcohol, composites were fabricated by particle-bonding and their mechanical property was measured with a Universal Testing Machine. Test results showed that gliadin is a good substitute for zein in the production of composites.

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1. Introduction

Many research groups have concentrated on the development of biodegradable polymer blends or composites from starch (Grazuleviciene et al., 2007; Ma et al., 2008), corn gluten meal (Beg et al., 2005; Samarasinghe et al., 2008), and wheat gluten (Olabarrieta et al., 2006; Ye et al., 2006; Zhang et al., 2007). In most cases, agricultural biopolymers contain a significant amount of unwanted materials that remain after the extraction/isolation process. Purification of these materials is very costly for the practical utilization of these materials as a component of useful final products. If these agricultural surplus products can be used without further purification, we can minimize the use of toxic chemicals, simplify the fabricating process, save energy, and lower the production cost.

In previous papers, a new technology that produces biopolymer composites at room temperature was introduced (Kim, 2008; Kim and Xu, 2008; Kim et al., 2010). This technology requires neither extrusion nor processing at high temperatures. Instead, micrometer-scale raw materials (powders) are coated with zein, and processed to form a rigid material. Zein is a class of prolamin protein found in maize. It is usually manufactured as a powder from corn gluten meal. Zein is utilized because it has good adsorption to hydrophilic surfaces and strong adhesive properties. Since this technology does not require purification of raw materials except zein, various types of compounds can be used as component materials.

Because of its high proportion of non-polar amino acid residues, zein is not soluble in pure water. Conventionally, 70–90% aqueous ethanol has been used as a solvent for zein in many experiments (Dickey et al., 2003; Fu and Weller, 1999; Guo et al., 2005; Kim et al., 2004; Parris and Coffin, 1997; Xu et al., 2007). In the previous paper, it was shown that the size variation of zein aggregates depends on the composition of the solvent and there is a structural inversion of the micelle-like structure of zein aggregates at around 90% aqueous ethanol (Kim and Xu, 2008). According to this basic research, aggregation number of zein is minimum in 90% aqueous ethanol, but it increases rapidly as the percentage of ethanol increases to 92%. Further increase in ethanol percentage causes precipitation. Any particles with a hydrophilic surface could be coated with zein during this process. This behavior led us to the development of a new technology for the production of polymer composites by particle-bonding whereby zein was used as an adhesive. In short, the newly developed technology includes three steps: (1) Suspending the matrix materials in zein solution. (2) Coating the matrix materials with zein molecules and induction of aggregation. (3) Removal of the solvent. In a subsequent paper, the mechanical property of gluten/zein composite that was fabricated by the aforementioned particle-bonding technology was introduced (Kim, 2008).
Zein belongs to the characteristic class of proteins known as prolamins, which occur specifically in cereals (Shukla and Cheryan, 2001). Most prolams are enriched in glutamine, proline and hydrophobic amino acids; they are insoluble in water or buffered salt solutions but soluble in solutions containing alcohols. A wheat protein, gliadin also is classified as a prolamin (Bietz, 1983). Gliadin is one of the main fractions of gluten found in the endosperms of wheat. Gliadins are mainly monomeric proteins with molecular weights around 28,000–55,000 and can be classified according to their different primary structures into the α/β-, γ- and ω-type. Each gluten protein type consists of two or three different structural domains; one of them contains unique repetitive sequences rich in glutamine and proline. Non-covalent bonds such as hydrogen bonds, ionic bonds and hydrophobic bonds are important for the aggregation of gliadins and implicate structure and physical properties of dough (Wieser, 2007).

Traditionally, 70% (v/v) aqueous ethanol has been used as a solvent for gliadin (Jackson et al., 1983; Robertson and Cao, 2004). In this paper, it will be shown that 70% (v/v) aqueous ethanol, which corresponds to 62% (w/w), dissolves gliadin more efficiently than other ethanol/water mixtures by both turbidity measurement and Size-Exclusion Chromatography. Since both gliadin and zein show high solubility in aqueous ethanol (the only difference is the peak solubility composition, i.e., 62% (w/w) for gliadin and 90% (w/w) for zein), similar aggregate-forming behavior is expected. In this paper, therefore, the aggregate-forming behavior of gliadin in aqueous solution was examined and it is shown that gliadin can be used for the fabrication of the composites in a similar process as was done with zein. The major advantage of utilizing gliadin is a much lower production cost.

