

Thermoplastic Films from Plant Proteins

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ABSTRACT: Films developed by thermoprocessing of plant proteins have better mechanical properties and are relatively inexpensive and environmentally friendly compared to casting films from solutions. Common plant proteins such as soyproteins, wheat gluten, and corn zein and proteins from lesser grown cereal crops such as peanut, barley, sunflower, and sorghum have all been injection or compression molded into films. Since plant proteins are non-thermoplastic, it is necessary to chemically or physically modify the proteins and make them thermoplastic. Extensive studies on the addition of plasticizers, pre-treatment of proteins with alkali, steam, chemical modifications such as acetylation and blending of the proteins with other biopolymers and synthetic polymers have been done to modify proteins and develop thermoplastics. Despite the extensive work, thermoplastic films obtained from plant proteins do not have the properties that can meet or exceed properties of films made from synthetic polymers. Poor water stability and brittleness are the two major limitations of plant protein films. This review presents an overview of the methods and processing conditions used to develop thermoplastic films from plant proteins and the properties of the films. A critical assessment of approaches that have been used so far, limitations of these approaches and potential considerations for future studies to overcome the current limitations have been discussed. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 130: 729–738, 2013

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INTRODUCTION

Plant proteins such as soyproteins, wheat gluten, and corn zein have been extensively studied to develop inexpensive and biodegradable films that are intended to be alternative to films made from synthetic polymers. Plant proteins are derived from annually renewable resources, mostly, as coproducts of cereal processing that have limited industrial applications and are relatively inexpensive. It has been reported that plant proteins have unique properties such as preventing the diffusion of oxygen that make them preferable over synthetic polymer films for food packaging and other applications.^{1,2} However, films developed from biopolymers, especially proteins, usually have considerably low elongation and are therefore brittle. Plasticizers, most commonly glycerol, are used to improve the elongation of protein based films. Although addition of plasticizers provides good elongation, the strength of the films significantly decreases, and the protein films also become more susceptible to moisture.

Traditionally, plant proteins have been made into films by dissolving the proteins in solvents or by hydrolyzing the proteins using alkaline solutions and later wet casting to form films.

There are considerable limitations in the approach of making films by wet casting. Only a limited number of plant proteins (gliadin and zein) are soluble in common solvents. Other plant proteins such as soyproteins and wheat gluten do not dissolve in common solvents and are made into films by heating in alkaline solution. However, hydrolysis that occurs during alkaline dissolution results in proteins with low molecular weights leading to films with poor mechanical properties and/or inadequate stability in aqueous environments. In addition, using solvents or alkaline solutions to dissolve proteins increases the cost and also makes the process environmentally unfriendly.

Compression and injection molding are the most common methods of developing thermoplastic films. Although plant proteins are inherently non-thermoplastic, plant proteins change their structure, are denatured and become thermoplastic when subject to physical and/or chemical modifications and also by adding additives such as plasticizers. Therefore, attempts have been made to develop thermoplastics from plant proteins using various approaches. Developing plastics by compression or injection molding is more convenient and also less expensive.

Table I. Major Amino Acids (g/100 g Protein) in Proteins from Cereal Crops That Have Been Used to Develop Thermoplastic Films

Amino acids	Wheat			Corn	Soy	Sunflower ^{12,13}	Barley ¹⁵	Peanut	Sorghum ¹⁴
	Gliadin	Gluten	Glutenin						
Glutamic acid	30.2	28.7	27.4	24.2	19.0	22.0	27.3	22.8	21.6
Leucine	7.8	7.2	7.1	17.7	8.1	6.8	3.7	6.6	13.1
Proline	17.1	14.1	12.4	8.9	5.1	5.1	11.8	3.7	7.7
Alanine	3.0	3.5	4.1	8.4	4.2	3.7	3.7	2.8	9.2
Phenylalanine	5.1	4.4	4.0	6.0	5.2	5.2	5.2	6.0	4.8
Serine	4.3	5.7	8.0	6.3	5.2	5.1	4.5	3.9	4.1
Tyrosine	2.6	2.8	3.1	4.3	3.8	3.2	2.9	4.5	1.6
Histidine	1.7	1.7	1.7	0.9	2.6	3.0	2.3	2.2	2.1
Arginine	2.9	3.2	3.1	2.4	7.5	9.4	5.1	11.0	2.7
Lysine	0.6	1.4	2.0	0.5	6.2	2.6	3.6	2.5	2.0
Glycine	2.2	3.9	5.6	1.3	4.1	4.5	3.4	4.2	3.0
Aspartic acid	1.7	4.2	2.5	4.2	11.5	9.3	5.8	12.6	6.3
Valine	4.0	2.9	4.3	3.4	5.0	4.1	4.9	4.8	4.9
Cysteine	1.8	1.7	1.5	1.0	1.3	1.0	1.3	0.2	2.0
Isoleucine	3.4	3.0	4.4	3.8	4.8	3.9	3.6	4.0	3.8
Acidic	31.9	32.9	29.9	28.4	30.5	31.3	33.1	35.4	27.9
Basic	5.2	6.3	6.8	3.8	16.3	15.0	11.0	15.6	6.8
Hydrophilic	4.3	5.7	8.0	6.3	5.2	5.1	4.5	3.9	4.1
Hydrophobic	43.6	40.5	42.1	51.0	36.8	33.6	36.9	32.8	46.4

Amino acid compositions of proteins are highly dependent on the source, conditions during growth and methods of analysis. Values presented in Table I may vary significantly compared to literature. We have attempted to provide the most representative numbers using data from multiple sources.

Solvents required for preparing solution cast films are eliminated making molding environmentally friendly than solution casting. In addition, molding is faster and provides a better opportunity to preserve the properties of the proteins compared to solvent casting. For instance, molding does not damage or degrade the proteins that occur during hydrolysis of proteins. Similarly, molding does not create unpleasant chemical smells or safety concerns related to handling and disposal of chemicals.

