Biodegradable Packaging Made from Cottonseed Flour: Formation and Improvement by Chemical Treatments with Gossypol, Formaldehyde, and Glutaraldehyde

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A process for the preparation of lipoprotein and protein films from glandless and glanded cottonseed flour (delipidated or not) is described. Chemical modifications of cottonseed proteins by gossypol, formaldehyde, and glutaraldehyde were used to increase puncture strength and decrease solubility of the films within the scope of biodegradable packaging applications. Promising results were obtained with glandless flour. Formaldehyde was effective in film formulations using glandless delipidated cottonseed flour at a molar ratio of 6 with regard to the potential reactive amino acids. Glutaraldehyde was a better cross-linking agent for glandless flour film when used at molar ratio (glutaraldehyde/lysine) also equal to 6. Gossypol, a naturally occurring dialdehyde in cotton, is also a useful agent for decreasing solubility and increasing puncture strength of films. Utilization of cottonseed varieties with a high gossypol content could be suitable.

Keywords: *Biodegradable packaging; cottonseed flour; gossypol; formaldehyde; glutaraldehyde*

INTRODUCTION

The use of nonbiodegradable and nonrenewable packaging materials and their accumulation is responsible for severe environmental problems. Biopolymers could be used to formulate biodegradable packagings, e.g. to replace short-life plastics, in conjunction with plastic recycling programs. These biopolymers can be categorized according to the main constituent in their formulation: polyosides, lipids, proteins, and polyesters (Gontard and Guilbert, 1994). Film formation involves intermolecular and intramolecular associations or cross-linkings of polymer chains to form a semirigid three-dimensional matrix that entraps and immobilizes the solvent (Kester and Fennema, 1986; Guilbert and Biquet, 1989). The degree of cohesion of the film depends on the biopolymer structure, the preparation procedure, different physical parameters (temperature, pressure, solvent type and dilution, solvent evaporation technique, application technique, etc.), and the presence of plasticizers and cross-linking additives (Gontard and Guilbert, 1994). Proteins, which are nonmonotonous polymers, offer high potential for forming numerous linkages. Higher performances are obtained by either choosing low-solubility proteins (such as corn zein, wheat gluten, and myofibrillar animal proteins) or using cross-linking or tanning treatments and/or adding lipidic compounds (composite films). Films primarily composed of proteins have suitable mechanical and optical properties but are generally highly sensitive to moisture and exhibit poor water vapor barrier properties (Gontard and Guilbert, 1994; Gontard et al., 1992, 1993, 1994).

The sole utilization of cottonseed proteins for film formulation was reported by Wu and Bates (1973). The process involves soaking cottonseed kernels (containing about 30% proteins) in hot water to make an "oilseed milk". The film is formed at the surface of the liquid during heating ("Yuba-like process"). Due to their poor mechanical properties, films made by using this process should be used only as edible film, not as packaging.

The protein content of delipidated cottonseed flour (dry matter basis) is from 50 to 60% (w/w). Cottonseed flour and delipidated cottonseed flour are naturally abundant and of economic interest ($55 U.S./ton for cottonseed and $110 U.S./ton for cottonseed delipidated flour).

Cottonseed proteins are mainly composed of water-soluble globulins and albumins (Saroso, 1989). To improve the mechanical properties and decrease the solubility of cotton film, we planned protein chemical treatments with bifunctional reagents capable of reacting with amino acid side chains, particularly with the ϵNH_2 group of lysine.

Only simple criteria (puncture strength, puncture deformation, and film solubility) were used to assess the efficiency of chemical treatment.

MATERIALS AND METHODS

Raw Materials. The compositions of the raw materials are presented in Table 1. Glandless flour (which contains no gossypol) was made by grinding kernels (variety CIRAD 411) obtained in Madagascar, and glanded flour (which contains pigments enclosing gossypol) was made by grinding kernels (variety CIRAD 151) obtained in Mali. Glandless delipidated flour was obtained after oil extraction (extrusion–solvent process) from glandless cottonseed kernels (variety CIRAD 411) at the Trituraf factory (Ivory Coast).

Preparation of Film-Forming Solution. Cottonseed flours (glanded or glandless) or glandless delipidated cottonseed flour were soaked for 1 h in water (30 or 20% w/v, respectively), at pH 10, with either triethylammonium or ammonia at 40 °C. The mixture was then centrifuged (500*g*,

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Table 1. Composition of Cottonseed Flours Used for the Film-Forming Preparations

material	crude composition (g/100 g, dry basis) protein[a]	lipids	free gossypol
glandless cottonseed flour	34.4	38.8	0.002
glandless delipidated cottonseed flour	49.3	2.6	0.004
glanded cottonseed flour	32.9	38.7	0.530

[a] Protein content of the material was calculated using the nitrogen conversion factor of 5.3 given by De Rham (1982).

2 × 5 min), and glycerol, used as a plasticizer, was added to the supernatant at the required concentration (10–30 g/100 g of dry matter content of the film-forming solution).

Chemical Treatments. Protein modification treatments with formaldehyde (40% w/v, Merck) or 5.6 M glutaraldehyde (Merck) were conducted in the centrifuged film-forming solution, at room temperature for 1 h, up to 12:1 molar ratios with reference to reactant amino acids (namely Lys, Cys, and His). According to Saroso (1989), Bourély (1990), and Marquié (1994), the Lys, Cys, and His contents in 100 g of cottonseed protein are 30, 15, and 20 mmol, respectively. Protein modification treatments by gossypol (Sigma) were conducted in the centrifuged film-forming solution made from a mixture of glandless cottonseed flour and pure gossypol (4.4 and 5.6 mmol/100 g of protein dispersed in the film-forming solution). Reaction is carried out at room temperature for 1 h. Glycerol was added until it represented 20% (w/w, dry matter basis).

