

Treatments of protein for biopolymer production in view of processability and physical properties: A review

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The development of bio-based polymers from proteins has gained attention for their large availability and renewable and biodegradable nature. However, protein-based plastics have limited commercial applications because of several drawbacks, such as poor processability, brittleness, moisture sensitivity, and inferior mechanical and thermal properties. Extensive studies have been conducted to solve or ameliorate these issues by pretreatment or modification of proteins or protein-derived biopolymers before or during wet processing or dry processing at elevated temperatures. This review provides an overview of research efforts conducted in the area of physical and chemical treatment of proteins to achieve better processability, mechanical properties, and other physical performance based on a literature review in this subject. © 2016 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2016**, *133*, 43351.

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INTRODUCTION

Biopolymers derived from agricultural resources such as wool, cotton, leather, and silk have been extensively used by humans for a long time. Industrial use of agricultural commodities for fuels and consumer products began in the 1920s. They were soon replaced by petroleum-based chemicals after World War II because these synthetic polymers were cheaper, more durable, and more versatile. Advances in petroleum-based fuels and polymers have benefited mankind in numerous ways. However, sustainability of their production and use has become a societal concern. Besides, petroleum resources are finite, and their prices are likely to continue to rise in the future. The disposal of petroleum-based plastic has also been causing environmental concerns.¹ Since the early 1990s, the use of renewable biopolymers as a substitute for nonbiodegradable petrochemical-based polymers has again attracted considerable attention because of their environmental benefits such as renewability and biodegradability. Nowadays, natural polymers can even be tailored to meet specific needs.

Protein is one of three main categories of renewable biopolymers aside from polysaccharides and polynucleotides that are derived from plant or animal resources. Plant proteins that can be used for bio-based plastics include soy protein, corn zein, wheat protein, cottonseed protein, and sunflower protein. Animal proteins such as blood meal, gelatin and collagen, keratin and feather quills, egg protein, whey protein, and meat and

bone meal can also be used as a feedstock for such bio-based polymers.² Despite their environmental friendliness, protein-based biopolymers have many shortcomings like lower mechanical properties when compared to petroleum-based polymers.³ Due to the hydrogen bonds between C=O and NH groups, protein-based polymers have poor processability and exhibit high sensitivity to moisture. As a result, the protein-based polymers are very difficult to process without plasticizers or pretreatment. To date, overcoming these shortcomings of protein-based bioplastics has proven difficult. Numerous methods have been investigated, including physical treatment, denaturation of the original protein in crystalline form, cleavage of disulfide bridges, grafting, and crosslinking.

The objective of this article is to review the literature available on pretreatment or modification of protein-based biopolymer resources, either physical or chemical, before or during processing, and on the effects of pretreatment on processability or properties of the resultant biopolymer. A recent review of plasticizing modifications of protein-based polymers is available,² so that is not covered in this paper.

DENATURATION AND PHYSICAL OR CHEMICAL TREATMENT

The protein network is stabilized by covalent (disulfide) bonds as well as by noncovalent interactions, i.e., hydrogen bonds and

hydrophobic interactions.⁴ The native conformation of protein can be irreversibly denatured with any treatment that cleaves hydrogen bridges or ionic or hydrophobic bonds. This can be accomplished by changing the temperature, adjusting the pH, increasing the interface area, or adding organic solvents, salts, urea, guanidine hydrochloride, or detergents such as sodium dodecyl sulfate.⁵ These methods have been used for pretreatment of protein prior to biopolymer processing. Cleavage of disulfide bonds by reducing agents to reduce viscosity for better thermal processability has also been used.

Heat Treatment

Heat treatment was believed to promote the formation of covalent bonds between wheat gluten proteins, which induces the thermosetting effect.⁶ Selling and coworkers also observed that heat treatment during the extrusion process triggered disulfide bond formation, which led to a molecular weight increase.^{7,8} Domenek *et al.* measured the gluten extractability and activation energy of a protein polymer hot-molded with 35 wt % glycerol at five temperature levels: 71, 82, 104, 124, and 150°C.⁴ Initially, approximately 85% (w/w) of the wheat gluten protein was soluble in a sodium dodecyl sulfate (SDS) buffer. Hot-molding of the gluten/glycerol blend significantly reduced its solubility in the SDS buffer, and the solubility kept decreasing as the temperature and hot-molding time increased. The drop in solubility was a result of the formation of disulfide crosslinks involved in the gluten protein aggregation because SDS disrupted only noncovalent interactions. The samples pressed at 150°C showed a maximum in insoluble protein. The activation energy of the polymer processed with the two-blade counterrotating mixer was 34 kJ/mol, significantly lower than that of the simply hot-molded gluten/glycerol blend, 149 kJ/mol. The mechanical scission of disulfide bonds may have contributed to the formation of free radicals in the system. Pereda *et al.* also found that the total soluble mass of the heat-treated wet-cast sodium caseinate/glycerol plastic film dropped to 25%, as compared to 100% of the samples without heat treatment.⁹

In terms of mechanical properties, heat treatment was reported to cause an increase in tensile modulus and strength but a reduction in elongation for extruded soy protein biopolymer.¹⁰ Pereda *et al.* observed the positive effect of heat treatment on the mechanical properties of the wet-cast sodium caseinate/glycerol plastic film.⁹ The wet-cast film was heat treated in an air-circulating oven at 105°C. After heat treatment, the modulus, strength, and elongation of the plastic film were improved 64%, 9.5%, and 42%, respectively. This heat-treated specimen also showed reduced moisture sorption and water vapor permeability and improved thermal stability. Zein-based extruded sheets and films, plasticized with oleic acid, were processed by single-screw or twin-screw extruders through a slit die or blowing head.¹¹ After heat treatment by hot rolling or pressing, extrudates from the twin-screw extruder showed higher tensile strength, lower elongation at break, and higher Young's modulus. Extrudates processed by a single-screw extruder followed the same trend, though changes were not significant.

Heat treatment was also reported to be able to control the crystal structure of an additive (potassium nitrate) in wet-cast

sodium caseinate-based biopolymer films.¹² The characterization and control of the additive's solubility and the mechanical properties of the resultant biopolymer film are of great interest to be studied as mulching films for its fertilizing capability. Two crystal structures, the typical orthorhombic crystal system (phase III KNO₃) and the trigonal structure (phase II KNO₃), were observed. The film drying is preferably conducted at low relative humidity to avoid the time-dependent formation of acicular crystals (phase III), which may result in irregularities and weak spots where film specimens can preferentially rupture during mechanical testing and eventually lead to inferior mechanical properties.

Acid

It is well known that the solubility of protein can be increased by adjusting the solution's pH value away from the isoelectric point. Khansari *et al.* used formic acid to dissolve protein to produce a nanofiber mat using the electrospinning process.¹³ With the addition of acid, two plant-derived proteins, soy protein and zein, and two animal-derived proteins, silk protein sericin and bovine serum albumin, were successfully copun into nanofiber with polyamide-6 (nylon-6) and poly(ethylene terephthalate) (PET). The incorporation of protein into synthetic polymers reduced the mechanical properties of the nanofiber mat but could increase its biocompatibility and biodegradability, which is of interest for applications such as wound dressing or drug carriers.