Soyproteins, wheat gluten, and zein are the common proteins that have been made into thermoplastic films using various approaches. In addition, proteins from lesser known cereal crops such as sunflower, barley, peanut, and sorghum have also been made into films. However, the processing conditions used, additives and modifications of proteins, testing conditions, and properties of the films reported in literature are vastly different. In fact, different studies using the same protein and identical processing and testing conditions report films with considerably different properties. It is therefore difficult to compare and analyze results among the various studies and obtain meaningful conclusions. Despite numerous studies, progress in developing protein films with properties comparable to those of synthetic polymer films has been limited. This review provides an analysis of the processing and properties of thermoplastics developed from plant proteins. Limitations of current studies and approaches necessary to obtain plant protein films with properties suitable to replace films from synthetic polymers have been presented.

AMINO ACID COMPOSITION OF PLANT PROTEINS

Table I provides a comparison of the major amino acids in cereal proteins that have been used to develop thermoplastic films. Considerable variations in the amount of the different types of amino acids can be seen within and between the proteins leading to variations in the processability and properties of the products developed. Among all the proteins in Table I, gliadin and zein are prolamin proteins that dissolve in aqueous ethanol and have a distinct amino acid composition. Zein contains about 18% leucine whereas gliadin contains about 17% proline, substantially higher compared to other proteins. Similarly, soyproteins contain much lower levels of glutamic acid but contain high levels of aspartic acid. Such differences should be due to the inherent properties of the proteins, growth conditions, and functions of the proteins. Although there is a wide variation in the amount of each type of amino acids among the cereal proteins, a grouping of the amino acids as acidic, basic, hydrophilic, and hydrophobic reveals that the proteins contain similar amounts of acidic and hydrophilic groups but considerably different basic and hydrophobic groups. Soy, sunflower, barley, and peanuts contain more than twice higher basic groups than the other proteins. Wheat glutenin had considerably higher amounts of hydrophilic amino acids whereas zein contains the highest amount of hydrophobic amino acids. Composition of the amino acids in the proteins may not influence the thermal processability of the proteins but the tensile properties and water stability will be dependent on the amino acid composition. However, an analysis of the influence of amino

Table II. Tensile Properties of Thermoplastics Films Made from Soyproteins Under Various Conditions

Additives, modifications, and processing conditions	Tensile properties			References
	Strength (MPa)	Elongation at break (%)	Modulus (MPa)	
Soyprotein with 10–50% glycerol ^a	40.6–7.1	3–185	1226–144	7
0–10% methyl glycoside, 20–30 parts glycerol	12.5–14.7	105–125	305–380	7
2.8–26% water and 30 parts glycerol	2.4–41.1	13–159	17–1220	7
0–0.4% Epichlorohydrin, 30 parts glycerol	13.7–16.7	101–148	257–389	7
0–2% ZnSO ₄ and 30 parts glycerol	14.3–16.9	107–162	257–586	7
0–0.6% Glutaric aldehyde, 30 parts glycerol	14.3–17.3	119–148	257–550	7
Soyprotein with 0.5–10% SDS, 10% moisture ^b	5.3–26.5	1.4–2.4	758–1667	12
Soyprotein films with 20–40% glycerol ^c	15.8–2.6	4.2–74.5	–	17
Soyprotein films with 30–50% glycerol ^d	7.8–2.9	132–137	7.8–2.9	18
Films with 30–40% glycerol, pH 1.4–10.0	1.5–7.5	10–160	–	18
Films with 0–30% stearic acid, 30% glycerol ^e	9.0–6.0	168–25.6	120–221	19
Films with 5–15% bovine gelatin and 30–40% glycerol ^f	7.2–11.3	–	136–146	20
Acetylated soyprotein films	1.8–2.5	73–113	–	21
Steamed soyproteins with 15% glycerol	5.0 ± 0.8	14.5 ± 3.0	193 ± 60	22

^a Sheets (0.35–1.5 mm) extruded at 100–120°C, screw speed of 20–25 rpm.

^b Dog-bone shaped specimens compression molded at 140°C for 5 min at 20 MPa.

^c Films compression molded at 150°C, 2 min at 10 MPa.

^d Films compression molded at 150°C, 2 min at 12 MPa.

^e Compression molded at 120–130°C, 5–25 min at 2.8–11 MPa.

^f Compression molded at 150°C, 2 min at 12 MPa.

acid composition on the film forming and performance properties has not been done.

THERMAL BEHAVIOR OF PLANT PROTEINS

Thermal behavior of proteins is dependent on the temperature, pH, pressure, plasticizers, salt content, and many other factors.^{3,4} Physical states of cereal proteins at various temperatures have been represented by what are called “State diagrams”.^{5,6} The physical state of the proteins is mainly influenced by the moisture/plasticizer content and temperature. For instance, the T_g of the soyprotein components was directly dependent on the amount of moisture. Conglycinin (7S) fraction of soyproteins had T_g ranging from 114 to -67°C when the moisture content was between 0–35% and the T_g of the glycinin (11S) proteins ranged from 160 to -17°C when the moisture content was between 0–40% and the T_g decreased continually with increasing moisture content.⁷ Similar observations have also been made for wheat proteins.⁶ Molecular weights and extent of denaturation of proteins during thermal processing have been reported to affect T_g .⁷ Lower molecular weight proteins such as gliadin had considerably lower T_g (121.5°C) compared to gluten or glutenin (145°C).⁶ Based on these phenomenon, altering the molecular weights or physical state of the proteins could be useful to develop thermoplastics. For instance, steaming of soyproteins and alkali treatment to reduce the molecular weights have been used to make proteins thermoplastic.⁸ Researchers have proposed several mechanisms for the changes in the behavior of proteins under heat. Heating above the glass transition temperature (T_g) causes the cereal proteins to change from a “glass” to

a “rubber” state that is accompanied by increased molecular mobility and decreased viscosity.⁶ Other researchers have suggested that temperature causes protein unfolding which exposes the hydrophobic protein zones leading to protein aggregation. In the unfolded state, proteins undergo thiol/disulfide interchanges which stabilized the denatured state.⁹ Several phenomenon have been proposed to understand the behavior of proteins during heat but there is a lack of general consensus, partly due to the complicated mechanisms and constrained information published by individual authors. A comprehensive examination of the proteins under identical conditions will be necessary to have a clear understanding of protein behavior under heat.

