

Review

# A Review of Cottonseed Protein Chemistry and Non-Food Applications

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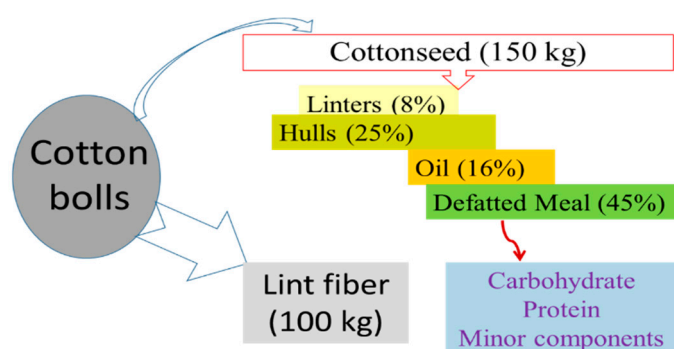
**Abstract:** There has been increasing interest in recent years in the use of agro-based raw materials for the production of bio-friendly and sustainable products. Plant-based proteins are among the popular materials being studied. In particular, cottonseed protein (a byproduct of cotton fiber production) is widely available and has useful properties. Although not as well-known as soy protein, cottonseed protein has been shown to be a potentially valuable raw material for numerous applications. In this review, the latest developments in isolation, composition and molecular weight, chemical and enzymatic modifications, and non-food applications are delineated. Among these applications, films and coatings, interfacial and emulsifying applications, adhesives, and bioplastics seem to attract the most attention. A particular effort has been made to cover the literature on these topics in the past 10 years.

**Keywords:** cottonseed protein; isolation; composition; molecular weight; modifications; applications; bioplastics; films; coatings; adhesives

## 1. Introduction

Cotton is a major industrial plant with about  $28 \times 10^9$  kg or 124 million bales produced annually [1], accounting for 2.3% of the world's arable land [2]. It has been estimated that the cotton fiber accounts for 85–90% of the value of the crop; the cottonseed and its products (primarily the oil) account for the rest [3]. Cottonseeds are typically covered by cotton fibers, which are “ginned” and used to make staple cotton and textile products. Typically, for every 100 kg fiber ginned there are also 150 kg of cottonseed produced (Figure 1). After ginning, the cottonseed comprises cotton linters grown from its outer cover, the hull (seed coat), and the kernel. In general, the seed consists of approximately 16% oil, 45% meal, 25% hull, and 8% linters [3]. The cotton linters can be removed to give chemical cotton. The hull is cracked to give the kernel. The hull can be used, for example, as animal feed [4] and growth substrate for mushrooms [5]. Chemically, the hull has been reacted to form cellulose acetate [6], mixed esters of cellulose [7] and carboxymethyl cellulose and carboxymethyl xylan [8], and nanocellulose [9].

The kernel is pressed or extracted to produce cottonseed oil, which is commercially significant; it is currently ranked sixth among the world's edible oils [10]. The leftover material (meal) is used mostly as an energy and protein source for domestic animals and fish [11–13]. The meal consists of over 40% protein. Through further processing, protein flour can be obtained with about 50% protein, protein concentrate with about 70% protein, and protein isolates with about 90% protein [14].



**Figure 1.** Composition of cottonseed (for 250 kg of cotton bolls).

Over the years, there has been a recurring interest in finding new applications for cottonseed protein. In an earlier review, Lusas and Jividen [15] covered the research and the utility of glandless cottonseed protein up 1987. The use of cottonseed and cottonseed meal as animal feed has been previously reviewed by Nagalakshmi et al. [16], Arieli [17], Coppock et al. [18], and Harper and Smith [19]. The formation and properties of cottonseed films and coatings have been covered by Marquie and Guilbert [20]. Some aspects of the bioplastic applications of glandless protein have been reported by Yue et al. [21]. The use of cottonseed protein for adhesives has been reviewed by He and Cheng [22].

This paper provides a review of selected research, development and applications of cottonseed protein with a particular emphasis on the publications that appeared in the past 10 years. Hopefully it will stimulate further interest in expanding the utility of this widely available, sustainable, and agro-based raw material.

## 2. Isolation Procedures

The extraction of protein from cottonseed meals has been ongoing for many years; Fontaine [23] has reviewed the early work in this area. Fractions of  $\alpha$  globulin,  $\beta$  globulin, pentose protein, and glutelin were isolated followed by component analysis and nitrogen content. Two helpful procedures were reported by Berardi et al. [24], where isolation was carried out by either a one-step extraction procedure for whole cottonseed protein isolate or a two-step sequential extraction procedure for water- and alkali-soluble cottonseed protein isolates (fractions), separately. The two extractants in the two-step isolation system were water and 0.027 M sodium hydroxide. Isolate 1 (water-soluble protein) was produced by precipitating the first clear extracts at pH 4, and isolate 2 (alkaline-soluble protein) was produced by precipitating the second clear extracts at pH of 7. Isolate 1 had the lower molecular weight of the two isolates and differed in nitrogen content, structural and spectroscopic properties [14,25]. Recently, both extraction procedures were further examined regarding the effects of blending conditions [25], and cottonseed protein isolate was produced in a pilot scale with other cottonseed products for application development [26].

In addition, cottonseed protein was isolated using water and sodium hexametaphosphate (SHMP) as a solvent and precipitation with acid. The highest yield isolate of 94.8% cottonseed protein was generated under the following conditions: temperature, 60 °C; time, 30 min; 2% SHMP; pH 7 [27]. Moreover, salt extraction was employed to isolate proteins from cottonseed meal. The extraction did not alter the cottonseed's structure and makeup, and it produced a 16% protein yield [28]. In another study, ammonia was used to isolate proteins from cottonseed meal [29]; this process produced a protein yield of 14% along with a nitrogen yield of around 14%. Furthermore, an aqueous and anhydrous extraction method isolated cottonseed protein with low levels of gossypol [30]. After this procedure, the cottonseed protein concentrate contained around 72% protein along with a very minute value of gossypol. This allowed the cottonseed protein concentrate to be used in foods.