Jansens *et al.* compared acid and alkaline pretreatments on the molecular network and the mechanical properties of wheat gluten bioplastics.¹⁴ Commercial wheat gluten has a protein content of 77.8%. Gluten was pretreated by acid (0–25 mmol/L sulfuric acid or 25 mmol/L hydrochloric acid) with 70% ethanol. The control or pretreated wheat gluten was molded into bioplastic at elevated temperatures for 5 min. In contrast to alkaline pretreatment, the formation of disulfide and non-disulfide crosslinks during compression molding was hindered by acid pretreatment. The flexural modulus showed little dependence on either alkaline or acid pretreatment. The acid pretreatment even caused a slight decrease in flexural strength.

Alkali

The mechanical properties of wet-cast plastic films from soy protein isolate and wheat gluten at various pH values were evaluated.¹⁵ The protein was dissolved into distilled water with glycerol (and ethanol for wheat gluten) with the pH values adjusted with hydrochloric acid or sodium hydroxide. The resultant solutions were heated, poured onto Teflon-coated glass plates, and dried to produce soy protein (SPI) and wheat gluten (WG) films. Film formation was inhibited by poor protein dispersion around the isoelectric pH region, 4.5 for soy protein isolate and 7.6 for wheat gluten. At pH values away from the isoelectric region, the protein denatures, unfolds, and solubilizes, exposing sulfhydryl and hydrophobic groups. Such groups associate upon drying to form disulfide bonds and hydrophobic bonding forces. The tensile testing results showed that protein biopolymer films prepared with pH values higher than the isoelectric pH region had significantly higher tensile strength than films processed under acidic conditions.

Similar results were obtained with compression-molded gluten pretreated by alkali or acid.¹⁴ Alkali-pretreated bioplastic samples had higher strengths than acid-pretreated ones. Gluten crosslinking was evaluated by protein extractability in an SDS-containing medium, by the amino acid composition, and by the free sulfhydryl and dehydroalanine contents. The results showed that, in addition to compression molding, alkaline treatment also enhanced both disulfide bonds and other types of non-disulfide crosslinks, possibly isopeptide bonds or ester bonds. Although alkali pretreatment has been found to be an effective method for mechanical property enhancement, extremely high alkali concentrations should be avoided because they inhibited protein film formation¹⁵ and protein degradation.¹⁴

Reducing Agents

Reducing agents have been widely used to dissociate the disulfide bonding within the protein chains, which cause large aggregates of protein, so that better processability can be achieved. Song and Zheng studied the effect of three reducing agents, sodium bisulfite, sodium sulfite, and thioglycolic acid, on the properties of the glycerol plasticized glutenin dough and the resultant crosslinked compression-molded bioplastics.¹⁶ The Young's modulus of the reducing agent-treated dough significantly decreased, suggesting possible better thermal processability. This pretreatment of glutenin by reducing agents led to a significant increase in Young's modulus but reduction in elongation at break of the resultant crosslinked bioplastic as compared with the control samples.

Li *et al.* studied the effect of sodium bisulfite during isolation of canola protein from canola meal with the alkali solubilization and acid precipitation methods.¹⁷ The isolation was carried out in either control or reducing conditions by NaHSO₃ at different concentrations (0–15 g/L). The protein recovery rate from canola meal increased slightly from 31.33 to 34.37% as the sodium bisulfite concentration was increased from 0 to 15 g/L. The sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) results showed that the intensity of the polypeptide bands of modified canola protein at 17 kDa, attributed to napin subunits, almost disappeared when the concentration of NaHSO₃ reached 15 g/L, and the density of smaller polypeptides at 9 kDa increased concomitantly, suggesting that sodium bisulfite caused disassociation of the disulfide bonds in napin protein molecules. The reducing effects of sodium bisulfite could also be reflected by the attenuated bands at 55 kDa, belonging to the cruciferin subunits stabilized by disulfide bonds, and by the intense bands at 30 kDa, belonging to the disassociated polypeptides. The viscosities of modified proteins decreased notably as sodium bisulfite concentration increased, 1200 Pa s (0.1 s⁻¹) for the canola protein treated with 15 g/L sodium bisulfite solution, as compared to 9000 Pa s (0.1 s⁻¹) for unmodified canola protein. The decrease in viscosity of the protein improved the handling and flowability of the canola protein adhesive. The adhesion properties of canola protein as an adhesive for plywood were evaluated. The wet shear strength decreased slightly as the concentration of NaHSO₃ increased from 0 to 6 g/L, then increased as NaHSO₃ concentration rose from 9 to 15 g/L.

The use of sodium sulfite to break down covalent crosslinks was found effective in achieving a better processability for extrusion of blood meal protein.¹⁸ Observations were based on the torque and pressure readings during extrusion, as well as other observations such as the surface of the extrudate and whether a consistent and continuous extrudate would form. Sodium sulfite assisted in forming a flowable melt with plasticizer (water) and other chemical additives. Sodium sulfite was further observed to cause significant changes in blood meal-based biopolymer water absorption, mechanical properties, thermal stability,^{19,20} and injection-molding performance.²¹ Sodium sulfite was also used as a thermal processing aid for blending soy protein with other synthetic polymers, for example, poly(butylene adipate-co-terephthalate)²² and PLA.²³

Fatty Acid

Fatty acid is mainly used to improve the moisture resistance of protein-based biopolymer because of its hydrophobic nature. Consequently, the mechanical properties of the resultant biopolymer would be affected too. For instance, rapeseed oil was homogenized and then was added to a heat-denatured whey protein isolate/glycerol mixture at a percentage up to 4 wt % relative to the weight of whey protein isolate.²⁴ The incorporation of rapeseed oil led to a 2.5-fold increase in surface hydrophobicity of the cast whey protein film, as shown by the contact angle observations. Water vapor permeability decreased consequently. Research on zein plasticized by oleic acid has been conducted by Padua and coworkers.^{25–27} Zein-based films or sheets plasticized by oleic acid showed increased elongation and decreased Young's modulus and water absorption. Palmitic or stearic acids were also used as plasticizers solely for protein-based biopolymers in the research of Padua *et al.*²⁸ As expected, water absorption on a corn zein/fatty acid polymer sheet decreased continuously as the fatty acid content increased. Tensile modulus, strength, and elongation of zein sheets increased as well with the addition of low levels of these two fatty acids. When the stearic acid was used with glycerol for soy protein isolate, in addition to the reduction in moisture sensitivity, the Young's modulus increased with increasing stearic acid content, reaching the maximum value at about 25% of stearic acid.²⁹ However, fracture stress and strain decreased on increasing the stearic acid concentration from 0 to 25% in the SPI resin with 30% glycerol. The effect of addition of fatty acid on the mechanical properties of a protein-based biopolymer could depend on the chemical structures of the fatty acid or the protein sources.

Fatty acid was also reported to induce differences in thermal properties of a protein-based biopolymer. TGA measurements showed that the thermal degradation of stearic acid–modified soy protein isolate resin took place at a higher temperature than that of the control samples, indicating improved thermal stabilities.²⁹ Replacing glycerol with olive oil led to increased glass-transition temperatures of wet-cast whey-zein biopolymer film.³⁰ The authors ascribed it to the higher molecular weight of olive oil and better compatibility between zein and olive oil due to their more hydrophobic nature in comparison to glycerol.

Compatibilizers and Coupling Agents

Compatibilizers were mainly used when protein-based biopolymers were blended with another polymer. For instance, the compatibility between soy protein and poly(lactic acid) (PLA) was improved by adding 1–5 phr poly(2-ethyl-2-oxazoline) (PEOX) in the blends.³¹ The addition of PEOX led to improved miscibility of the two phases and improved thermal processability of the polymer blend. Compatibilized SP/PLA blends showed better mechanical and water resistance properties than the uncompatibilized ones. Soy protein also induced and accelerated cold crystallization of PLA in the blends. SPI/polycaprolactone (PCL) blends exhibited improved compatibility with the addition of methylene diphenyl diisocyanate (MDI).³² The mechanical properties of 50/50 (SPI/PCL) blends increased with increasing MDI concentrations. The water resistance of SPI/PCL blends was improved significantly by increasing PCL concentration and by incorporating MDI.