Researchers have also studied the most suitable processing temperature window to develop thermoplastics from plant proteins. Wheat gluten is reported to have a considerably narrow temperature (90–130°C) range to be processed into bioplastics with good properties. Similarly, soyproteins were reported to have a thermal degradation temperature at about 180°C and it was suggested that the processing temperature limit for soyproteins was about 150°C.¹⁰ The reader is referred to several in depth studies on the behavior of proteins during thermal processing and the influence of various parameters on the state of the proteins.^{5,6,10} Most of the studies in literature have focused on understanding the influence of heat on properties of proteins during thermal processing and very limited investigations have been done on the properties of the proteins after being processed into products. In a unique study, the digestibility and bioavailability of soyproteins and zein after compression molding

was investigated both *in vivo* and *in vitro*.¹¹ Heat setting made corn less digestible *in vitro* whereas heat setting did not affect the *in vivo* digestibility of both soyproteins and corn zein.¹¹ Based on the effects of moisture and molecular weights on the thermal behavior, it seems prudent to control and have consistent levels of moisture before thermal processing. Molecular weights of the proteins, however, have contrasting effect on film processability and properties. Higher molecular weights would provide better tensile properties and water stability but would be difficult to melt and form good films. Therefore, it would be necessary to know the properties of the proteins to be processed and the desired film properties before choosing the processing conditions.

PROPERTIES OF COMPRESSION MOLDED PROTEIN FILMS

Thermoplastic Soyprotein Films

Soyproteins are one of the most widely studied plant proteins to develop thermoplastic products mainly due to their larger availability, lower cost, and better properties of products obtained compared to other plant proteins. In addition, soyproteins are much purer (>90% protein) compared to wheat gluten (80% protein) that makes soyproteins easier to process and control the quality of the products. As seen from Table II, thermoplastic films developed from soyproteins with strength as high as 40 MPa to as low as 1.5 MPa have been reported depending on the conditions used to process the films and type and amount of plasticizers. Zhang et al. developed soyprotein sheets by extrusion and have studied the effects of water, glycerol, methyl glucoside, zinc sulfate, epichlorohydrin, and glutaric dialdehyde on the mechanical and thermal properties of the extruded sheets.⁷ Glycerol added as plasticizer increased elongation but decreased strength and modulus. When glycerol content was changed from 10% to 50%, tensile strength at break decreased from 40.6 to 7.1 MPa, elongation increased from 3% to 185% and modulus decreased from 1226 to 144 MPa.⁷ Glycerol as plasticizer reduced the interaction between protein molecules and increased the flexibility, extensibility, and processability and T_g of soyproteins was also found to decrease with the increase in the concentration of glycerol. In the same research, methyl glycoside (10 parts) was included as plasticizer instead of glycerol and was found to increase tensile strength and toughness by 24.4% and 33.3%, respectively. Similar effect was also observed when the moisture content was increased from 2.8% to 26%. Among the various plasticizers studied, glycerol is most widely used mainly because it is cheaper, derived from a bio-based source and approved for food use and is biodegradable. Most studies on developing films have intended to use the films for food packaging applications and glycerol has been the most suitable choice. It may be beneficial to study the potential of using non-glycerol plasticizers for films intended for non-food applications.

Because of the relatively poor properties of plant protein based films compared to synthetic polymer based films, external crosslinking agents are used to improve the mechanical properties and water stability. Crosslinking agents such as $ZnSO_4$ and epichlorohydrin were used to improve the tensile properties of the films. $ZnSO_4$ was found to increase the modulus but did not

significantly change the elongation. Crosslinking with epichlorohydrin or glutaric aldehyde was found to increase the modulus of the films to a higher extent compared to films crosslinked with $ZnSO_4$. Bovine gelatin was found to interact with soyproteins via hydrogen bonding, electrostatic, and/or hydrophobic interactions and improve the tensile properties and resistance to UV light.²⁰ In addition to using chemicals for crosslinking, physical crosslinking using UV light, and heat have also been used to improve properties of the films. Chemical crosslinking provides better properties than physical crosslinking. However, use of chemical crosslinkers raises concerns on safety and some chemical crosslinkers also cause undesirable changes and may also be difficult to use. In addition, it may not be suitable to use chemicals for crosslinking thermoplastic films because of the high temperatures required for film formation.

Effect of processing conditions such as molding temperature and pressure on properties of soyprotein polymers were studied by Mo et al.¹⁶ Under the various conditions studied, it was found that the soyprotein polymers provided maximum strength and strain when the molding was done close to the phase transition temperature or about 40°C lower than the exothermic temperature (185–192°C).¹⁶ Another study had also reported that soyproteins molded at 140–160°C provided the strongest plastics.²³ Although studies have suggested the optimum processing temperature, many other parameters including the composition of the proteins, moisture levels, condition and type of equipments, and analytical methods used significantly affect results. Therefore, researchers should use the suggested temperatures as a guide but develop their own conditions to obtain films with optimum properties.

Soyprotein isolates were mixed with sodium dodecyl sulfate to denature the soyproteins by disrupting the hydrophobic and electrostatic interaction leading to partial unfolding that would make the proteins thermoplastic.¹⁶ Addition of SDS (5%) was found to completely denature the proteins based on the denaturation temperature determined by DSC thermograms.¹⁶ T_g of the samples decreased from 87.2°C (0% SDS) continuously to -6.0°C with increase in SDS content (10%) and tensile strength of the samples increased at low levels of SDS but decreased when the SDS was higher than 5% due to the plasticizing effect of the surfactant. Chemical modifications of soyproteins have also been done to develop bioplastics with improved mechanical properties and water stability. Environmentally green plastics were developed from stearic acid modified soyprotein isolates.¹⁹ Addition of stearic acid was found to increase modulus, decrease strain, and fracture stress when higher than 25% stearic acid was used. Changes in the tensile properties due to the addition of stearic acid were attributed to lower moisture sorption of the films, plasticization, and crystallization. Soyprotein isolates were grafted with methyl acrylate and methyl methacrylate to develop a thermoplastic copolymer but no products were developed.²³ In another study, two types of soyprotein isolates were acetylated and made into thermoplastic films without adding any plasticizers. Acetylation helped to improve the tensile strength and water stability of the soyprotein films. Chemical modifications will help to make the proteins thermoplastic and also to improve the properties. However, it should be noted