Zhang et al. [31] employed an alkaline medium to extract cottonseed protein at varying pH values. The best extraction (70% cottonseed protein extractability) was obtained under these conditions: temperature, 60 °C; time, 40 min; pH, 12.5; solvent: flour ratio, 12:1. Enzymatic hydrolysis followed by ethanol extraction was another isolation method used, where 64% protein concentrate was obtained with a protein yield of 78% [32]. Arzu et al. [33] achieved enzyme hydrolysis via microbial proteases, which was able to produce around 50% protein solubilization. Lawhon et al. [34] reported a process of ultracentrifugation and reverse osmosis for the production of protein isolates. Later, Lawhon et al. [35] used isoelectric precipitation and ultrafiltration to isolate glandless cottonseed protein, with a pH of 10 being the most effective. Yao et al. [36] showed that a response surface method used after alkaline extraction provided accurate and optimum results with an 80.22% protein extraction rate. Marshall [37] published a detailed method explaining the isolation and purification of the cottonseed 7S storage protein along with its subunits. Liadakis et al. [38] used organic solvent extractions in order to remove gossypol, with 1-butanol hydrochloride producing the best results.

Cottonseed proteins were isolated from hot-pressed solvent-extraction cottonseed meal, cold-pressed solvent-extraction cottonseed meal, and subcritical fluid-extraction cottonseed meal [39]. These materials exhibited different hydrolysis, high water/oil absorption capacity, emulsifying abilities, surface hydrophobicity and fluorescence intensity.

Protein isolates were prepared from meals recovered from both glandless and glanded cottonseed [40]. Isolate yield was 2.5-fold greater from the glandless meal than from the glanded meal, indicating that the gossypol in the glanded meal likely promoted the formation of protein aggregates that were more difficult to extract. Both isolate preparations were very high in protein (>96%). These protein isolates demonstrated considerable solubility in both acidic and alkaline environments.

Cottonseed meal was extracted with water or phosphate buffer [41,42], and the resulting cottonseed meal was found to be a lower cost alternative to cottonseed protein isolate for adhesive applications. The washing process was recently scaled up and shown to be feasible for production and practical application [43].

In a different process, Meyer et al. [44] used hexane in a liquid cyclone process as well as high energy milling to produce cottonseed protein isolates.

### 3. Composition and Molecular Weight

The amino acid composition of cottonseed meal and isolate was reported by Fontaine [23]. There have been further reports of the protein composition over the years, e.g., Li et al. [45] and Martinez et al. [14] (Table 1). The amino acids with the highest levels are arginine (about 13%), glutamic (ca 12%) acid, and glutamine (ca. 10%). Amino acids with aliphatic side chains account for 17.5%, and with phenyl side chain 5.4%. Relative to soy protein, it has 4% more arginine and 4% less histidine. For cottonseed meal and isolates, arginine is the most abundant essential amino acid while glutamate is the most abundant nonessential amino acid [46]. In addition, there may be some non-amino acid impurities in cottonseed protein products as the protein content varied from 64.4% to 100% (Table 2) [26]. These impurities include, but are not limited to oil, hydrocarbons, and minerals. In the meantime, a commercial cottonseed protein only has 55.0% crude protein content [47]. Thus, the collection of comprehensive composition of cottonseed protein products should be helpful in developing industrial standards and quality control of cottonseed protein products in commercialization.

In an early work, cottonseed protein was separated into different fractions, such as globulin, pentose protein, glutelin, and phosphoproteins [23]. From SDS-PAGE analysis, cottonseed protein was separated into several distinct protein components with different molecular weights [48]. The molecular weight changes with maturation [49]. Recently, Singh et al. [50] applied one- and two-dimensional gel electrophoresis to explore the cottonseed protein characteristics. They compared the relative distribution of four protein fractions (i.e., albumins, globulins, prolamins and glutelins) of cottonseed in eight lines of two *Gossypium* species and found that globulins represented the dominating fraction in

both species followed by albumins, glutelins and prolamins. There was a significant positive correlation between albumins/globulins and seed protein content in *G. arboreum*/*G. hirsutum*, respectively. They also observed the intraspecific electrophoretic variation in seed protein extracts in the region of molecular weight 22–27 kDa in the lines of both species. A major protein, made up of seven non-homogenous subunits, was isolated in cottonseed meal.

**Table 1.** Amino acid profile for cottonseed meals (CSM), cottonseed flour (CSF) and cottonseed protein isolate (CSPI), normalized to 100%.

	CSM <sup>a</sup>	CSF <sup>b</sup>	(CSPI) <sup>b</sup>
Ala	3.8	4.1	4.1
Arg	12.1	13.6	13.2
Asn	4.2		
Asp	5.2	9.9	9.8
Cys	1.9		0.4
Gln	10.2		
Glu	11.7	22.3	22.1
Gly	5.7	4.5	4.3
His	2.9	3.2	3.5
Hyp	0.1		
Ile	3.2	3.7	3.6
Leu	6.0	6.3	6.8
Lys	4.4	4.8	3.5
Met	1.8	1.4	1.2
Phe	5.4	6.0	7.4
Pro	5.0	3.9	3.6
Ser	4.6	4.5	5.3
Trp	1.2		
Thr	3.3	3.4	3.2
Tyr	2.9	3.4	3.0
Val	4.5	5.1	5.1

<sup>a</sup> Reported by Li et al. [45], <sup>b</sup> Reported by Martinez et al. [14], where Glu and Gln were reported together and Asp and Asn were reported together.

**Table 2.** Selected organic and mineral components of pilot produced cottonseed protein products. WCSM: water-washed defatted cottonseed meal. CSPw, CSPa, and CSPI: water-soluble, alkali-soluble, and total cottonseed protein isolates. Adapted from He et al. [26].