Maleic anhydride-grafted poly(butylene adipate-co-terephthalate) (PBAT-g-MA) was used as a compatibilizer for soy protein concentrate (SPC) and PBAT.³³ Using PBAT-g-MA as a compatibilizer resulted in finer domain sizes, better dispersion of SPC in the PBAT matrix, and improved tensile and dynamic mechanical properties of the PBAT/SPC blends. In the presence of compatibilizer, an elongated SPC thread structure was observed in the blends when the SPC content was 25% or higher. This percolated SPC network structure resulted in drastic changes in the rheological properties and the mechanical and dynamic mechanical properties of the blends.

A titanate coupling agent was used for a soy protein/cornstarch mixture plasticized with glycerol and soy oil.³⁴ The viscosity of the polymer was determined by a screw-driven capillary rheometer. The viscosities of the mixture fit a power-law model. The mixture was found to be pseudoplastic, with a flow behavior index (n) of 0.18–0.46 and a consistency index (m) of 1.1×10^4 to 1.0×10^5 . Addition of the titanate coupling agent resulted in an increased flow behavior index and a decreased consistency index, suggesting that the compatibilized mixture has a lower viscosity and is closer to a Newtonian fluid.

Compatibilizer was also used to improve the dispersion of natural fiber in a protein-based polymer-containing matrix with soy flour/glycerol/polyester amide (weight ratio: 7/3/10).³⁵ The biopolymer blend was extruded with pineapple leaf fiber (PALF) with polyester amide-grafted glycidyl methacrylate (PEA-g-GMA) as a compatibilizer. The dispersion of PALF fiber in the soy-based bioplastic matrix became worse with increasing fiber content, but it improved upon the addition of the compatibilizer. It was also found that both tensile and flexural strength and modulus improved with the addition of compatibilizer to the 30 wt % PALF fiber-reinforced biocomposites, indicating that the PEA-g-GMA is an effective compatibilizer for this system. Consequently, the heat distortion temperature and storage modulus of the composites increased with the addition of PEA-g-GMA, due to the increase in modulus. In addition, the water absorption of compatibilized composites decreased when compared to their uncompatibilized counterparts. The Fourier transform infrared (FTIR) spectra show evidence of interactions

between PALF and PEA-g-GMA in the diminishing epoxy peak at 908 cm^{-1} as a result of the reaction between the hydroxyl group in PALF and the epoxy group in PEA-g-GMA.

Denaturation Agents

SDS is the most commonly used denaturing agent for protein. Sessa *et al.* used SDS to control the viscosity of zein during thermal processing.³⁶ When zein is blended with water and triethylene glycol (TEG) and subjected to heat and shear in a torque rheometer, an increase in viscosity was observed. Upon addition of SDS, the increase in viscosity of the zein/water/TEG was significantly delayed in a linear relationship to the amount of SDS added. As the amount of SDS increased, a decrease in tensile strength and Young's modulus and an increase in elongation of zein-based bioplastic were observed, suggesting improved ductility of the resultant polymer.

Verbeek and Van Den Berg used SDS and urea as processing aids for extrusion of thermoplastic blood meal bioplastic.¹⁸ It was found that the consolidation of the bioplastic was strongly dependent on the amount of SDS. SDS was required for processing and consolidation, but it may restrict the formation of new intermolecular forces if used at higher concentrations. The use of SDS also ensured a ductile material,¹⁹ such that processability is improved and the processing window is enlarged. The blood meal could even be processed by injection molding with the addition of SDS and urea.²¹

SDS had drawbacks too, such as increased water absorption and solubility after processing²⁰ and a permanent reduction in transition temperatures,³⁷ as compared with the unmodified blood meal, whose glass-transition temperature was restored upon drying.

Urea is another widely used denaturing agent for protein. It forms strong hydrogen bonds with the water molecules that surround the protein. Urea may disrupt the protein hydrogen bonds, resulting in partially unfolded protein structures and flexible peptide chains.³⁸ This may promote more entanglements and crosslinked structures during the molding process, resulting in strong, ductile, and low-water-absorption plastics. Mo and Sun evaluated the effect of urea treatment of soy protein on the properties of the resultant bioplastic.³⁹ Defatted soy protein isolate was modified with various urea concentrations (1, 2, 4, 8M) at a weight ratio of 1:6 for 6 h. The differential scanning calorimetry results showed that the denaturation enthalpy decreased as the concentration of urea increased. No peak was observed for samples with 8M urea modification, indicating that the soy protein was denatured completely. The modified soy protein was compression-molded into plastics. Plastic made with 1M urea-modified soy protein become soft and stretchable first, and it became rigid again as the urea concentration increased further from 2M to 8M. All plastics made from urea-modified SPI had lower glass-transition temperatures than that of the unmodified soy protein.

Urea was also used as a thermal processing aid for extrusion of a bovine blood meal-based polymer.¹⁹ The urea was added into bovine blood meal at a ratio of 10 or 20 phr. Increasing urea content resulted in decreased tensile strength and Young's

modulus and increased elongation of the biopolymer. However, the amount of urea should be carefully controlled because increasing the level of urea reduced the onset of thermal degradation like sodium sulfite, causing a reduction in thermal stability.²⁰

The effects of the addition of salts from the Hofmeister series on hydrophobic interactions between zein and salts were evaluated.⁴⁰ The addition of NaI and NaSCN altered the properties of viscoelastic materials made from zein, rendering them softer and more extensible, as did urea and ethanol. The addition of NaCl and Na₂SO₄ negatively impacted the ability of zein to form a viscoelastic material and completely disrupted the formation of viscoelastic material at higher salt concentrations. These results indicated that manipulating noncovalent interactions in zein could alter and, in some cases, completely disrupt the formation of a viscoelastic material.

Selling *et al.*⁴¹ found that the addition of ammonium, potassium, guanidine, and magnesium thiocyanate salts affected the zein solution's rheology and intrinsic viscosity, demonstrating that the salts interacted with the protein. With the addition of ammonium thiocyanate salt (ATC), there was a modest reduction in viscosity versus shear rate and an increase in intrinsic viscosity. These results suggested that, in the presence of ATC, the zein protein opened up, leading to a higher mean end-to-end distance and a higher radius of gyration.

GRAFTING

Proteins have several reactive groups, such as NH₂, NH, COOH, SH, and OH, on the polypeptide backbone or pendant side chain. Some of these functional groups can serve as suitable sites for being grafted to other species to achieve better processing performance or physical properties.

Acylation and Esterification

Shalaby *et al.* invented a method to treat proteins and synthetic polypeptides with a peracylation reaction.⁴² The acid-catalyzed acylation of soy protein was conducted with different amounts of anhydrides in glacial acetic acid. Acylated soy protein isolate differed from the parent molecule in being less hydrophilic, water insoluble, and melt-processable. The modified soy protein isolate can be subsequently extruded or compression-molded into fibers or films. The melt flow rate of two soy protein isolate thermoplastics modified using this method was determined to be 175.5 and 208.9 mg, respectively (150°C, 2.16 kg).⁴³ The method significantly widened the thermal processing window, and films with a thickness as thin as 0.1 mm can be produced.