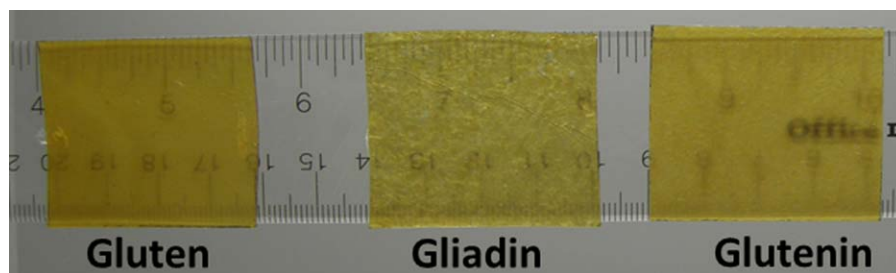


Figure 1. Thermoplastic films from wheat gluten, gliadin, and glutenin developed by compression molding. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that chemical modifications not only add to the cost but could also make the proteins less biodegradable depending on the type of chemical modification done.

Steaming of soyproteins was found to increase thermoplasticity by cleaving disulfide bonds and denaturing the proteins. Thermoplastics developed from steamed soyproteins were found to have better tensile properties than many soyprotein thermoplastics reported earlier.²¹ Treating soyprotein films with benzoic acid resulted in a lotus leaf like structure and films also exhibited higher mechanical properties and water stability compared to untreated films.²⁴ Similarly, pH of soyproteins was also found to affect the tensile properties. Films prepared under acidic conditions and those prepared at pH 7.5 and 10 had the best mechanical properties.¹⁷ If similar properties can be achieved, modifying the properties of the proteins using steam, pH or

other means is probably a better approach than chemical modifications in view of the simplicity, potential cost savings, and biodegradability concerns.

Thermoplastics from Wheat Gluten

Wheat proteins are composed of three main components gluten, glutenin, and gliadin which have all been made into thermoplastic films as seen from Figure 1 and with varying properties as seen from Table III. Presence of distinct components with vastly different properties and relatively less purity make it difficult to process wheat gluten into films. However, the elasticity, binding ability, and oxygen barrier properties offered by wheat gluten are not found in other plant proteins. Also, the allergenic effects of gliadin in gluten make it a less desirable source of protein compared to soyproteins for edible applications and therefore more suitable to develop thermoplastic products for

Table III. Comparison of the Tensile Properties of Thermoplastic Films Made from Wheat Proteins

Type of wheat protein and conditions used	Tensile properties			References
	Strength (MPa)	Elongation at break (%)	Modulus (MPa)	
Wheat gluten with 36% glycerol ^a	0.3-1.0	30-352	1.3-10.2	30
Gluten with 35% glycerol ^b	6.69	240	36	2
Glutenin reduced with sodium bisulfite ^c	1.54	87.8	-	34
Glutenin reduced with sodium sulfite ^c	1.67	100.2	-	34
Glutenin reduced with thioglycolic acid ^c	1.8	109.8	-	34
Gluten crosslinked with aldehydes ^d	2.5-3.3	110-115	-	33
Gluten crosslinked with L-cysteine ^d	2.6	200	-	33
Gliadin with 10-40% glycerol ^e	22-0.8	8.5-301	4.6-0.04	28
Gluten crosslinked with thiol-terminated molecule ^f	15-35	1.5-7.5	-	26
Gluten reacted with thiol modified PVA ^g	74-89	2-2.7	4-4.3	27
Gluten with 20% glycerol ^h	6.7	118	51	35
Gliadin with 20% glycerol ^h	2.2	46	33	35
Glutenin with 20% glycerol ^h	6.1	20	166	35

^a Thermomoulded at 38-125°C, 9 MPa for 10 min.

^b Compression molded at 125°C for 15 min at 15 MPa.

^c Compression molded at 125°C for 10 min at 10 MPa.

^d Thermomoulded at 100°C for 12 min at 10 MPa.

^e Thermomoulded at 120°C for 15 min at 15 MPa.

^f Compression molded at 150°C for 5 min at 0.5 MPa.

^g Thermomoulded at 150°C for 10 min at 20,000 lbs.

^h Compression molded at 120-150°C for 2-6 min at 40,000 lbs.

non-food industrial applications. Researchers have examined the role of heat, plasticizers, additives, crosslinkers, and effect of various wheat protein components on properties of the thermoplastics developed. A fundamental study on the molecular basis of processing wheat gluten to develop bioproducts was done by Lagrain et al. They reported that wheat gluten aggregates upon heating due to the direct covalent crosslinking in and between its protein components glutenin and gliadin. Oxidation of sulfhydryl groups and sulfhydryl/disulfide interchange reactions leading to the formation of disulfide crosslinks were reported to be responsible for the thermoplastic nature of wheat gluten.⁵ Using this approach, the authors claim to have developed gluten based materials with properties similar to materials made from polypropylene and epoxy.⁵ Similarly, unlike most other researches where the wheat gluten bioplastics developed had low tensile properties compared to commercially available synthetic bioplastics, sheets formed by chemically reductive thermoforming were claimed to have elasticity comparable to commercial polymeric materials.²⁵ Incorporation of thiol-terminated star-branched molecules into wheat gluten was found to provide tough, plastic-like substance.²⁶ In that research, incorporation of the thiol terminated star branch molecule increased work of fracture four folds and tensile strain doubled compared to unmodified gluten due to crosslinking. Interestingly, tensile properties of the samples were found to increase with time when stored under ambient conditions.²⁶ In a similar approach, a multifunctional macromolecular thiol (TPVA) obtained by esterification of poly(vinyl alcohol) with 3-mercaptopropionic acid was used as reactive modifier for wheat gluten. The modified wheat gluten was compression molded into bars and had increased strength, elongation, and modulus unlike the plasticizers commonly used for wheat gluten. About 76% increase in fracture strength, 80% increase in elongation, and 25% higher modulus was observed.²⁷