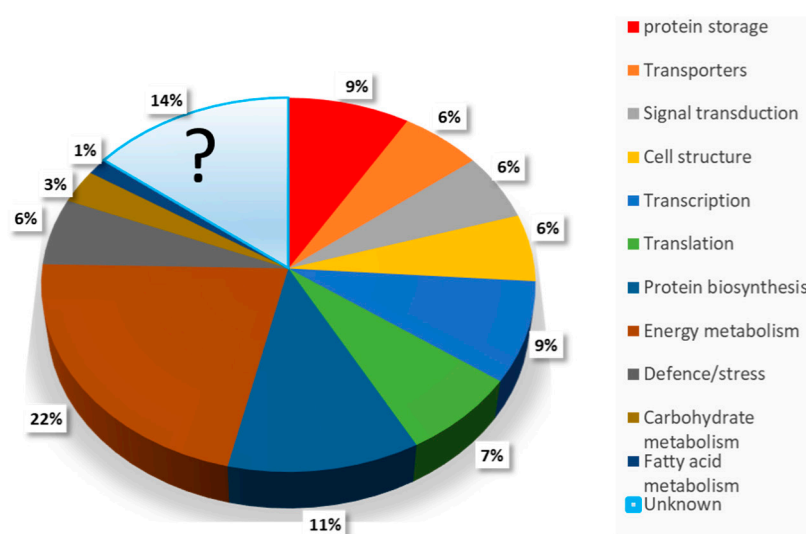
	Protein	Oil	Cellulose	P	Ca	K	Mg	Na	S
% of Product Weight									
WCSM	46.3	1.0	17.6	1.2	0.3	1.0	0.7	0.1	0.5
CSPw	64.4	3.4	1.6	1.1	0.1	0.9	0.2	0.1	0.8
CSPa	101.2	0.1	0.3	0.3	0.1	0.2	0.1	0.2	0.6
CSPI	94.8	0.2	0.6	0.5	<0.1	0.3	0.1	0.2	0.7

The protein's secondary structure was reported to contain mostly  $\beta$  domains and random coils [51]. Per FT-IR spectral features, He et al. [25] reported that  $\alpha$ -helix content ranged from 13.4% to 14.3%,  $\beta$ -sheet from 40.2% to 41.4%,  $\beta$ -turn from 32.2% to 32.5%, but random coils accounted

for only 11.9–12.2% of the total secondary structures of cottonseed protein isolate and their water- and alkali-soluble fractions. Further intrinsic fluorescence excitation–emission matrix (EEM) spectral investigation revealed that the tryptophan residues (fluorescence source) in the native cottonseed protein and its alkali fraction were protected within the micro hydrophobic environment and gradually became water accessible with progressive denaturation. On the other hand, the tryptophan residues in native water-soluble protein were already in contact with water [52]. In addition, a disulfide-bonded polypeptide was discovered in cottonseed protein, along with evidence that the major polypeptide bands of cottonseed protein were homogenous [53].

The surface properties of protein are related to numerous functional properties for practical uses, such as wetting, dispersibility, oxidative stability, flowability and rehydration properties. Thus, He et al. [54] investigated the surface characteristics of cottonseed protein products by scanning electron microscopy (SEM), SEM-energy dispersive spectrometer, X-ray diffraction, and X-ray photoelectron spectroscopy. One-step total cottonseed protein isolate and two-step sequentially extracted water and alkali soluble cottonseed fractions all showed compact crystalline structures while the residues (hydrocarbon dominated) after protein extraction either by one-step or two-step procedures showed more loose and porous structures. Gamero-Barraza et al. [55] examined the microstructures of cottonseed meal and its extruded products with corn flours by advanced microscopic techniques. Their SEM images showed surface characteristics similar to those observed by He et al. [54].

The detailed protein profile of cottonseed was reported recently by proteomic analysis [56]. First, water-soluble cottonseed proteins (CSPw) and alkali-soluble cottonseed proteins (CSPa) were sequentially extracted from defatted cottonseed meal. Through SDS-PAGE, 7 and 12 polypeptide components were found for CSPa and CSPw, respectively. Through mass spectrometry, 70 polypeptides were identified, with molecular weights ranging from 10 to 381 kDa. Thus, cottonseed protein contains a complex mixture of polypeptides with different biological functions per gene ontology terminology (Figure 2). These include storage proteins, transporters, signal transduction, cell structure, transcription, translation, protein biosynthesis, energy metabolism, antimicrobial activity, defense/stress, carbohydrate metabolism, and fatty acid metabolism, with 14% protein species of unknown functions. Quantitative analysis of the abundance of the polypeptides showed that these functional proteins account for only small or even tiny fractions of the whole cottonseed protein, while vicilin- and legumin-related polypeptides overwhelmingly dominate as the major storage proteins.



**Figure 2.** Putative biological functions and percentage of each class of proteins identified in cottonseeds. Adapted from [56].

#### 4. Improved Proteins through Agriculture and Genetic Engineering

In an early review, Tharp [57] covered an extensive amount of information on the effects of geographical source, weather, maturity, and nutrition on cottonseed composition. Nitrogen and potassium in soil were found to increase seed index while phosphorus had mixed effects. In terms of cottonseed oil produced, potassium and phosphorus showed an increase while nitrogen had mixed effects. Nitrogen showed an increase in cottonseed protein content while potassium showed a decrease in cottonseed protein content. He et al. [58] reported that protein and fiber profiles of cottonseed were impacted by different fertilization management practices. They found the seed content of crude protein to increase in this order: no fertilizer  $\leq$  poultry litter  $\leq$  chemical fertilizer. However, the amino acid composition and the levels of total carbon and acid detergent lignin were less affected by the management practices. He et al. [59] further noticed that both essential and nonessential amino acids were enriched in cotton leaf blades and reproductive parts in mid growth, but both nutritional carbohydrates and amino acids were later accumulated in seeds at pre-defoliation before harvest.