2. Materials and methods

2.1. Materials

Wheat gluten, normal maize starch, potato (Solanum tuberosum L.) starch, iron powder, glass spheres, graphite powder, and ethyl ether were purchased from Sigma (St. Louis, MO). Wheat gliadin was a gift from MGP Ingredients, Inc (Atchison, KS). Oil impurities of gliadin were extracted with ether. Ethanol was from Aaper (Benton City, WA) and Millipore water was used without further treatment.

2.2. Preparation of purified gliadin

To purify gliadin, 200 g of crude gliadin was immersed in 700 g ethyl ether and stirred for 1 h followed by filtration. The defatted crude gliadin was dried in the hood overnight. From the recovered crude gliadin, 100 g was suspended in 1200 g of 65% (w/w) aqueous ethanol, stirred for one day, stood overnight, and supernatant was collected. Extraction of gliadin with 65% (w/w) aqueous ethanol was repeated twice. After the second process, the residual supernatant was collected by filtration. Most ethanol in the collected supernatant was removed by using a rotavapor, and the concentrated gliadin solution was freeze-dried and weighed. This analysis was duplicated and the amount of gliadin in gluten was found to be 40 ± 0.5%.

2.4. Transmittance measurement

Transmittance of the gliadin solution in aqueous ethanol was measured with a custom-built turbidimeter composed of a He–Ne laser, temperature-controlled sample block, stirrer, neutral density filter, laser powermeter, and laptop computer. The 633 nm beam from the He–Ne laser passes through the sample solution in a scintillation vial, the diameter of which is 2.5 cm. During the transmittance measurement, the sample solution was continuously stirred with a digitally controlled magnetic stirrer. The intensity of the transmitted laser beam was monitored with a laser powermeter, the output of which is interfaced with a laptop computer.

2.5. Size-exclusion chromatography (SEC)

Size variation of gliadin aggregates in aqueous ethanol was measured using SEC. An SEC instrument (Shimadzu, VP series, Tokyo, Japan) equipped with an SEC column (Asahipak, GF-510HQ) maintained at 25 °C was employed. Injection volume was 50 μL. Eluent was 45–65 wt% aqueous ethanol with a flow rate of 0.5 mL/min. Two detectors, refractive index (Optilab DSP Interferometric Refractometer, Wyatt Technology, Santa Barbara, CA) and light scattering (DAWN EOS, Wyatt Technology) were used to detect and record SEC output.

2.6. Preparation of protein composites

Test specimens from protein composites were prepared by using molds fabricated from Teflon and aluminum. Typical procedure for test specimens was as follows (example composites with 20% gliadin). Both 6 g of matrix material and 1.5 g of gliadin powder were dispersed and mixed thoroughly in 12.4 g of absolute ethanol. Subsequently, 7.6 g of distilled water were added and stirred until the gliadin was fully dissolved. After that, 60 g of ethanol were added with vigorous stirring. During this time, proteins aggregated to form a large chunk. Supernatant was decanted, and the aggregate was poured into a Teflon mold. Compression pressure was adjusted to 75 lbs/cm² for 2 min. The fabricated specimens were air-dried for 72 h, and stored at 50 ± 5% relative humidity until the mechanical property was measured. For other percentages of gliadin, the content of gliadin was calculated by (wt. of gliadin)/(wt. of both matrix and gliadin) × 100. In such cases, the amounts of both matrix and gliadin were adjusted, leaving the total weight (7.5 g, matrix material + gliadin) intact. If x times larger composites need to be fabricated, the above procedure can be modified by simply multiplying the amount of the materials and chemicals by x.