Palmitic acid chloride or alkenyl-substituted succinic anhydride was used as an acylation reagent to ameliorate two main problems of protein-based materials: the inapplicability for thermoplastic shaping and the low water resistance.⁴⁴ The plant proteins studied were wheat gluten, soy protein, corn zein, and pea protein. The hygroscopic properties of the original protein could be strongly reduced by acylation in all cases. Acylation combined with plasticizer overcame the problem of brittleness, leading to improved thermal processability. Acylation was also found to be able to slow down biodegradation of the resultant polymer. Thermoplastic shaping could be successfully performed by extrusion to produce filaments, threads, and sheets.

Liu *et al.* modified soy protein with maleic anhydride through an esterification reaction during reactive extrusion.⁴⁵ Soy protein was blended with maleic anhydride and bis(*tert*-butyl peroxy)-2,5-dimethyl hexane at a ratio of 100:5:0.5 for 20 min using a kitchen blender. Then glycerol was added as a plasticizer to the mixture at a ratio of 70:30, and they were mixed for 30 min. The extruded maleic anhydride-modified soy protein plastic (MASPP) had a new peak at 1720 cm⁻¹ assigned to the ester bond formed between maleic anhydride and soy protein, which suggested that esterification took place during the processing of modified soy protein plastic. MASPP had improved tensile strength and modulus and reduced denaturation temperature and glass-transition temperature.

In the research works of Biswas *et al.*, 2-octenyl succinic anhydride (OSA) was used to react with the hydroxyl/amine groups of zein in a microwave reactor.⁴⁶ The microwave-assisted method provided a tremendously fast reaction and expanded the use of microwave technology in the area of zein chemistry. The OSA-modified zein had comparable mechanical properties according to a preliminary evaluation. In an ionic liquid, zein was acylated with anhydrides in the presence of pyridine to give benzoyl zein with various degrees of substitution.⁴⁷ The acylation reaction did not proceed without pyridine.

Shi *et al.* grafted zein with lauryl groups through an acylation reaction.⁴⁸ Triethylamine (3, 9, or 15 μmol) and lauryl chloride (1, 3, or 5 μmol) per gram of zein were added to a 5% w/v solution of zein in dimethyl sulfoxide. The reaction was conducted at 65°C under stirring for 3 h. In comparison with the ¹H NMR spectrum of unmodified zein, the ratio of the number of H in CH₂ (δ 1.21) to that in CH₃ (δ 0.83) of the modified zein derivatives significantly increased from 0.26:1 to 0.32:1, 0.47:1, and 0.51:1, which suggested more alkyl chains in the molecule of lauryl group-grafted zein. Lauryl group-grafted zein experienced a drop in glass-transition temperature, which consequently improved its thermoplasticity and broadened the processing windows. The modification also resulted in an increase in the water contact angle of 75.6° for zein to 86° for the modified zein, due to the increased surface hydrophobicity through the acylation reaction, implying possible decreased water permeability and moisture sensitivity. A decrease in Young's modulus and an increase in elongation at break were also observed for the film from cast, modified zein, indicating that the brittleness was reduced.

Wet-spun zein fibers were modified by soaking in acetic anhydride or its acetic acid solution at room temperature for 30 min.⁴⁹ The modification of wet-spun zein fibers by acetylation blocked the polar groups and increased fiber tenacity. The addition of acetic acid promoted the swelling of zein fibers and provided acetic anhydride with easy access to the zein fiber matrix. The highest fiber modulus was obtained by 95% acetic anhydride–5% acetic acid treatment.

Acrylates

Graft copolymerization with acrylates has been used to weaken the intermolecular bonds between proteins. The synthesis of casein-g-poly(methyl acrylate) was conducted using the potassium peroxydisulfate–ascorbic acid redox system or with

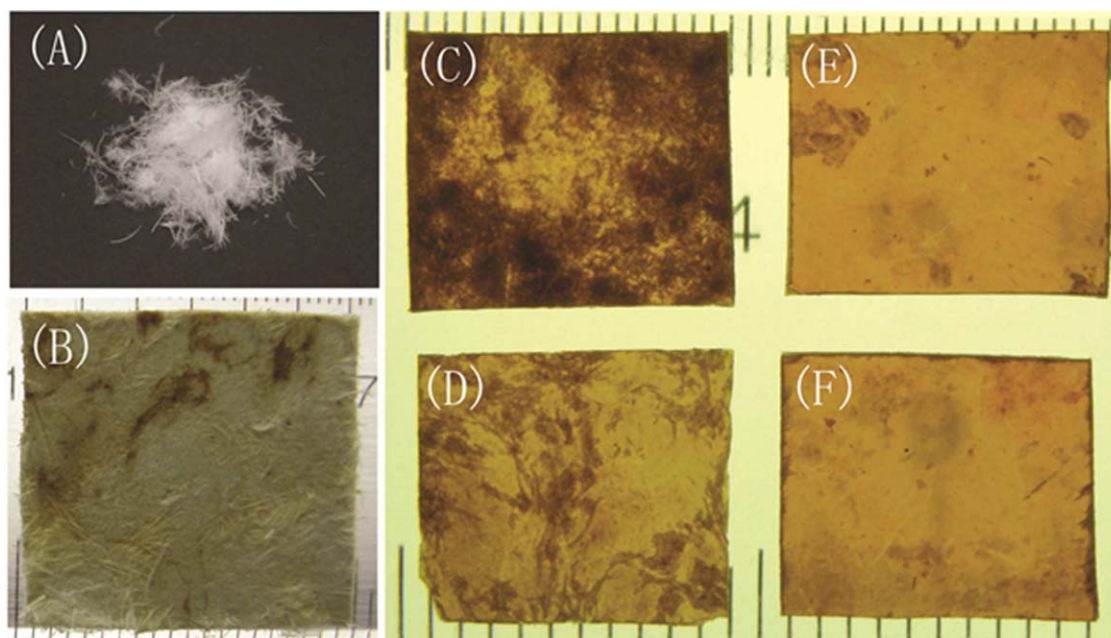


Figure 1. Images of raw feathers (A), feathers compression-molded with 20% glycerol (B), and feathers grafted with methyl acrylate (C), methyl methacrylate (D), butyl acrylate (E), and butylmethacrylate (F) with similar grafting ratios (Copyright 2013 Elsevier). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

potassium peroxydisulfate alone.⁵⁰ The effects of concentrations of methyl acrylate, potassium peroxydisulfate, ascorbic acid, and casein and the reaction temperature on the rates of conversion of monomer, graft copolymerization, homopolymerization, percentage of grafting, and grafting efficiency were investigated. The results from a thermal gravimetric analysis illustrated that casein grafted with poly(methyl acrylate) was thermally more stable than pure casein. Casein-g-poly(methyl acrylate) produced films with high elongation, good dry-rub fastness, and fair wet-rub fastness. The casein-g-poly(methyl acrylate) copolymer may be applied on leather as a top coat.