Several reports were published by Sawan et al., testing how certain chemicals affect the agriculture and growing yield of cottonseed. Nitrogen, phosphorus, and growth regulators were found to improve seed index, cottonseed protein content, and oil yield [60]. In a later study, potassium, zinc, and growth retardants were also found to increase seed index, cottonseed protein content, and oil yield [61]. These results were confirmed in two later studies [62]. In the latest report [63], they described the use of fertilizers and a plant growth retardant (Pix), on cottonseed, protein, oil yields, and oil properties of Egyptian cotton.

Two integrated management strategies were found to give significantly higher cottonseed, oil and protein yields than conventional management practices in a two-year study. The results suggested that increased sucrose/H<sup>+</sup> symport, sucrose hydrolysis, hexoses synthesis, and cumulative photo-thermal product (PTP), especially in the early stage of embryo growth, play a dominant role in the high productivity of cottonseed oil and protein [64].

In a two-year study of five sets of near-isogenic mutant cotton lines, seed protein was shown to be higher in the fuzzy genotype in all sets, but seed oil was higher in fuzzless genotype in all sets [65]. This information is beneficial to breeders to consider fussy or fuzzless cottonseed for respective protein or oil use.

Gossypol, a terpenoid compound found naturally in cotton, is an insect repellent but also a toxin for non-ruminant animals. Cottonseed protein concentration increased significantly from 287 g·kg<sup>-1</sup> to 357 g·kg<sup>-1</sup> after 112 kg·ha<sup>-1</sup> of nitrogen in a form of urea/ammonium nitrate was added. However, gossypol decreased 14% and the oil yield 9% after the same amount of nitrogen fertilization was applied [66].

Much effort has been devoted to using plant breeding and genetic engineering methods to produce glandless cotton, thereby minimizing the level of gossypol, which has undesirable physiological effects. Cai et al. [67] wrote a review that illustrated the importance of glandless cottonseed for food/feed, and discussed strategies to enhance cottonseed via methods such as genetic engineering. The earlier work using plant breeding has been reviewed [15]. Sunilkumar et al. [68] demonstrated that a targeted genetic modification could significantly reduce gossypol levels in a stable and heritable manner. The benefit of gossypol-free cottonseed protein, obtained through genetic engineering to satisfy the protein needs in human diet, has been pointed out [69].

There have been other publications on the genetic enhancement of cottonseed traits (protein content, oil content, seed index, and others). A genetic model was created with crossed cottonseed chromosome substitution sections. After a simulation of this model, the results showed positive outcomes with an increase in cottonseed traits that can be applied to industrial use, such as adhesives [70]. Recently, He et al. [71] comparatively evaluated the mineral and protein contents of the seeds of 21 parental cotton lines and their 177 recombinant inbred lines produced with a multi-parent advanced generation inter-crossing (MAGIC) approach. This work reported the data of the top five cotton lines of the highest and lowest contents of seed mineral and protein, thus providing the opportunities for researchers and



breeders to select better and/or preferred seed quality, in addition to the assurance of lint yield and quality, for further development.

## 5. Chemical and Enzymatic Modifications of Cottonseed Protein

Because unmodified cottonseed protein does not have the functional properties that are optimal for some applications, a lot of work has been done to find ways to improve these properties. A simple method was enzymatic hydrolysis. This was reported by Arzu et al. [33] using ten proteolytic enzymes. Two enzymes were then chosen for detailed studies of pH, temperature, enzyme and substrate concentrations. In this way, about 40–60% of the protein could be solubilized.

Most of the other modifications reported thus far were done chemically. Succinylation was shown to be effective in enhancing the functional properties of cottonseed protein [72]. After succinylation, the modified protein isolates showed superior functional properties (e.g., emulsion capacity, gel strength, viscosity) compared to unmodified protein isolates. Acid anhydride treatment was used on protein extraction to see how it affected the isolate's functional properties [73]. With the exclusion of one sample, the acylating agents caused approximately 20% more protein extracted from the flour medium. Solubility in the pH 6–7 range also increased with the protein being approximately 90% soluble when treated with acylating agents compared to 60% for the ones not treated with the agents in the extraction medium. For the most part, oil absorption capacities remained around the same after treatment with the acylating agents.

SDS-catalyzed deamidated cottonseed protein was tested for improved functional properties [74]. During the hydrolysis of these amide groups, there was only a negligible amount of peptide bond destruction. After deamidation, the functional properties (e.g., solubility and emulsion capacity) of the cottonseed proteins were enhanced. It may be noted that denaturants (e.g., SDS, guanidine HCl and urea) made the cottonseed protein more hydrophilic by rendering the protected amino groups' micro hydrophobic environment more water accessible [52]. Ravindran et al. [75] found the lysine in cottonseed protein to be difficult to undergo guanidination reaction. They had to use high pH and long reaction times to optimize this reaction.

Chemical modifications of cottonseed proteins by gossypol, formaldehyde, and glutaraldehyde were used to increase puncture strength and decrease solubility of the films made from cottonseed flour [76]. A more detailed study of the crosslinking reaction was done by using HPLC to determine the reactive lysine content [77]. In a comparative study involving formaldehyde, glutaraldehyde, and glyoxal, formaldehyde treatment gave the highest film puncture force and glutaraldehyde the lowest. However, glutaraldehyde was shown to react with 100% of the lysine while formaldehyde with 50% of the lysine. The results were interpreted as due to the impact of the molecular structure of the crosslink bridges on the mobility between protein chains [77]. These results were supplemented in a later study, where acid-resistant lysine derivatives were found; lysine was shown to play a major role in protein crosslinking by dialdehydes, tyrosine was involved in formaldehyde reaction, and arginine in glyoxal reaction [78].

## 6. Cottonseed Protein Applications

### 6.1. Films and Coatings

Cuq et al. [79] provided a review of the use of proteins (including cottonseed protein) for packaging applications. They noted that the macroscopic properties (including solubility in water, mechanical properties, and barrier properties) of agricultural packaging materials based on proteins are dependent mainly on the structure of the macromolecular three-dimensional network and on interactions between proteins, plasticizers, and crosslinkers.