2.7. Mechanical property measurement

The sample specimens were prepared by the conditions specified in the American Society for Testing and Materials (ASTM) standard, D695. The test specimens were conditioned at 23 ± 2 °C and 50 ± 5% relative humidity for no less than 40 h before testing in accordance with Procedure A of Practice D618. The samples were tested with an Instron Universal Testing Machine (Model 55R1122, Canton, MA) equipped with 500 kg reversible load cell. Merlin software handled data recording and manipulation. For all the tested samples, a crosshead speed of
1.3 mm/min was used. Compressive yield strength, modulus of elasticity, and yield strength at 0.2% offset were compared between sample specimens.

3. Results and discussion

3.1. Aggregation of gliadin in aqueous ethanol

While zein dissolves in 70–90% aqueous ethanol, gliadin is known to dissolve in 65% (w/w) aqueous ethanol (i.e., 70% (v/v) aqueous ethanol) (Bietz and Wall, 1980). For better understanding of the solubility behavior of gliadin, solution turbidity was measured. Overall behavior of gliadin was very similar to that of zein (Fig. 1a). Zein was highly soluble in 70–90% aqueous ethanol but rapidly precipitated when the ethanol content exceeded 90%. On the other hand, gliadin favored lower ethanol content: it was soluble in 45–62% aqueous ethanol and precipitated at higher than 62% ethanol. Comparing gliadin turbidity data with that of zein, it was expected that the size of aggregates would increase as the content of ethanol decreases from 62% to 45%; this issue will be discussed later in this section. The aggregate size also is expected to be smallest at approximately 62% ethanol and structural inversion of the aggregate is expected at the same time. Disintegration of aggregates at 62% ethanol, structural inversion of aggregates, and aggregate formation on the surface of hydrophilic powder particles at higher ethanol content are the required conditions for gliadin to be used as a substitute material for zein in the production of composites by particle-bonding.

During the collection of turbidity data, it was noted that the transmittance of the gliadin solution changes with time when the content of ethanol is higher than 62%. Fig. 1b shows the transmittance variation of gliadin solution from the moment gliadin powder was dissolved in 62–70% aqueous ethanol solutions. The initial stage of the data was randomly scattered because of the floating gliadin particles. As the solution was stirred, gliadin particles were solubilized and formed aggregates. In the recorded data, this process appeared as an initial decrease and subsequent increase in the transmittance. The reason for showing a minimum transmittance point is as follows. Upon immersion of gliadin in higher than 62 wt% aqueous ethanol followed by stirring, gliadin powders began to dissolve and form aggregates. Therefore, there existed two types of particles in the solution; undissolved gliadin patch and solubilized aggregates. During this time, transmittance decreased with time, showing scattered detector output (Fig. 1b). Once the transmittance reached a minimum, it increased again because aggregates grew to form large agglomerates which occasionally blocked the laser beam. A scattered signal was observed by the detector. At this time, transmittance increased because the concentration of gliadin in the solution decreased. If stirring was stopped at that moment, aggregates precipitated at the bottom of the vial. Since the observed transmittance showed initial decrease and subsequent increase, the lowest transmittance point was used for the construction of Fig. 1a. The lowest transmittance point decreased as higher ethanol content was used, indicating that the thermodynamic parameters favored the formation of larger aggregates in that ethanol content. From this experiment, it was concluded that higher than 62 wt% ethanol content in the solvent induced precipitation and faster precipitation was observed as the content of ethanol increased. This means that gliadin can be used as a substitute for zein in the production of composites by a particle-bonding technique.

In the case of 45–62 wt% aqueous ethanol, it was expected that the degree of aggregation (or aggregate size) would increase as ethanol content in the solvent decreases. SEC chromatography was employed to observe the size variation of gliadin aggregates. The

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**Fig. 1.** (a) Transmittance of Gliadin in a series of ethanol solutions. 0.5% gliadin solutions was examined at 25.0 ± 0.2 °C. A 15 mW He–Ne laser (λ = 633 nm) was used as a light source. (b) Time-dependent transmittance: Dissolution of gliadin and subsequent aggregate formation are shown. Weight percentage of ethanol is specified on each curve.