Effect of glycerol and other plasticizers on properties of wheat protein films have also been studied by several researchers. Wheat gluten was mixed with glycerol and compression molded into films at different temperatures at a pressure of 9 MPa for compression time of 10 min. Sun et al. studied the effect of molding temperature on wheat gluten plastics plasticized with glycerol and reported that increasing molding temperature from 25°C to 125°C significantly increased the crosslinking density and therefore mechanical properties.²⁸ The transport and tensile properties of wheat gluten plastics compression molded with 25–40% glycerol were studied by Gallstedt et al. It was reported that water vapor and oxygen permeability increased in the presence of a plasticizer.²⁹ Tensile properties of the wheat gluten films were considerably influenced by humidity and storage time that was varied from 3 to 24 days. Bioplastics developed from wheat gluten were studied for their controlled release and hydrophilic properties.³⁰ It was reported that addition of plasticizers with higher molecular weights results in stiffer and more elastic materials and that inclusion of polyethylene glycol reduced the release of potassium chloride (KCl) from the bioplastics. Influence of water, glycerol, 1,4-butanediol, lactic acid, and octanoic acid on the functional properties and reactivity of wheat gluten materials was studied.³¹ At the same molar

content, the plasticizing effect of water, glycerol, and 1,4-butanediol were found to be similar whereas lactic acid had higher and octanoic acid had lower plasticizing effect. Water provided lower but lactic acid provided higher extensibilities to the thermoplastics. Mangavel et al. compared the properties and microstructure of wheat gluten films developed by compression molding and solution casting.¹ Glycerol was added into both the solution cast and compression molded films and was found to have similar role but the compression molded films had higher tensile stress than solution cast films.¹ Compression molded films were found to have large starch granules but had considerably less water uptake and swelling compared to the cast films. Considerably higher amount of research appears to have been conducted on understanding and developing wheat proteins into thermoplastic products mostly due to the complexity of wheat proteins and their unique behavior after chemical and physical modifications. However, the potential of chemically modifying wheat proteins and developing thermoplastic products has not been studied.

Unlike the common approach of using glycerol and other plasticizers, wheat gluten was plasticized with fatty acids containing 6–10 carbon chain lengths and it was found that the longer the length of the fatty acid chain, poorer was the compatibility with proteins.³² Water vapor permeability was highest for glycerol and lowest for palmitic acid plasticized wheat gluten suggesting that fatty acids were able to provide better water resistance to the films. Mechanical properties of the films were not studied in that research.³²

Wheat gluten films have also been crosslinked using crosslinking agents such as L-cysteine, glutaraldehyde and formaldehyde to improve mechanical properties. It was found that crosslinking with L-cysteine resulted in higher glass transition temperature (59°C) compared to 38.8 and 23.6°C for glutaraldehyde and formaldehyde crosslinked samples due to high degree of phase separation.³³ Crosslinking with aldehydes was found to increase strength but decrease elongation whereas cysteine crosslinking improved both the strength and elongation.³³ Most studies on developing thermoplastic films from wheat proteins have focused on reporting the dry mechanical properties. However, properties of the films when treated in water or under high humidity conditions are also important. It will be necessary to understand the ability of the films to withstand actual use conditions before the films can be developed for commercial applications.

Instead of using commercially available gluten, glutenin-rich fractions were compression molded into bioplastics by Song and Zheng. When added into glutenin, reducing agents such as sodium bisulfite and sodium sulfite were found not to affect tensile strength but increased water vapor permeability and decreased elongation.³⁴ Similarly, gliadin extracted from wheat gluten was also compression molded to form plastics. Samples with strength ranging from 0.8 to 22 MPa were obtained depending on the concentration of glycerol which decreased the T_g in the gliadin rich and glycerol rich domains.² A higher activation energy (227–356 kJ mol⁻¹) was necessary to process the

Table IV. Comparison of the Tensile Properties of Thermoplastic Films Made from Corn Zein

Processing parameters	Tensile properties			References
	Strength (MPa)	Elongation at break (%)	Modulus (MPa)	
Blow molded zein films ^a	0.04–3.6	42–270	4.1–383	36
Extruded zein sheets with 70% oleic acid	7.1 ± 1.0	50.9 ± 7.0	145.6 ± 16.3	37
Blow molded zein sheets with 70% oleic acid	3.3 ± 0.2	79.2 ± 7.3	81.3 ± 8.4	37
Zein films coated with oils ^b	2.9–6.0	19–78	75–200	41
Films crosslinked with 1–8% glutaraldehyde ^c	23.2–42.5	19.8–30.7	291–423	39
Zein plasticized with 25% poly(ethylene glycol) ^d	0.7–18.0	–	24–866	38

^aBlow molded at 70°C for 10 min at 10 bar.

^bCompression molded at 60–140°C, 3 min at 3 MPa.

^cCompression molded at 99°C, 20 min at 12,500 PSI.

^dCompression molded at 120°C, 5 min at 5.5 metric tons.

gliadins into thermoplastics in this research compared to glycerol plasticized wheat gluten. We have recently studied the properties of thermoplastic films developed from wheat gluten, gliadin, and glutenin with and without starch. Under the optimized conditions for each protein, gluten, and pure glutenin (without starch) had higher tensile strength than gliadin and glutenin with starch. However, gluten had much higher elongation (118%) compared to 46% for gliadin when the proteins were compression molded with 15% glycerol.³⁵ Gliadin films were unstable and disintegrated when immersed in water whereas gluten and glutenin were stable but retained only 10% of their tensile strength when immersed in 21°C water for 24 h.³⁵ Gliadin is toxic to humans and therefore using gliadin for non-food applications seems to be a sensible approach. However, films developed from gliadin have poor mechanical properties, especially water stability.