In the previous section, the crosslinking of cottonseed protein with dialdehydes (e.g., formaldehyde, glutaraldehyde, glyoxal, and gossypol) to reduce solubility and enhance the puncture force of packaging films was noted [76–78]. In another study [80], cottonseed protein isolate was plasticized with glycerol

and incorporated into biodegradable products through extrusion and thermomolding. The results showed that glycerol could make cottonseed protein a thermoplastic through a 54 °C increase in thermal denaturation temperature.

Another study [81] tested the effect of crosslinking with glutaraldehyde and montmorillonite on the properties of the bioplastics. Tensile strength was 19.1 MPa and water absorption resistance was 27.8% when there was a 30% mass content of glutaraldehyde and a 6% mass content of montmorillonite. In a different study [82], cottonseed protein was mixed with polyurethane prepolymer in order to increase cottonseed protein's mechanical properties to be used for packaging. As the percentage of polyurethane prepolymer increased, properties such as elongation and water resistance increased, thus leading to improved mechanical properties.

Compression molding, along with glycerol and aldehydes, were involved in order to generate cottonseed protein plastic sheets. This crosslinking process increased the functional properties of the bioplastics [83]. Likewise, chemical additives (urea, aldehydes and glycerol) were applied to cottonseed meal during a heat press in order to form cotton protein bioplastics [84]. These chemicals provided crosslinking which enhanced the thermal stability, water absorption resistance and mechanical strength of the products.

In another approach [85], nanoclay and carvacrol were added to cottonseed protein films. The nanoclay improved the mechanical characteristics of the film giving it a tensile strength of 4.07 MPa at 3% nanoclay. Carvacrol, an antimicrobial agent, increased the film's antimicrobial activity when the film was used for packaging bacon.

Chen et al. [86] prepared blend films by casting cottonseed protein with poly(vinyl alcohol) (PVA) and modified them with different plasticizers. Plasticizers altered the degree of interaction between protein and PVA. Such interactions changed the secondary structure of the cottonseed protein but had little effect on the crystallinity of protein/PVA blend films. Those blend films exhibit the potentials as promising plastics for food packaging and flower planting applications.

Filho et al. [87] studied blends of hydrolyzed cottonseed proteins and alginate as packaging films in terms of their physical, chemical, barrier, optical, antioxidant and antimicrobial properties and the release of peptides in two different alginate-film food simulants. In migration tests in aqueous media, the active films released more than 60% of their peptides in 30 min, and there was a controlled and gradual diffusion of the compounds embedded in the film when fatty foods were simulated. The results showed that alginate films with protein hydrolyzates are promising as active packaging for the preservation of fatty foods.

Edible protein films appear to be a trendy topic these days. A review on this topic was written by Dursun and Erkan [88], where cottonseed protein was mentioned as a possibility. Obviously, glandless cottonseed protein needs to be used for this purpose.

## 6.2. Interfacial and Emulsifying Applications

Moure et al. [89] published a review on the functional properties (solubility, water and oil retention capacity, foaming capacity and stability, emulsion capacity and stability, viscosity, gelation) of oilseed proteins during processing and storage. Chemical and enzymatic treatments could modify these properties. Data corresponding to diverse oilseeds and from different defatting and extraction processes were compiled and grouped according to the protein content into meals, concentrates, and isolates.

Tsaliki et al. [90] examined the foaming abilities of cottonseed protein isolates. A pH of 7 exhibited better foam ability than a pH of 6. Xanthan gum increased foaming abilities while pullulan decreased foaming abilities of cottonseed protein isolates. In addition, the emulsifying properties of cottonseed protein isolates were studied [91]. A pH of 7 produced higher emulsion stability than a pH of 6. The addition of both xanthan gum and pullulan increased emulsion stability. Applying NaCl to a concentration limiting around 0.2 M helped emulsion stability as well.



Tunc and Duman [92] obtained the moisture adsorption isotherms of different types of cottonseed protein, which were found to be type II sigmoidal. Thermodynamic properties such as differential enthalpy and entropy were determined, using Clausius–Clapeyron equation.

Delgado et al. [93] extracted glandless cottonseed proteins and determined their properties. They showed major protein bands between 13,273 and 56,564 Da with an isoelectric point of 5.1. The protein isolate showed lower water-holding and oil-holding capacity, but similar gelation properties as soy protein. It had a high foaming capacity at high pH values and high emulsion stability.

### 6.3. Adhesives

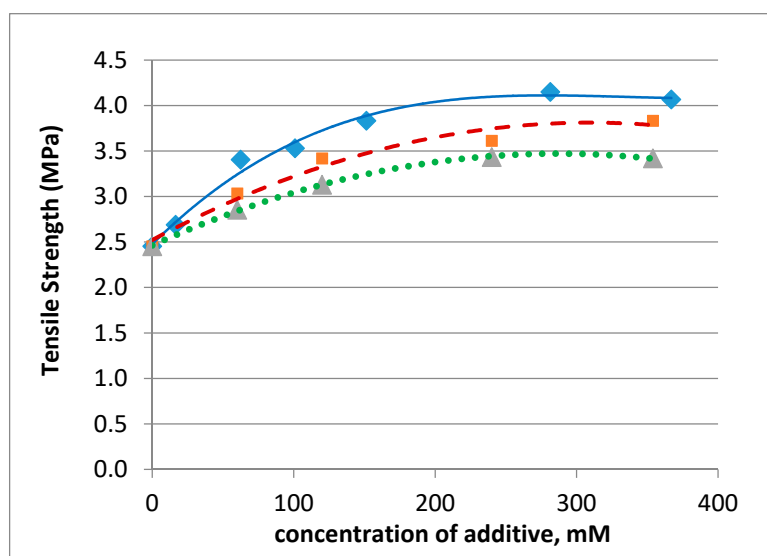
Earlier studies of cottonseed protein as wood adhesives were reported by Hogan and Arthur [94–96]. There has been a revived interest in recent years. Cheng et al. [97] showed cottonseed protein to provide equivalent or greater adhesive strength than soy protein when tested on maplewood veneer. Further testing [97] was done with soy and cottonseed proteins with four chemical additives (urea, sodium dodecyl sulfate, alkali, and guanidine hydrochloride); sodium dodecyl sulfate was shown to have favorable adhesive and hot water resistance properties with cottonseed protein. More detailed studies were reported later on these denaturants [98]. Mixtures of cottonseed with soy protein were blended for testing results [99]. As the proportion of cottonseed protein in the blends increased, the adhesive strength on wood increased. Polysaccharides (e.g., starch and cellulose) was shown to be a helpful blend component in cottonseed protein or soy protein [99]. A formula containing up to the 75% polysaccharides still maintained the same adhesive shear strength of the 100% protein, which led to a cost-efficient way of making adhesives because the cottonseed/soy proteins are relatively more expensive. These and other related blends have been reviewed [100]. A U.S. patent [101] has been granted to cover the adhesive compositions containing cottonseed protein, and one or more of the following components: (i) soy protein, (ii) a polysaccharide, or (iii) at least one modifier selected from a carboxylic acid, a dicarboxylic acid, a phosphorus-containing acid or ester, a cationic amino acid, a quaternary ammonium salt, or their mixtures.