Jin *et al.* grafted methyl acrylate onto chicken feathers to improve their thermoplasticity for thermoplastic applications.⁵¹ The effects of the graft polymerization conditions such as molar ratio of NaHSO_3 to $\text{K}_2\text{S}_2\text{O}_8$, initiator and monomer concentrations, pH, temperature, and time of polymerization on monomer conversion rate, grafting percentage, and grafting efficiency were evaluated. As shown by the results from an NMR analysis, methyl acrylate was successfully grafted onto functional groups on the surfaces of the chicken feathers. The poly(methyl acrylate)-g-feather produced using a compression-molding process had a tensile strength range from 206.3 to 55.7 MPa depending on the amount of plasticizer (glycerol) used (0–30 wt % to grafted feathers with the homopolymer). In their subsequent research, the feasibility of bioplastics developed from chicken feathers grafted with acrylate or methacrylate as scaffolds for tissue engineering applications was studied.⁵² Images of raw chicken feathers, compression-molded raw chicken feathers with glycerol, and the thermoplastics developed from the feathers grafted with various acrylates and methacrylates are illustrated in Figure 1. The existence of grafted branches on the feathers was confirmed by $^1\text{H-NMR}$. The bioplastic properties could be

controlled by various acrylic monomers with different molecular structures. The films produced from feathers modified by monomers with α -methyl groups containing shorter alkyl ester groups possessed higher strengths. However, better water stability and cytocompatibility were observed on feathers modified by monomers with α -methyl groups having longer chains of alkyl ester groups. The result of a cytocompatibility analysis revealed that cell viabilities on all of the grafted feather films were higher than that on the PLA film.

Other advantages of grafting acrylates onto a protein polymer included improved wet tensile strength and retarded collagenase activity of acrylic latex-enhanced glutaraldehyde-crosslinked collagen film for wound dressing applications,⁵³ improved mechanical properties of a glycidyl methacrylate-modified soy protein plastic,⁴⁵ and higher sorption capacity for petroleum contaminants of methyl methacrylate-grafted benzoylated soy protein.⁵⁴

Polycaprolactone

PCL is one of the few synthetic polymers that are biodegradable. PCL has been compounded with protein into polymer blends, such as wheat gluten with anhydride-modified PCL⁵⁵ and soy protein with PCL.³² PCL was grafted onto zein protein with hexamethylene diisocyanate (HDI) as a crosslinker.⁵⁶ The reaction pathway is shown in Figure 2. PCL-HDI (PCLH) prepolymer was synthesized with 0.12 wt % of tin (II) 2-ethylhexanoate as a catalyst. The reaction was carried out until the NCO group content reached about 3.6%. PCLH reacted with zein in *N,N*-dimethylformamide (DMF). The unreacted PCL was removed from a filter thimble by Soxhlet extraction with toluene for 24 h. The NMR results showed that at least four amino acids (Glu, Gln, Tyr, and His) of zein were involved in the reaction with PCLH. The powder samples were compression-molded into sheet. The increasing PCLH content

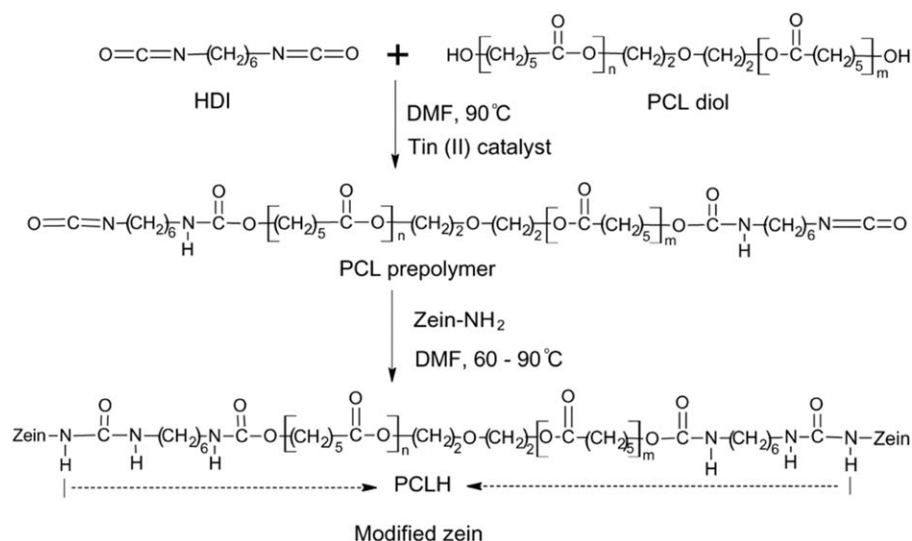


Figure 2. Reaction pathway for grafting PCL onto zein (Copyright 2003 Elsevier).

on zein protein caused reduction of the T_g of the resultant polymer and changed the brittle failure of the neat zein polymer to ductile failure of the PCLH-modified zein polymer. Incorporation of 50% of PCLH onto the zein protein dramatically increased the elongation of the resultant polymer to 522%, as compared to 4.3% of the neat zein polymer.

Silanes

Two alkoxy silane compounds, glycidoxypopyl trimethoxy silane (SiA) and glycidoxypopyl methyldiethoxy silane (SiB), were grafted onto plasticized wheat gluten via condensation reactions.⁵⁷ Either SiA or SiB was mixed with the plasticizers at varying amounts from 1 to 8 wt % of silane to the total mass of wheat gluten and plasticizer. Polymer samples were produced by compression molding on a heated press at a pressure of 5.6 MPa at 130°C for 5 min. The storage modulus data were lower than those of the control neat WG when applying 1% and 2% alkoxy silane to the systems, recovered when 4% of alkoxy silane was used, and then exhibited a significant E' improvement with 8% of alkoxy silane in the system. As compared with the control wheat gluten, the tensile strength at 50% relative humidity decreased when 1% alkoxy silane was incorporated into the wheat gluten system, and then the tensile strengths had different amounts of increases when 2, 4, and 8 wt % of alkoxy silane were in the systems. The authors ascribed the differences in mechanical properties at different alkoxy silane concentrations to two mechanisms. A lower amount of alkoxy silane caused the alkoxy silane molecules to predominantly graft onto the WG chains without forming linkages between the WG segments, which produced an additional plasticizing effect on the WG systems with a longer elongation value and weaker tensile strength as compared to the WG system. Higher amounts of alkoxy silanes in the system led to the formation of crosslinked wheat gluten-siloxane networks via linkages between alkoxy silane segments, producing higher tensile strength, higher T_g , a reduction in elongation, and improved hydrostability as compared to neat wheat gluten samples.

Acrylonitrile

The acrylonitrile and *n*-butyl methacrylate binary mixture was grafted onto casein with 9.7×10^{-3} mol/L potassium persulfate solution as the initiator.⁵⁸ The polymer films were produced by casting the grafted copolymer solution on a mercury surface. The film formed from the control casein solution was brittle, giving a high tensile strength of 26.7 MPa and a small elongation of 4.3%. The grafted casein polymer presented elastomeric-like behavior, with a significant reduction of 94 to 79% in strength and an 11-fold to 49-fold increase in elongation. The film strengths were almost linearly proportional to the binary monomer concentrations, and the elongations were inversely linearly proportional to the binary monomer concentrations. The effect of acrylonitrile monomer concentrations on grafting efficiency was further evaluated between 0.8 and 3.0 mol/L.⁵⁹ The optimum monomer concentration for best percent grafting and grafting efficiency was 2.0 mol/L.

The effects of initiator concentrations and reaction times on percent grafting and grafting efficiency of acrylonitrile on casein were evaluated.⁶⁰ Graft copolymerization of casein with acrylonitrile was carried out with a method similar to Somanathan *et al.*⁵⁸ The graft copolymerization was stopped by precipitation in 8% glacial acetic acid at 25°C. The crude product was filtered off, washed thoroughly with distilled water, then dried in vacuum at room temperature and weighed. Percent grafting and grafting efficiency increased with increasing initiator concentrations up to 1.7×10^{-2} mol/L. The FTIR spectra confirmed the formation of the acrylonitrile-*g*-casein copolymers. The number-average molecular weight (M_n) of the polyacrylonitrile side chain was characterized between 15.8 and 58.8 kDa by gel permeation chromatography. The properties of acrylonitrile-*g*-casein copolymers, such as decomposition exothermic peak and solubility, were between casein proteins and polyacrylonitrile homopolymer.