Protein Films from Corn Zein

Unlike most plant proteins, corn zein is a prolamin that dissolves in aqueous ethanol. Therefore, most of the attempts on developing films from zein have used the solution casting approach. Nevertheless, some reports are available on thermoprocessing of zein to develop plastics as seen from Table IV. Thermoplastic zein films were developed by blow molding using poly(ethylene glycol) as the plasticizer.³⁶ Tensile properties of the films were considerably influenced by the processing conditions and also by the amount of α -helix content. Oleic acid plasticized zein was extruded using a single and twin-screw extruder and also blow-molded into ribbons and later compression molded into sheets and the tensile properties were studied.³⁷ No major difference was observed in tensile properties for the single or twin screw extruded samples but heat treatment (80°C) increased the tensile properties for samples obtained by twin screw extrusion. Films were also developed from zein after addition of polyethylene glycol (PEG), lactic acid, lauric acid, and stearic acid as plasticizers and the effect of mixing temperature and temperature on tensile properties were investigated.³⁸ Mixing process and temperature during mixing were found to play a critical role on film properties and a

processing temperature between 60–100°C was found to be most suitable for zein.

Zein crosslinked with glutaraldehyde was compression molded into films at 99°C.³⁹ Increasing concentration of glutaraldehyde from 1% to 4% increased the tensile strength and elongation of the films. Crosslinked zein films were found to have good resistance to soaking and boiling. After soaking in room temperature water for 24 h, the tensile strength decreased by 73% whereas elongation of the films increased nearly 3.5 folds. Films boiled in water for 10 min also had similar decrease in strength but had an even higher increase in elongation by about five folds.³⁹

Several studies have also been done on developing zein films blended/coated with other proteins, biopolymers and synthetic polymers. Soyprotein films were coated with zein to decrease the water vapor permeability.⁴⁰ It was also found that coating zein provided soyprotein films higher strength and modulus but lower elongation. A single coat of zein increased strength of the soyprotein films from 1.7 to 3.3 MPa, modulus from 45 to 101 MPa and the elongation decreased from 79% to 43%. Double coating of zein further increased the strength and modulus to 6.2 and 316 MPa, respectively, and significantly decreased the elongation to 2.6%. Lower molecular weight and ductile nature of zein coated on the surface were attributed for the decreasing tensile properties after coating of zein. Coating of zein sheets with vegetable oils (tung, linseed, and soybean) was found to increase tensile strength and elongation but decreased water vapor permeability.⁴¹

Blends of zein and starch were compression molded into films with 20–40% glycerol as plasticizer.⁴² Modulus and strength of films were found to increase with increasing zein content whereas elongation at break decreased sharply, a phenomenon also observed by other researchers. Zein was blended with poly(ϵ -caprolactone) in an effort to reduce cost and improve biodegradability.⁴² Blends of PCL/zein were found to be incompatible resulting in lower tensile strength and elongation at break but had increased modulus compared to films made from neat polymers. Unlike soyproteins and wheat gluten which are inevitably generated during processing, zein is deliberately

Table V. Comparison of the Tensile Properties of Thermoplastic Films Made from Peanut, Sorghum, Sunflower, and Barley Proteins

Type of protein	Tensile properties			References
	Strength (MPa)	Elongation at break (%)	Modulus (MPa)	
Peanut protein ^a	8.0 ± 0.6	63.0 ± 13.5	147 ± 17	48
Kafrin (sorghum) with 25% plasticizers ^b	6.3–9.0	–	126–752	38
Sunflower proteins, 50% glycerol, 0–30% water ^c	2.2–1.5	43–36	–	45
Sunflower proteins with plasticizers (40–70%) ^d	6.4–9.6	2–140	–	47
Barley proteins with 20–40% glycerol ^e	65–17	5–97	500–1840	44

^a Compression molded at 150–175°C, 2–8 min at 40,000 PSI.

^b Compression molded at 60°C, 3 min at 3 MPa.

^c Extruded between 85–160°C.

^d Compression molded at 150°C, 3 min, 10 MPa.

^e Compression molded at 100–140°C, 10 min, 10,000 lbs.

extracted from corn and is available to a lesser extent and also has much higher cost (\$15–18 per lb) compared to soyproteins (\$1–1.20) and wheat gluten (\$0.80–1.20). Based on the reports so far, there are no specific properties that make zein preferable over the other plant proteins for thermoplastic applications. However, zein (up to 20%) can be extracted from distillers dried grains (DDG) which are the coproducts of ethanol production and are available at about \$100–125 per ton which would make zein price-wise competitive to soyproteins and wheat gluten. Ability of zein extracted from DDG to be thermoprocessed and properties of the products developed in comparison to zein extracted from corn need to be explored.

Thermoplastic Films from Lesser Known Plant Proteins

In addition to soy, wheat, and corn, proteins from lesser known cereal grains have also been studied for their potential to be made into thermoplastic films. A comparison of the properties of thermoplastic protein films obtained from barley, sunflower protein isolates, peanut, and sorghum proteins are provided in Table V. Although these cereal crops or their proteins are not available at quantities and cost comparable to soyproteins or wheat gluten, these lesser known proteins are regionally based and are the primary crops in specific region. Researchers have therefore explored the possibility of developing films from these proteins with a view of finding exquisite properties and/or to add value to the crops. Barley protein films were prepared by compression molding with 20–40% glycerol as the plasticizer.⁴³ Barley proteins were reported to have good cohesive and elastic properties similar to wheat gluten and are hydrophobic with about one-third of hydrophobic amino acids. Films made from barley proteins were reported to have water vapor permeability values similar to wheat gluten films and the films were also found to be biocompatible to Caco-2 cells which are the intestinal cells used as models for studying the absorptive and defensive properties of the intestinal mucosa.^{44,45} Sunflower protein isolates containing 11S globulin and 2S albumin were extruded into films at 160°C. Films were sensitive to water and swelled about 180% when soaked in water for 24 h.⁴⁶ In another report, the effect of crosslinking agents, plasticizers, and other additives on the mechanical properties and water stability of compression

molded sunflower protein isolate films were studied.⁴⁷ Aldehydes used to crosslink the films provided higher strength than tannins and gallic acid because of their covalent interactions with the proteins. Octonal, decanol, or dodecanol added to increase the surface hydrophobicity and decrease solubility of the films was found to improve the plasticity of the films without affecting the tensile properties.⁴⁷ In another report, it was found that addition of various plasticizers (40–60%) resulted in sunflower protein films with strength ranging from 6.2 to 9.6 MPa and elongation ranging from 2% to 140%.⁴⁸ Similar to compression molding, sunflower protein isolates were injection molded on a single screw extruder at 85–160°C with 10–70 parts glycerol.⁴⁶ Sheets obtained after extrusion had relatively low tensile strength ranging from 0.6 to 1.9 MPa and elongation of 9.3–36%.