**Fig. 2.** Normalized RI signal output from SEC chromatography. 0.5% gliadin dissolved in a series of aqueous ethanol to show the growth of aggregates. Weight percentage of ethanol is specified in the inset.
refractive index (RI) detector clearly showed the most abundant size in the aggregates increased as the content of ethanol decreased. To show this trend more clearly, RI signal output was normalized (Fig. 2). In all ethanol contents, gliadin existed in three sizes. According to the light scattering (LS) detector output, the middle peak in the RI output was the major species (Fig. 3). The rightmost peak did not indicate a polymer as it did not register a signal in the LS detector output. It was an unexpected finding that there existed two kinds of aggregates in the solution. RI signal for 62 wt% ethanol showed a minimum degree of aggregation. Less ethanol content induced formation of aggregates that appeared as a leftmost peak in each curve. From Fig. 2, it was readily concluded that the average size of aggregates increased as the content of ethanol decreased. This trend is more clearly visualized in Fig. 3. As was expected, gliadin in 62% ethanol showed minimum degree of aggregation. The particle size difference between 60 wt% and 62 wt.% was huge, and comparable to the abrupt change in the particle size of zein at around 90 wt % ethanol. From these two sets of chromatography data and transmittance data, it was concluded that gliadin behaved in the same way as zein did in aqueous ethanol except that the optimal solubilization ethanol composition was different.

3.2. Principle of fabrication

The fabrication process of gliadin composites was basically the same as that of zein composites except that the suspension medium was ca. 60% aqueous ethanol. Gliadin was dissolved in 60% aqueous ethanol and matrix particles that did not dissolve in the same solvent were dispersed by stirring. As more ethanol was added, the amount of aggregate decreased and inversion of the micellar structure of gliadin occurred at ca. 62% ethanol. More addition of ethanol induced precipitation of gliadin on the surface of matrix particles. Matrix particles were coated with gliadin molecules in this way. Since the surface of gliadin-coated particles is sticky, these particles stuck together and formed large precipitates. These precipitates were put in a mold, solvent molecules were squeezed out, and stored in a ventilated place until fully dried.

3.3. Mechanical properties

Provided the size of the particle that will be suspended is not too large, any type of particles can be used as a matrix material of composites. The function of gliadin is to bind the particles together. Starches are micron-sized particles unless they are gelatinized. Therefore, they can be used without further treatment. For other types of materials such as proteins, powders that range from a few microns to several hundred microns can be produced by spray drying.

Since zein was replaced with gliadin, the other factors such as optimum percentage of gliadin and mechanical strength were also examined. In the case of zein composites, it was observed that too little or too great of an amount of zein yielded a mechanically weak product. In the previous paper, a wheat protein mixture, gluten, had been used to find an optimum percentage of zein in the composite and composites with ca. 20% zein showed highest mechanical strength (Kim, 2008). In this paper, glass spheres were used for the same purpose. Gluten was not used because gluten itself contains some amount of gliadin. As was anticipated, too little or too great an amount of gliadin yielded a mechanically weak product (Fig. 4). The strength of the composites was increased until the composition reached 20% and was weakened thereafter. Repeated experiment revealed that there was variation in the overall profile of the compressive stress vs. strain curves, but the maximum strength was always obtained with ca. 20% gliadin, at which composition the compressive strength was comparable to that of polypropylene (Northolt, 1981).

To show that the aforementioned technology can be applied to most of the biopolymer particles, various types of materials were used to fabricate the composites (Fig. 5). Since the suspension medium of the matrix material(s) contained equal to or higher than 62% ethanol, matrix materials that dissolved in this concentration could not be used. In the case of fine sugar, fabrication of composite with gliadin was not possible because it formed a gel in 62% aqueous ethanol while its composite could be fabricated with zein. Other requirements of the particles to be used for fabricating composites are the micrometer-scale size and hydrophilicity of the particle surface. It was speculated that the maximum compressive strength of the composite would be proportional to both the bond strength between the matrix particle and gliadin and hardness of the matrix particle itself. Weak bond strength between the matrix particle and gliadin could be caused by the difference in the hydrophilicity of the surface of matrix particles. In the previous paper, it was shown that the inclusion of graphite which is less hydrophilic than average biopolymers weakened the strength of produced composites (Kim et al., 2010).
require isolation/purification of gliadin. Thus less expensive composites can be fabricated.