Somanathan and Sanjeevi investigated the effect of temperature on the mechanical properties of poly(acrylonitrile)-*g*-casein films.⁶¹ The mechanical properties of poly(acrylonitrile)-*g*-

casein were tested at temperatures from 10 to 140°C. The tensile modulus and strength of the grafted casein increased as the temperature reached between 30 and 40°C and then decreased as the temperature kept increasing to 140°C. The tensile elongation changed in an opposite way to tensile modulus and strength. It decreased until the lowest value at 40°C and then increased as the temperature increased to 120°C. The morphology examination showed that at 40°C both crazing and shear-banding failure mechanisms took place. The mechanical testing and morphological studies showed that the plastic strain increased rapidly after 60°C, suggesting a transition took place in the system around 60°C. The copolymer polyacrylonitrile-*g*-casein had intermediate glass-transition temperatures, between the 50°C of casein films and the 80–100°C of polyacrylonitrile.

Vinyl Monomers

Ten vinyl-type monomers were graft polymerized onto chromium-tanned collagen.⁶² Based on the weight of treated leather, only 13–16% of the product was grafted polymer, and the rest of it was extractable. The tensile elongation values increased in most cases for the treated leathers. The tensile strength of treated leather decreased. However, since the thickness of the samples also increased, the tensile load at break essentially increased in all samples but one case, with a maximum increase of 70%. With this technology, it is practical to make leather that is more uniform in thickness, stretchable, and able to withstand increased loads. The modified collagen is viable for use as a leather upper in shoes with injection-molded soles.

Styrene was successfully grafted on the SPI in an 8 mol/L urea aqueous solution initiated by ammonium cerous nitrate and potassium persulfate.⁶³ The influence of the reaction conditions on the grafting and efficiency percentages was investigated. The grafting and efficiency percentages initially increased and then decreased with the increase of the initiator concentration, monomer concentration, and reaction temperature. The optimum initiator concentration, monomer concentration, and reaction temperature for highest grafting and efficiency were found to be 20 mmol/L, 0.35 mol/L, and 50–60°C, respectively. The grafting and efficiency percentages increased with reaction time to 15 min and then leveled off as the reaction time reached 25 min.

Styrene could be grafted onto soy protein during the dry processing too.⁴⁵ Soy protein was blended with styrene and bis(*tert*-butyl peroxy)-2,5-dimethyl hexane according to a ratio of 100:5:0.5 for 20 min using a kitchen blender. The mixture was blended with glycerol at a ratio of 70:30. The whole mixture underwent a reactive extrusion and compression-molding process. The peak at 995 cm⁻¹ on the FTIR spectrum of styrene-modified soy protein plastic (StSPP) was assigned to the benzyl ring of polystyrene and indicated that the polystyrene had been grafted to the soy protein. StSPP had a higher carbon content and reduced nitrogen and oxygen contents in the X-ray photoelectron spectroscopy (XPS) results, due to the high carbon content of styrene. The decrease in nitrogen and oxygen content indicated by the XPS results suggested that the amino group in

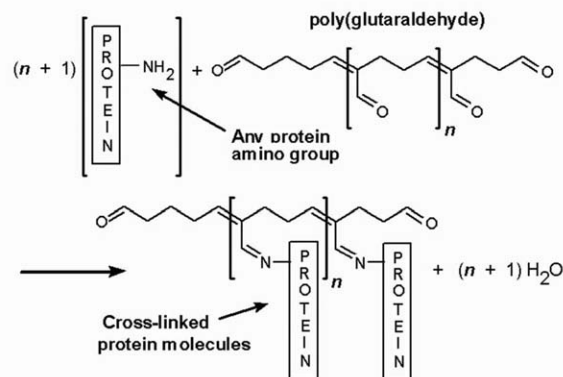


Figure 3. Reaction of poly(glutaraldehyde) with amino groups of proteins (Copyright 2000 Cambridge).

soy protein was buried by the interaction between the hydrophobic group in soy protein and polystyrene.

CROSSLINKING

The hydrogen bridges between the CO and NH groups of protein contribute to the crosslinking required for stability. In addition to hydrogen bonds, disulfide bonds formed from released sulfhydryl groups of cystine and methionine can also contribute to crosslinkage. Due to the large quantity of polar amino acids and limited cystine and methionine content in most proteins, the protein-based polymer is sensitive to moisture and has lower mechanical properties. Crosslinking has been used to improve the water resistance and mechanical properties of protein-based polymers.

Aldehydes

Aldehydes are the most effective protein crosslinking agents. Protein can be crosslinked by dialdehyde through the condensation reaction. A typical reaction between protein and dialdehyde is shown in Figure 3 in which glutaraldehyde was used.⁶⁴

The molecular weight increase in glutaraldehyde-crosslinked SPIs in comparison to a control SPI are illustrated in SDS-PAGE patterns, as shown in Figure 4.⁶⁵ Glutaraldehyde was added into the SPI suspension at four concentrations: 4, 20, 40, and 80 μ M. The characteristic polypeptide bands were still visible at a glutaraldehyde concentration of 4 μ M. These bands became dim as the glutaraldehyde concentration increased to 20 and 40 μ M, then completely disappeared as the glutaraldehyde concentration reached 80 μ M, suggesting that the crosslinked protein molecules have a molecular weight greater than 97.4 kDa. The crosslinking by glutaraldehyde could improve the water resistance of the protein-based polymer, as shown by the significant reduction in total soluble matter up to 30% of the wet-cast sodium caseinate/glycerol film.⁹ The glutaraldehyde-introduced crosslinking could improve the mechanical properties as well, according to the experimental results with SPI adhesive at dry, wet, and soaked conditions,⁶⁵ wet-cast silk-like, elastin-like protein,⁶⁶ wet-cast sodium caseinate/glycerol films,⁹ and wet-cast feather keratin films.⁶⁷

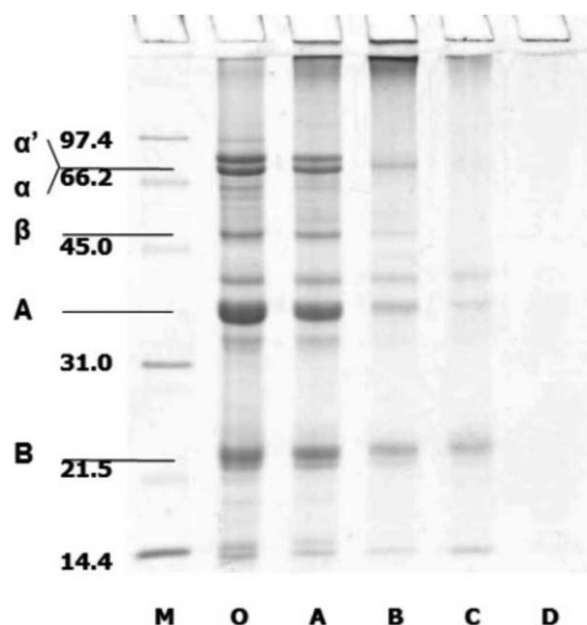


Figure 4. SDS-PAGE analysis for crosslinked soy protein samples. The numbers on the left are the molecular masses of marker proteins in kDa. The letters on the bottom are the crosslinking conditions. M: marker proteins; O: no glutaraldehyde; A: 4 μM glutaraldehyde; B: 20 μM glutaraldehyde; C: 40 μM glutaraldehyde; and D: 80 μM glutaraldehyde. Glycinin acidic and basic subunits are A and B (left), respectively. The β -conglycinin subunits are labeled α' , α , and β (Copyright 2007 John Wiley and Sons).