We have recently reported the development of compression molded films from peanut proteins that were extracted from peanut meal.⁴⁹ Similar to soy meal, peanut meal is the coproducts after processing the seed for oil. Oil-meals contain up to 50% protein and are inexpensive sources of protein compared to extracting proteins directly from the grains. However, the proteins in the meal may have to undergo physical and/or chemical modifications during extraction of oil and it is therefore necessary to understand the properties of the proteins in the meal before using the proteins for various applications. Peanut proteins were mixed with 20% glycerol and compression molded into films at 150–175°C. It was also found that compression molding provided better films than solution cast peanut protein films. Peanut protein films had considerably higher strength than similar protein films developed from wheat gluten or soyproteins. Proteins extracted from sorghum referred to as Kafrin proteins were compression molded into films using different plasticizers. It was found that the tensile properties of the kafrin protein films were similar to that of zein films.³⁸

CROSSLINKING TO IMPROVE MECHANICAL PROPERTIES

Inferior tensile properties and poor water stability are the major limitations of films developed from plant proteins. Crosslinking

is the most common approach used to improve the functional properties including strength and water stability. As seen from the discussions above, glutaraldehyde has been the most widely used protein crosslinking agent. In addition to glutaraldehyde, other aldehydes, enzymes, and physical and chemical means have been used to crosslink plant protein based films. Compared to thermoplastic films, wet cast films have been crosslinked with a wider variety of chemicals. Chemicals such as salicylic acid, carbodiimide, and enzymes including transglutaminase and horseradish peroxidase have been used to crosslink solution cast films.⁵⁰ Radiation and ultraviolet crosslinking has also been done to crosslink solution cast protein films. Since thermoplastic films are processed at high temperatures, crosslinking with enzymes, or chemicals that have lower boiling points are not ideally suited for crosslinking thermoplastic films. This could be the reason for the relatively fewer number of reports available on crosslinking thermoplastic protein films despite their poor tensile properties and water stability. Although glutaraldehyde has been successfully used to crosslink thermoplastic protein films, glutaraldehyde is reported to be toxic and is not convenient to use. Plant proteins also tend to be discolored after crosslinking with glutaraldehyde due to browning during Maillard reaction.

Compared to the crosslinkers previously studied, our research group has recently demonstrated that poly(carboxylic acids) such as citric acid are suitable to crosslink thermoplastic protein films.⁴⁹ Carboxylic acids are safe to use for food applications and are also inexpensive. More importantly, the carboxylic acid crosslinking occurs at high temperature (150–175°C) in the range of processing temperatures used to develop thermoplastic protein films. Poly(carboxylic acids) can be incorporated into the proteins and a one-step film formation and crosslinking can be accomplished leading to a simple and effective process. Further studies are necessary to understand the ability of poly(carboxylic acids) to improve the dry and wet tensile properties of thermoplastic films developed from various plant proteins.

Potential Considerations for Future Studies

Conventional approaches of examining the behavior of proteins during thermal treatments, understanding the influence of processing conditions and addition of various types of plasticizers on properties of plant protein films have culminated in limited new knowledge and have been unable to provide thermoplastic protein films with properties comparable to that of synthetic polymers. Since there is limited scope to change the method and/or equipment used for thermal processing, a significant change in approach in designing proteins and new chemical modifications of plant proteins may offer promise to develop high quality protein films. Except for one report on acetylation of soyproteins, there have been limited studies on chemical modifications of plant proteins to develop thermoplastic films. In addition to acetylation, etherification, grafting of synthetic monomers could be done to improve thermoplasticity and water stability. However, it should be noted that chemical modifications will add cost and may also reduce the biodegradability of the films depending on the extent of modification. Copolymerization of proteins with other biopolymers may also be

possible and provide films with unique properties. Similarly, addition of nanoclays and self-assembled structures obtained from nanoclays may also help to improve the mechanical properties and stability in aqueous environments.

CONCLUSIONS

Attempts to develop thermoplastic films from plant proteins that can replace synthetic polymer based films have met with limited success. Inferior tensile properties and poor water stability are the major limitations of thermoplastics developed from plant proteins. Extensive studies have been done on understanding the behavior of plant proteins and properties of the products obtained under various processing conditions and in the presence of plasticizers and other additives. Such studies have mostly resulted in similar findings or confirmation of previous results. Increasing availability of low cost cereal proteins as coproducts of biofuel production and the unique properties of plant proteins and environmentally friendly nature of thermoplastic products compared to solution cast products justifies further studies on developing thermoplastic products from plant proteins. However, a paradigm shift in the protein modifications and/or novel techniques in processing plant proteins will be necessary to successfully replace synthetic polymeric films with thermoplastic films made from plant proteins.

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REFERENCES

1. Mangavel, C.; Rossignol, N.; Perronnet, A.; Barbot, J.; Popineau, Y.; Gueguen, J. *Biomacromolecules* **2004**, *5*, 1596.
2. Sun, S.; Song, Y.; Zheng, Q. *Food Hydrocolloids* **2008**, *22*, 1006.
3. Sobral, P. A.; Palazolo, G. G.; Wagner, J. R. *J. Agric. Food Chem.* **2010**, *58*, 10092.
4. Guerrero, P.; Retegi, A.; Gabilondo, N.; Caba, K. *J. Food Eng.* **2010**, *100*, 145.
5. Lagrain, B.; Goderis, B.; Brijis, K.; Delcour, J. A. *Biomacromolecules* **2010**, *11*, 533.
6. Kokini, J. L.; Cocero, A. M.; Madeka, H.; Graaf, E. *Trends Food Sci. Technol.* **199**, *5*, 281.
7. Zhang, J.; Mungara, P.; Jane, J. *Polymer* **2001**, *42*, 2569.
8. Reddy, N.; Yang, Y.; *Indl. Crops Prod.* **2012**, *36*, 116.
9. Domenek, S.; Morel, M.; Bonicel, J.; Guilbert, S. *J. Agric. Food Chem.* **2002**, *50*, 5947.
10. Ogale, A. A.; Cunnigham, P.; Dawson, P. L.; Acton, J. C. *J. Food. Sci.* **2000**, *65*, 672.
11. Matthews, L. B.; Kunkel, M. E.; Acton, J. C.; Ogale, A. A.; Dawson, P. L. *Food Nutr. Sci.* **2011**, *2*, 1105.
12. Conde, J. M.; Escobar, M. M.; Jimenez, J. J. P.; Rodriguez, F. M.; Patino, J. M. R. *J. Agric. Food Chem.* **2005**, 8038.