4. Conclusion

The aggregate-forming behavior of gliadin is very similar to that of zein in aqueous ethanol. Therefore, it is possible to fabricate composites by a particle-bonding technique. Since the price of gliadin is far lower than that of zein ($3-4/lb vs. $25-30/lb), gliadin is a preferred binder to zein. When wheat gluten is to be used as a matrix material, isolation/purification of gliadin is not needed thereby production costs are reduced. The composites produced with gliadin have the same merits as those produced with zein. They do not require purification of matrix materials; the fabrication process can be performed at room temperature without heating; biodegradable composites can be fabricated when biopolymers are used as raw material; two or more types of raw materials can be used at any mixing ratio.

3.4. Gluten composites

Wheat gluten is a mixture of gliadin and glutenin (Lafandra et al., 2004). Since both components are insoluble in 90% aqueous ethanol, wheat gluten could be used as a matrix material in the zein composites (Kim, 2008). As was described in the previous sections, gliadin, which is one of the two components of gluten, can work as a bonding material when the matrix gliadin mixture is processed with 62% (w/w) aqueous ethanol. Since wheat gluten is already a mixture of glutenin (matrix) and gliadin, it is expected that we can process it with aqueous ethanol to fabricate composites. However, the wheat gluten used in this study contains ca. 40% of gliadin according to the analysis described previously in Section 2.3. Fig. 4 and the previous study showed that the content of gliadin or zein needs to be around 20% to fabricate mechanically sturdy composites, assuming the density of the matrix is not much different from that of gliadin or zein. Therefore, a second matrix material has to be added to the mixture to lower the percentage of gliadin in the final mixture. In Table 1, several examples are shown with mechanical properties. As a second matrix material, cornstarch, glass spheres, flour salt, potato starch, milk powder, buckwheat flour, or cornstarch were added to the mixture. Experimental data shown in Table 1 confirmed that mechanical strength of gluten composites fabricated by the aforementioned process was acceptable for practical usage. Needless to say, any material that does not dissolve in 62% aqueous ethanol can be mixed with gluten as a second matrix material. Unlike the procedure explained in the previous sections, fabrication of wheat gluten composites does not need isolation/purification of gliadin. Thus less expensive composites can be fabricated.

Table 1

<table>
<thead>
<tr>
<th>2nd Matrix material</th>
<th>Gliadin content in the final mixture (%)</th>
<th>Compressive strength (MPa)</th>
<th>Compressive modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>15</td>
<td>17.1 ± 4.4</td>
<td>391 ± 46</td>
</tr>
<tr>
<td>Glass spheres</td>
<td>15</td>
<td>28.1 ± 2.1</td>
<td>964 ± 63</td>
</tr>
<tr>
<td>Flour salt</td>
<td>15</td>
<td>32.3 ± 2.2</td>
<td>1440 ± 130</td>
</tr>
<tr>
<td>Potato starch</td>
<td>15</td>
<td>19.6 ± 3.6</td>
<td>450 ± 78</td>
</tr>
<tr>
<td>Milk powder</td>
<td>15</td>
<td>9.3 ± 2.9</td>
<td>570 ± 120</td>
</tr>
<tr>
<td>Buckwheat flour</td>
<td>15</td>
<td>17.9 ± 2.1</td>
<td>479 ± 87</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>20</td>
<td>19.6 ± 4.4</td>
<td>741 ± 102</td>
</tr>
<tr>
<td>Glass spheres</td>
<td>20</td>
<td>17.3 ± 0.5</td>
<td>1023 ± 74</td>
</tr>
</tbody>
</table>

Fig. 5. Compressive stress–strain diagram of various composite samples: flour salt, flour, cornstarch, potato starch, cocoa powder, amaranth starch, milk powder, and graphite. Each composite contains 18% (w/w) gliadin. This illustration shows that various materials can be used for the production of composites by particle-bonding technology.

References


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