Another widely used dialdehyde is glyoxal. Similar results introduced by crosslinking with glyoxal were observed, such as a significant increase in strength and modulus of compression-molded wheat gluten protein-based polymers at a relative humidity of 50%,⁶⁸ an increase in water-resistance properties and stiffness but a decrease in elongation of the wet-cast gelatin biopolymer films,⁶⁹ a decrease in the diameter of the electrospun zein fibers, and enhancement of the mechanical properties.^{70,71}

Monoaldehydes such as formaldehyde, cinnamaldehyde, and glycolaldehyde were also used for crosslinking of protein-based polymers, of which formaldehyde is the most commonly used. The physical properties of protein-based polymers following monoaldehyde treatment were similar to that of dialdehyde treatment. The following is a summary of the results obtained: increases in the tensile modulus and strength, reduction in elongation, and improved water-resistance properties of compression-molded soy isolate polymer after formaldehyde treatment⁷²; increases in tensile modulus and strength of formaldehyde-crosslinked feather keratin films⁶⁷; and reduced water vapor permeability and oxygen permeability at mild relative humidity conditions of wet-cast gliadin/glycerol films crosslinked by cinnamaldehyde.⁷³

Though the use of aldehyde generally improved the mechanical properties and water-resistance properties of protein-based biopolymers, the optimal aldehyde type and crosslinking density should be experimentally determined for two reasons: (1) the diverse amino acid group in the various protein sources may

selectively react with different aldehydes, and (2) deterioration in mechanical or moisture-resistance properties were reported in some studies, especially at high aldehyde concentrations.

For example, Spanneberg *et al.* reported that the mechanisms of protein modification by glyoxal and glycolaldehyde were different.⁶⁹ Arginine was the major target to be modified by glyoxal, while glycolaldehyde reacted almost exclusively with lysine and other amino groups. Zhang *et al.* reported that the Young's modulus and tensile strength could be improved as the glyoxal content increased from 0 to 1.4%, but the further increase in glyoxal content from 1.4 to 2.1% caused a significant reduction in mechanical properties.⁶⁸ Pereda *et al.* found that water vapor permeability and moisture absorption of caseinate/glycerol/glutaraldehyde films increased as crosslinking density increased, which was opposite to what was initially expected.⁹

Zárate-Ramírez *et al.* evaluated the mechanical properties of wheat gluten biopolymer samples with and without treatment by formaldehyde, glutaraldehyde, and glyoxal.⁷⁴ The mixture was compounded in a two-blade counterrotating batch mixer, and biopolymer test pieces were then prepared through the compression-molding process. No improvement in tensile strength was observed for the aldehyde-crosslinked samples as compared to the aldehyde-free samples. Vaz *et al.* found that both the tensile modulus and strength of the extruded soy protein isolate polymer decreased with the addition of glyoxal.¹⁰ The authors believed the probability of glyoxal crosslinking through two amine groups of soy located on two adjacent chains was smaller than along the same chain during extrusion. As a consequence, the covalent bonds were predominantly introduced within the polypeptide chains instead of between them (intramolecular crosslinking). This type of crosslinking did not affect the melt processing cycle but resulted in reduced chain mobility (with greater difficulty in unfolding and aligning during the extrusion process). The reduction in mechanical properties of aldehyde-modified protein-based polymer in these two studies may be ascribed to the high shear stress that the premixed materials were subject to during dry processing.

Reactive extrusion of zein with glyoxal was also reported by Selling *et al.*⁷⁵ Crosslinked zein was produced using glyoxal as the crosslinking agent via reactive extrusion using dilute sodium hydroxide as catalyst. Tri(ethylene glycol) was used as a plasticizer for various formulations. It was found that formulations with 2% or less glyoxal could be readily injection-molded. Other formulations, especially those with elevated amounts of glyoxal, could not be injection-molded under the same conditions.

Thiol

A thiol-terminated and star-branched molecule, tri-thiol (Figure 5), was used to toughen a gluten-based biopolymer.⁷⁶ Commercial wheat gluten was dispersed in 0.05M acetic acid at a ratio of 1:10 (w/v) at room temperature. Tri-thiol was added at a weight percentage of 5.8% with respect to gluten, which corresponded to an equimolar amount of thiol groups in tri-thiol and gluten. Mixing was continued overnight at 7°C. Subsequently, the mixture was freeze-dried, homogenized with a mortar and pestle, and passed through a 400- μm sieve. The powder

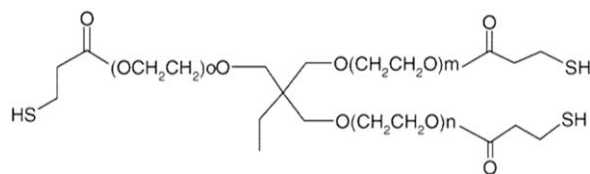


Figure 5. Schematic of the thiol-terminated, star-branched molecule ($m + n + o = 20$) used to toughen the wheat gluten protein polymer (Copyright 2004, American Chemical Society).

was compressed in a heated mold at 150°C under a pressure of 0.5 MPa for 5 min to consolidate the protein powder into the samples. The thiol-terminated, star-branched additive first served as a reducing agent, converting existing intramolecular disulfide bonds to free sulfhydryl groups, as shown by the similar rheological behavior of a wheat flour dough doped with the reducing agent dithiothreitol. Then the thiol-terminated, star-branched additive acted as a crosslinking agent during the molding process by forming disulfide linkages. The specimens of gluten in which the tri-thiol was incorporated failed at a strain of approximately 4.3%, compared with 2.0% for the unmodified gluten specimen. The yield stress increased to 26 MPa, as compared with 15 MPa for the unmodified gluten specimen. No evident difference in the stiffness was observed between modified and unmodified samples. The integral under the tri-thiol-modified gluten curve is approximately 1.3 MPa, representing a four-fold increase from 0.3 MPa of the integral under the plain gluten curve. The modified and unmodified samples showed similar short-range water-absorption properties (<80 h). However, at about 300 h, the unmodified specimens had absorbed more than twice as much water as the tri-thiol-modified specimens. The authors believed the reduction in water absorption of the specimen molded by modified protein was a result of an increase in the crosslink density.

Polyurethane and Polyisocyanate

Many active groups, such as the amino, hydroxyl, and mercapto groups that soybean protein contains, can react with polyisocyanate, which involves complicated reaction mechanisms. Two possible linkages that can be formed are shown in Figure 6.⁷⁷ This method was used to develop a soybean protein-based water-resistant adhesive for engineered wood composites. The wet strength was determined after the specimens were conditioned in boiling water for 4 h, oven-dried at 63°C for 20 h, and conditioned in boiling water for 4 h for a second cycle. The wet shear strength of the modified soy protein adhesive was 0.49 MPa, as compared to complete delamination of the control soy protein adhesive. Crosslinking by polyisocyanate increased the molecular weight and produced a soybean protein with a cross-linked network structure, which improved the water resistance. However, polyisocyanate-modified soybean protein adhesive had a shortened shelf life.