Additionally, several studies have discussed the adhesive effects of various additives combined with cottonseed protein. Small molecules bearing a carboxylic functionality [102] were shown to enhance the dry adhesive strength but not the hot-water resistance of cottonseed protein. Several phosphorus-containing compounds [103] were found to enhance both dry strength and hot water resistance of cottonseed protein but showed no effect on soy protein. Anionic polysaccharides (such as carboxymethyl cellulose, CMC, and low methoxy pectin) [104] displayed significantly enhanced cottonseed protein's adhesive properties; other anionic polysaccharides (alginate and carrageenans) showed intermediate effects, whereas anionic vinyl polymers exhibited even weaker effects. For illustration, the dry adhesive strengths for cottonseed protein isolate with different additive concentrations of carboxymethyl cellulose, low methoxy pectin, and alginate are shown in Figure 3. These enhancements found with anionic polymer additives occurred only for cottonseed protein, but not for soy protein.

More recently, nanocellulose (both cellulose nanofibers and nanocrystals) was found to enhance the adhesive performance of both soy- and cottonseed-based adhesives [105]. It was reported that addition of tung oil improved adhesion strength and water resistance of cottonseed meal and protein adhesives on maple veneer [106]. However, the impact became non-apparent when the bonding strengths of those protein-based adhesives were themselves high [42,107].

Cottonseed meal was extracted with two solutions: NaCl and water; NaCl and phosphate buffer [108]. The fractions that were water washed or phosphate washed had relatively the same adhesive strength as the initial meal. This implies that water- or buffer-washed cottonseed meal can be used as adhesives to provide similar adhesive strength as isolated cottonseed protein, leading to higher cost efficiency. In another study [41], the water resistant and adhesive shear strength properties of water-based, phosphate-based, and cottonseed protein isolate were tested on wood veneers. All three samples showed analogous adhesive characteristics of adhesive shear strength

and water-resistant qualities escalating with temperature, thus supporting the claim that water- and phosphate buffer-washed cottonseed meals can be used as cost-effective alternatives.



**Figure 3.** Dry adhesive tensile strength of cottonseed protein isolate with different additive concentrations of CMC (diamond markers, solid line), pectin (square markers, dashed line), and alginate (triangle markers, dotted line), Adapted from Cheng et al. [104].

Different drying methods were studied for water-washed cottonseed meal (WCSM) as wood adhesives [109]. The differences among the products with the three drying methods became smaller, and was even none with the press temperature at 150 and 170 °C. The adhesion performance could be further improved by pH 4.5 adjustment and removal of large residual hull particles. Spray-drying and freeze-drying were more suitable for making high-quality cottonseed meal-based adhesives for a variety of operation conditions. The more economical oven-drying may be applied to make WCSM product for bonding at higher press temperature (e.g., 170 °C) without undermining WCSM's adhesion performance. He et al. [110] comparatively investigated the effects of pH and storage time on the adhesive performance, water resistance and rheological properties of cottonseed meal water-washed cottonseed meal (WCSM) and cottonseed protein isolate (CSPI). They found an optimal pH at 6.0 for all three slurries. Storage time (up to 8 days) did not greatly impact the adhesive performance of WCSM slurries prepared at pH 6.0, 7.5, and 9.0, but slightly reduced the adhesive strength of CSPI slurries with the same pH. The viscosity of WCSM slurries increased with storage over 8 days, but not for CSPI slurries. Pradyawonga et al. [111] blended cottonseed-based adhesive slurries at different cottonseed protein contents. Their data demonstrated that the blends with 65–70% of protein content possessed the bonding performance and flowability comparable to highest protein product CSPI (94.8% protein) within the acceptable standard deviations; this is helpful in setting up industrial standard requirements and quality control for protein content in cost-effective adhesive-grade cottonseed products.

A greener adhesive composed of UF resin and cottonseed meal was successfully prepared via a common synthetic process of pure UF resins [112]. The raw materials (urea and formaldehyde) of UF resins were replaced by cottonseed meal with up to 40% on weight basis. The adhesive showed an improved mechanical strength as compared to pure UF resins in the tensile shear strength of bonded wood specimens, especially on the water-soaked strength. Cottonseed meal acted as a reinforcement for the adhesive other than a filler or an additive. This “greener” adhesive improved the performance of pure UF resins while retaining its outstanding features. In addition, cottonseed protein-based adhesives also tested with or without a synthetic glue Vinavil 2259 L [43]. The results showed that these adhesives might be used as the conventional D1 wood adhesives for nonstructural interior application per European standard EN204/205. Under the testing conditions, the pilot-scale produced

washed cottonseed meal possessed very high heat resistance according to European standard EN14257 (WATT 91). Furthermore, blending the cottonseed product with Vinavil 2259 L improved the water resistance of WCSM, allowing the classification of the cottonseed-protein adhesive as non-structural D3 type adhesives for protected outside use.