13. Villanueva, A.; Vioque, J.; Sanchez-Vioque, R.; Clemente, A.; Pedroche, J.; Bautista, J.; Millan, F. *JAOCs*, **1999**, *76*, 1455.
14. Deyoe, C. W.; Shellenberger, J. A. *J. Agric. Food Chem.* **1965**, *13*, 446.
15. Newman, C. W.; McGuire, C. F. In *Barley*; Rasmusson, D. C., Ed. Soil Science Society of America Publishers: Madison, Wisconsin, **1985**; pp 403–456.
16. Mo, X.; Sun, X. *J. Polym. Environ.* **2000**, *8*, 161.
17. Cunningham, P.; Ogale, A. A.; Dawson, P. L.; Acton, J. C. *J. Food Eng.* **2000**, *65*, 668.
18. Guerrero, P.; Caba, K. *J. Food Eng.* **2010**, *100*, 261.
19. Lodha, P.; Netravali, A.N. *Incl. Crops Prod.* **2005**, *21*, 49.
20. Guerrero, P.; Stefani, P. M.; Ruseckaite, R. A.; Caba, K. *J. Food Eng.* **2011**, *105*, 65.
21. Foulk, J. A.; Bunn, J. M. *Incl. Crops Prod.* **2001**, *14*, 11.
22. Paetau, I.; Chen, C.; Jane, J. *Ind. Eng. Chem. Res.* **1994**, *33*, 1821.
23. Lu, Y.; Luo, X.; Lin, X.; He, P. *Mater. Sci. Forum* **2010**, *658*, 125.
24. Kumar, R.; Zhang, L.; *Incl. Crops Prod.* **2009**, *29*, 485.
25. Pallos, F. M.; Robertson, G. H.; Pavlath, A. E.; Orts, W. J. *J. Agric. Food Chem.* **2006**, *54*, 349.
26. Woerdeman, D. L.; Veraverbeke, W. S.; Parnas, R. S.; Johnson, D.; Delcour, J. A.; Verpoest, I.; Plummer, J. G. *Biomacromolecules* **2004**, *5*, 1262.
27. Dicharry, R. M.; Ye, P.; Saha, G.; Waxman, E.; Asandai, A. D.; Parnas, R. S. *Biomacromolecules* **2006**, *7*, 2837.
28. Sun, S.; Song, Y.; Zheng, Q. *J. Cereal Sci.* **2008**, *48*, 613.
29. Gallstedt, M.; Mattozzi, A.; Johansson, E.; Hedenqvist, M. S. *Biomacromolecules* **2004**, *5*, 2020.
30. Zarate-Ramirez, L.; Martinez, I.; Romero, A.; Partal, P.; Guerrero, A. *J. Sci. Food Agric.* **2011**, *91*, 625.
31. Pommet, M.; Redl, A.; Guilbert, S.; Morel, M. *J. Cereal Sci.* **2005**, *42*, 81.
32. Pommet, M.; Redl, A.; Morel, M.; Guilbert, S. *Polymer* **2003**, *44*, 115.
33. Sun, S.; Song, Y.; Zheng, Q. *Food Hydrocolloids*, **2007**, *21*, 1005.
34. Song, Y.; Zheng, Q. *J. Cereal Sci.* **2008**, *48*, 77.
35. Chen, L.; Reddy, N.; Wu, X.; Yang, Y. *Incl. Crops Prod.* **2012**, *35*, 70.
36. Oliviero, M.; Maio, E. D.; Iannace, S. *J. Appl. Polym. Sci.* **2010**, *115*, 277.
37. Wang, Y.; Padua, G. W. *Macromol. Mater. Eng.* **2003**, *288*, 886.
38. Maio, E. D.; Mali, R.; Iannace, S. *J. Polym. Environ.* **2010**, *18*, 626.
39. Sessa, D. J.; Mohamed, A.; Byars, J. A. *J. Agric. Food Chem.* **2008**, *56*, 7067.
40. Pol, H.; Dawson, P.; Acton, J.; Ogale, A. *J. Food Sci.* **2002**, *67*, 212.
41. Rakotonirainy, A. M.; Padua, G. W. *J. Agric. Food Chem.* **2001**, *49*, 2860.
42. Corradini, E.; Mattoso, L. H. C.; Guedes, C. G. F.; Rosa, D. S. *Polym. Adv. Technol.* **2004**, *15*, 340.
43. Corradini, E.; Medeiros, E. S.; Carvalho, A. J. F.; Curvelo, A. A. S.; Mattoso, L. H. C. *J. Appl. Polym. Sci.* **2006**, *101*, 4133.
44. Xia, Y.; Wang, Y.; Chen, L. *J. Agric. Food Chem.* **2011**, *59*, 13221.
45. Sambuy, Y.; Angelis, I. De.; Ranaldi, G.; Scarino, M. L.; Stamatii, A.; Zucco, F. *Cell Biol. Toxicol.* **2005**, *21*, 1.
46. Rouilly, A.; Meriaux, A.; Geneau, C.; Silvestre, F.; Rigal, L. *Polym. Eng. Sci.* **2006**, *46*, 1635.
47. Orliac, O.; Rouilly, A.; Silvestre, F.; Rigal, L. *Polymer* **2002**, *43*, 5417.
48. Orliac, O.; Rouilly, A.; Silvestre, F.; Rigal, L. *Incl. Crops Prod.* **2003**, *18*, 91.
49. Reddy, N.; Chen, L.; Yang, Y. *Incl. Crops Prod.* **2013**, *43*, 159.
50. Wihodo, M.; Moraru, C. I. *J. Food Eng.* **2013**, *114*, 292.