Chen *et al.* used polyurethane as a crosslinking agent for soy protein and soy dreg.⁷⁸ The polyurethane had four levels of NCO/OH molar ratio (1.33%, 1.50%, 1.71%, and 2.0%) by adding different amounts of butanol. The polyurethane/soy protein mixture was compounded with extrusion, and composites were made by the compression-molding process. The absorption

at 2272 cm^{-1} on the FTIR spectrum of free NCO groups in polyurethane prepolymer disappeared in the resulting composite materials, indicating that NCO groups reacted with NH_2 and NH in the protein and OH groups in the cellulose and polysaccharide of the soy dreg during processing. The strong absorption peak at 1651 cm^{-1} in the spectrum of the original soy dreg, which was assigned to amide I, became weak in the blended materials. Meanwhile, the intensity of the peaks at 1532 cm^{-1} (amide II) and 1219 cm^{-1} (amide III) in the spectrum of the composites increased, suggesting the formation of new urea bonds as a result of the reaction between the NCO groups of polyurethane prepolymer with NH_2 and NH of soy protein. The polyurethane-crosslinked composites showed higher glass-transition temperatures, higher strengths, higher elasticity (lower $\tan \delta$), and better water-resistance properties at higher NCO/OH molar ratios. The only drawback of the increased crosslinking is the reduction in the materials' biodegradability.

A waterborne polyurethane (WPU) was used to modify SPI to develop composite films for biomaterial applications.⁷⁹ Soy protein thermoplastics were prepared by blending SPI with waterborne polyurethane through a wet-casting process. The SPI/WPU blend films exhibited fine compatibility due to the affinity of WPU to SPI. The blend films exhibited improved flexibility and water resistance compared with pure soy protein films. The introduction of WPU into the protein matrix dramatically improved their flexibilities and mechanical properties in both the dry and wet states. The SPI/WPU blend film was biodegradable and biocompatible and also possessed lower cytotoxicity than neat WPU.

Zein was melt-processed with methylene diphenyl-4,4'-diisocyanate (MDI) using trimethylamine (TEA) as a catalyst to facilitate the reaction of the isocyanate groups with the nucleophilic moieties present on zein.⁸⁰ As the amount of MDI increased, the molecular weight of the resultant protein increased, yielding a high viscosity. The presence of moisture in zein proved beneficial for melt processing and gave articles with improved physical properties.

Enzyme

The incorporation of microbial transglutaminase (MTG) was found to be able to induce crosslinking of the sodium caseinate film during the wet-casting process, as proven by the increase in the molecular weight shown in the SDS-PAGE results (Figure 7).⁸¹ Stolte *et al.* studied the effects of temperature and air humidity during the drying process on the mechanical properties of MTG-crosslinked sodium caseinate and the additive (potassium nitrate) crystalline structure in the resultant

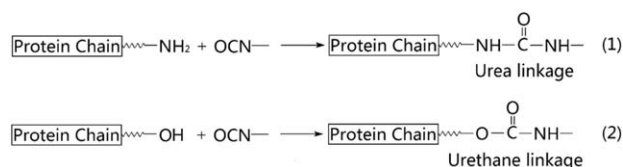


Figure 6. Illustrations of polyisocyanate reactions with soybean protein (Copyright 2014 Elsevier).

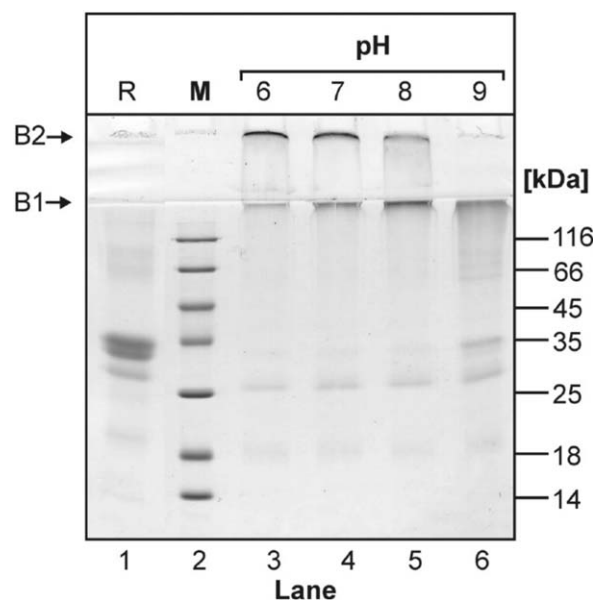


Figure 7. SDS-PAGE showing the crosslinking of sodium caseinate by microbial transglutaminase dependent on the pH values from 6 to 9. R: noncrosslinked sodium caseinate as reference; M: molecular weight marker. (Copyright 2010, American Chemical Society).

biopolymer films.¹² Sodium caseinate films showed the best mechanical performance when dried at 75°C and 50–75% relative humidity with and without MTG. The MTG stabilized and enhanced the flexibility of the polymer, inducing the highest elongation and tensile strengths. Higher air humidity lowered the water loss during film formation, which contributed to better diffusion of the enzyme molecules. MTG-induced crosslinking had a minor effect on the crystallized potassium nitrate, resulting in more opaque films with slightly larger crystals at low air humidity during drying.

Epoxide

1,2-Epoxy-3-chloropropane (ECP) was added into an acetone aqueous solution to crosslink the zein molecules to improve the breaking strength and water-resistant properties of the resultant film.⁸² The results showed that the water transmitted through the film was estimated to be about $20 \mu\text{l cm}^{-2}$ for the film from the aqueous acetone solution and about $10 \mu\text{l cm}^{-2}$ for the film with ECP modification; therefore, the crosslinked zein showed greater water resistance. The strength of the film prepared from the aqueous acetone solution increased linearly as film thickness increased; however, the breaking strength of the film with ECP modification had a sudden drop at the maximum value of 700 g at $60 \mu\text{m}$, which may be ascribed to the crosslinking of zein with ECP. The rigidity increase might have reduced the breaking strength. The crosslinked zein film also showed a lower oxygen gas permeability than the zein film without ECP modification.

Other Chemicals

Kim *et al.*⁸³ used mild crosslinking agents, 1-(3-dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) to crosslink zein molecules. The film-forming property was improved, and the aggregation in solution was suppressed. At the air/water interface, native zein

forms brittle films with a rough surface, whereas crosslinked zein forms rigid films with a very smooth surface. The tensile strengths of the films were significantly increased by crosslinking. The flexibility of the crosslinked films was also improved by the decrease in Young's modulus. The crosslinked films showed a small but significant increase in elongation. This is probably due to the high glass-transition temperature of zein in the absence of plasticizers. The crosslinking of zein by EDC and NHS seemed to be self-terminating because the crosslinking reaction did not proceed to precipitation.

CONCLUSIONS

There has been an increasing interest in the manufacture and use of ecologically friendly bio-based polymer materials over the past few decades, so it is no surprise that protein-based polymers have been extensively studied. Original protein has shortcomings in terms of processability and properties of the processed material, such as a narrow processing window, its hygroscopic nature, and inferior mechanical properties. These issues can be ascribed to the original secondary and tertiary structure of the protein, rheological properties not in a proper processing window due to the crosslinking by disulfide bonds, or strong intermolecular interaction. For better thermal processing, the physical or chemical treatment methods can be classified into two methods: breaking the original structure of protein molecules, and grafting more flexible functional groups onto protein molecular chains to increase mobility. Nevertheless, the fact that the protein is prone to crosslinking can also be used to increase the mechanical properties of the resultant polymer. In this case, protein must be processed with the methods that are being used for traditional thermosets. These methods reviewed here have proved effective in improving these disadvantages of protein-based biopolymers. With the continuous emergence of new technologies and processes, protein-based biopolymers will be suitable for use in a wide variety of commercial applications in the near future.

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