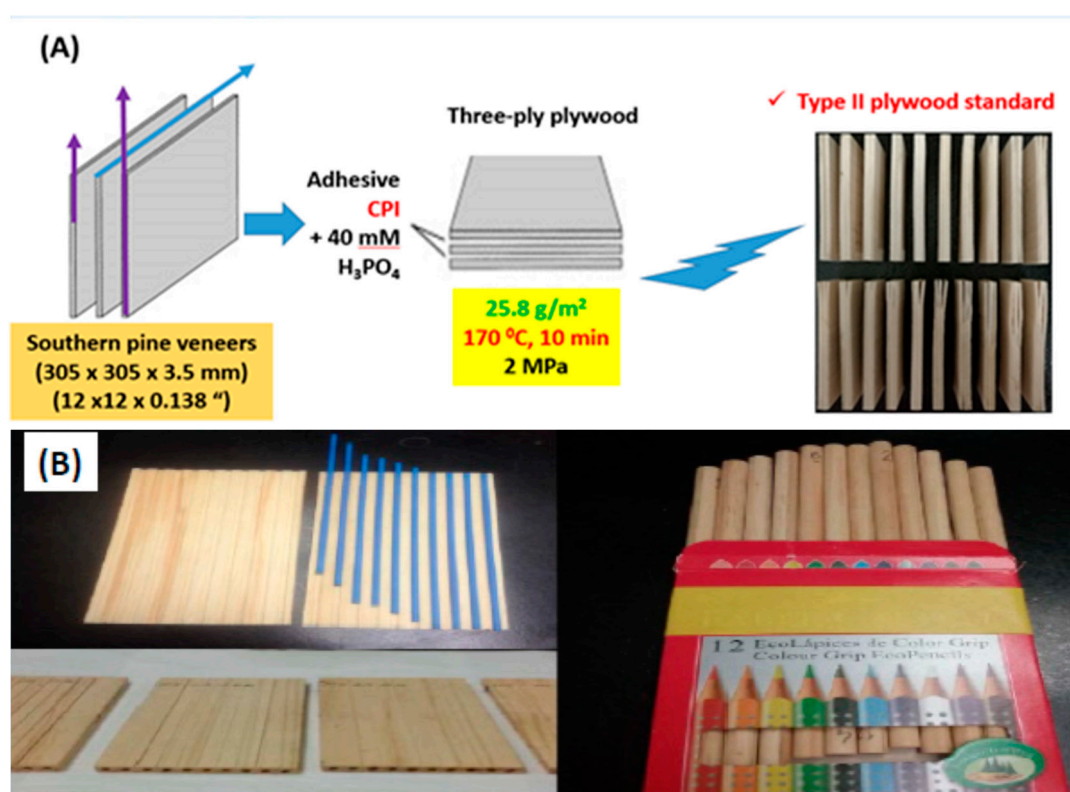
For practical application, Li et al. [113] assessed phosphorus/calcium-cottonseed protein adhesives for 3-ply plywood production (Figure 4A). They made the 3-ply 12 × 12" pine wood panels and tested their water resistance using a three-cycle soak as recommended by the American National Standard for Hardwood and Decorative Plywood-2016. Their data demonstrated that cottonseed protein-based adhesives have the capability to function as a conventional interior adhesive for Type II plywood for interior usage per the American National Standard ANSI/HPVA HP-1-2016. Three-ply plywood panels were also constructed with varying blends of cottonseed protein isolate (CSPI), soy protein, and phenol: formaldehyde adhesive [114]. Wet and dry shear testing revealed that while the novel adhesives did not perform as well as a commercial control, the CSPI and soy adhesives generated similar shear strengths. A comparison was made with commercial cottonseed meal (CM), water washed cottonseed meal (WW) and defatted cottonseed flour (DF). CSPI had a performance against termites that was not significantly different from guayule (a known antifeedant). Chen et al. [115] made efforts to develop cottonseed protein-based adhesives for interior plywood bonding. They made 5-ply yellow poplar plywood panels bonded by the adhesive slurries of defatted cottonseed meal and Kymene<sup>TM</sup> 736 resin. Similar to the 3-ply pine plywood panels [113], 5-ply poplar plywood panels also meet the American National Standard ANSI/HPVA HP-1-2016 as interior Type II plywood [113].

While the optimization of the bonding strength and water resistance seems appropriate in formulation of bio-based wood adhesives [116,117], certain operational parameters, such as adhesives' rheology and viscosity, are also important in practical applications of these adhesives [118]. More than that, certain industrial applications, such as furniture and domestic small utensil bonding, may not require the highest adhesive strength, but may need stricter requirements on the solid content and operational parameters. He et al. [117] reported that an industrial partner presented their current synthetic adhesive parameters, viz., 2000–5000 mPa.s, solid content 50–52%, pH 4–5, press temperature <60 °C, and press time up to 120 min. To adapt the potential bio-based bonding operation to the current production line, the industrial partner required the operational parameters of the WCSM adhesives close to those of the synthetic adhesive as many as possible. Thus, He et al. [117,119] tested and optimized the bonding performances of cottonseed protein-based adhesive formulations at higher solid contents, lower bonding temperatures and long bonding times. Four formulations with 20% and 30% of WCSM as well as 9.6% and 19.1% of SDS were used to glue "real world" pencil slat sandwiches for pencil making. All the pencils made from these sandwiches bonded at 40 °C and 1 MPa for 120 min passed the Industrial Temperature Cycle test (Figure 4B). The pencil sandwich bonding represents a specific application that could benefit from the non-toxic bio-based adhesive and is an example of an interior application.

Oxidation of several protein flours (including cottonseed flour) was carried out with periodate, permanganate, or iodate [120]. This treatment was found to improve their strength under wet conditions. Nitric acid, chlorate, perchlorate, and bromate were not effective in increasing wet strength. This mechanism was also supported by the improved wet strength with the addition of dialdehydes (glyoxal and glutaraldehyde).

#### 6.4. Other Applications

Thermomechanical relaxation events and different water states in cottonseed protein bioplastics were found during an investigation of the effects of aldehyde crosslinking agents [121]. From these measurements, three water states were noted in the water-absorbed bioplastics: strongly bound-to-polymer, weakly bound-to-polymer and bulk-like water.



**Figure 4.** Wood products bonded by cottonseed protein-based adhesives. (A) 3-ply pine wood boards, (B) EcoPencils. Adapted from Li et al. [114] and He et al. [120].

A cottonseed protein-poly (acrylic acid) copolymer (CP-PAA) hydrogel was formed for testing of specific sorption properties. Water absorbency increased with a decrease in crosslinking density and an increase in molecular weight. Peak water absorbency reached its peak at a pH around 7. Overall, manipulations in the polymerization reaction would lead to differences in water absorbency properties [122]. Metal cations could absorb readily into the hydrogel mixture [123].

The influence of pH and ionic strength on gel formation and gel properties of CSPI was studied using dynamic oscillatory rheometer, differential scanning calorimeter, and scanning electron microscopy [124]. The gelation temperature was influenced by ionic strength. The pH altered the denaturation temperature of the protein. Lower pH and higher salt concentrations resulted in dense networks. Gamero-Barraza et al. [55] evaluated the interaction between cottonseed protein and corn starch as they were processed through extrusion. They made extruded products from cottonseed meal and nixtamalized corn flour at weight levels of 10, 25, 50 and 75% of cottonseed meal. Their work demonstrated that cottonseed protein and corn starch had a favorable interaction and were miscible, confirming an earlier study on CSP/starch blends [100]. The interaction between cottonseed protein and corn starch was electrostatic in nature.

Blends of water-washed cottonseed meal (WCSM) and polycaprolactone (PCL) plasticized with cottonseed oil were made and analyzed for their mechanical, adhesive, and thermal properties [125]. The addition of WCSM to PCL increased the Young's modulus but decreased the tensile strength and elongation-at-break of PCL. The addition of cottonseed oil to the PCL/WCSM blend kept the tensile strength about the same but enhanced the elongation. The PCL blends with WCSM and cottonseed protein isolate gave about the same mechanical properties, both somewhat better than the PCL/soy protein isolate blend. As plasticizers, cottonseed oil performed slightly better than coconut oil, both better than poly(ethylene glycol). The addition of WCSM and cottonseed oil did not change the adhesive performance of PCL on fiberboard. Thus, the combination of PCL/cottonseed

protein/cottonseed oil seems to be a viable bioplastic, and one possible application for this material may be in the hot melt adhesive area.

Defatted cottonseed meal is an N-rich biomass that deserves valorized recycling. Slow pyrolysis was applied to defatted cottonseed meal to produce pyrolytic bio-oil [126]. The contents of C and S were much higher in the oily fraction than in the aqueous fraction. Furthermore, the composition and molecular formulas of the organic compounds in the oily fraction were much more complex and diverse. This information is useful if these N-rich types of products are used as a bioenergy resource and as an industrial feedstock.

Cottonseed protein isolate was studied as a paper additive, and the tensile strength of paper was found to vary with the amount of the protein applied [127]. With the application of an 11% protein solution to the paper, the dry and wet strength increased by 33 and 16% compared with the paper by itself, respectively. The combined use of cottonseed protein and an acid (acetic, adipic, aspartic, and citric acids) resulted in even greater dry paper strength but not in greater wet paper strength. Analytical characterization suggested that the protein interacted with acid and that both components interacted with paper fibers to produce increased strength.

In another application, cottonseed protein was used as an additive to increase the dry strength of cotton-based nonwovens [128]. The tensile strength of the nonwovens was found to increase as the concentration of protein applied increased. At 11% protein concentration, the tear strength and burst strength increased significantly (relative to the nonwoven by itself) by 288% and 295% (machine direction), respectively. Further characterization by infrared and thermogravimetric analysis suggested that cottonseed protein interacted with the cotton fiber in the nonwoven fabric to produce the increased dry strength.

## 7. Conclusions and Future Prospects

Cottonseed constitutes a potentially important agro-industry residue with biotechnological applications due to its chemical composition (fiber, proteins, carbohydrates, and lipids) [129]. Although cottonseed protein is a major plant-based protein, it has attracted only a moderate amount of attention in research and development in the past. There have been occasional spurts of publication activities from time to time, but these activities cannot compare with those of other plant proteins, notably soy protein. In the past ten years, there seems to be a resurgence of interest in this material, and many more papers have appeared. As shown in this review, cottonseed protein has many interesting and useful properties and can be a good raw material for numerous applications.

A major problem that hinders the commercialization of cottonseed protein concentrate and isolate for industrial uses is its lack of availability at large commercial scales. This is due in part to the fact that the gossypol present in cottonseed protein prevents its use as food or feed for non-ruminants. (In comparison, soy protein is being sold at a high volume primarily as food and feed, and only a small fraction of soy protein is channeled to industrial uses. A welcome development is the approval by the FDA of gossypol-free cottonseed protein for food and feed use in 2020 [130]. Recently, He et al. [131] conducted four antioxidant tests for two water-soluble protein samples (GI-L and GD-L, i.e., gossypol-free and gossypol-associated) isolated at a lab scale from glandless and glanded cottonseed meal, respectively, and one soluble protein samples (gossypol-associated) at a pilot scale from glanded cottonseed meal. Song et al. [47] also measured the antioxidant and antibacterial activity and in vitro digestion stability of cottonseed protein hydrolysates. These data could be helpful in understanding the beneficial functions of cottonseed-protein food products. If the consumption of cottonseed protein increases, hopefully the availability and the price will both become more favorable. Moreover, with increasing number of applications for cottonseed protein, the demand-versus-supply situation will further improve in the coming years. It may be noted that the production of cottonseed protein will also depend on market forces, competitive pressures, and commercial considerations in the future.



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