Film Preparation. Defined volumes of film-forming solution (6–9 mL, according to the dry matter content of the film-forming solution) were poured into polystyrene crystal Petri dishes to obtain 20 mg/cm^2 of dry matter. The solvent was first evaporated at room temperature for 24 h and then at 60 °C for 24 h.

Chemical Analyses. Total nitrogen content was determined by combustion with a Leco FP 428 analyser (Leco Corp. 3000).

Total lipids were determined according to the method described by Wu and Bates (1972a). The aqueous fraction was used for carbohydrate determination by thin layer chromatography.

Gossypol content was determined by HPLC using a method described by Marquie and Bourély (1991).

The film pH was determined by the dilution method: Two film disks of 2 cm diameter (approximate weight 100 mg) were introduced in a tube and dispersed in 2 mL of water. After pH measurement, the solution was successively diluted with 1 mL increments of water, and pH was measured after each addition. The film pH was determined by extrapolation of the linear portion of the pH versus volume curve.

Mechanical Tests. The puncture strength and puncture deformation of the film were evaluated with a RHEO TA.XT2 texturimeter. Films for measurement were equilibrated in a 56% relative humidity atmosphere (NaBr saturated solution, 25 °C, 72 h), cut in 6 cm diameter disks fixed in an annular device. A cylindrical probe (12.6 mm^2 surface) was moved perpendicularly at the film surface at constant speed (1 mm/s) until it passed through the film, and the force versus deformation curve was recorded. The film thickness was measured at several points with a micrometer. Puncture strength and puncture deformation were determined and expressed in newtons (N) per 100 μm of thickness and in millimeters, respectively.

Water Solubility. The water solubility of the film was measured according to the method of Gontard et al. (1992). It was expressed as a percentage of dry matter of the film solubilized after immersion for 24 h in water at 25 °C under agitation using a magnetic barrel at 250 rpm. The dry matter content of each film, before immersion and after 24 h of immersion (pieces of film not dispersed in water) was determined by drying at 105 °C for 24 h. By subtracting the weight of dry matter not dispersed in water from the weight of initial dry matter of the film, the weight of dry matter dispersed in water after 24 h of immersion was obtained. Solubility was calculated without taking into account the glycerol content in the dry matter of the film (glycerol, which is soluble in water, is automatically exuded from the film during the solubility test).

Table 2. Chemical Composition of Films Obtained from Three Different Cottonseed Flours

material	film composition (% dry basis without glycerol[a]) protein[b]	lipids	carbohydrates	ash	gossypol
glandless flour	44.5	31.1	7,6	1.8	0.00
glanded flour	42.4	29.4	6,2	2.3	0.79
glandless delipidated flour	60.4	1.8	6,5	2.2	0.00

[a] The glycerol content in the dry matter of the film was not taken into account in calculating the nitrogen, lipid, carbohydrate, ash, and gossypol contents. [b] Protein content of the material was calculated using the nitrogen conversion factor of 5.3 given by De Rham (1982).

RESULTS AND DISCUSSION

Film Formation. The conditions to obtain films from cottonseed flour based solutions are difficult to determine because of the complexity of the raw material, which contains proteins, lipids, ash, cellulose, and carbohydrates (Table 1), in contrast to protein isolates that are generally used in this domain. Preliminary experiments were carried out to determine the feasibility range of the films, i.e. best pH (8–12), temperature (20–60 °C), solid/solvent ratio (10–50%), use of dispersive agents, plasticizer content (10–50%, w/w on dry basis).

The compositions of films obtained from cottonseed flour are given in Table 2. The protein and lipid contents of films made from glandless or glanded flour were nearly the same (i.e. protein/lipid ratio close to 1.4) as those of soybean films obtained by the "yuba-like process" (Wu and Bates, 1972b). The main difference between glanded and glandless films was the presence of gossypol in films made from glanded cottonseed flour, a compound that modified the physical and mechanical properties of the film, as discussed below. Films obtained from defatted glandless flour were richer in protein than those obtained from nondefatted flour, and they contained less than 2% lipids.

Table 3 lists the characteristics of films made from cottonseed flour (delipidated or not). Films containing lipids were less resistant than those made from delipidated flour. This could be explained by the protein content, which was lower in lipoprotein films than in films made from delipidated cottonseed flour. Gossypol in glanded flour led to less soluble films. Lipoprotein films made from cottonseed flour were opaque. This could have been due to the lipid distribution within the protein matrix. Lipid drops were immobilized inside the protein network, and some were exuded to the surface of the film, conferring particular surface characteristics. Film color varied from light yellow (in the absence of gossypol) to brown (in the presence of gossypol). Films made from glandless delipidated cottonseed flour were smooth and transparent.

Choice of Bases. As expected from the overall amino acid composition (Bourély, 1990; Lawhon et al., 1977; Harden and Yang, 1975), solubility of cottonseed proteins varied with pH, with a minimum value around pH 5 (Dumay et al., 1986), while solubility increased in the basic pH range (nearly 100% of the protein content

Table 3. Physical Properties of the Films Made from Three Different Cottonseed Flours[a]

	raw material used		
	glandless flour	glanded flour	glandless delipidated flour
protein dispersion in the film-forming solution (%, w/w)[b]	5.9	6.0	6.7
thickness (μm)	124 (8)	73 (8)	106 (12)
puncture strength (N) for a thickness of 100 μm	0.25 (0.01)	0.77 (0.08)	1.77 (0.07)
puncture deformation (mm)	8.7 (0.6)	6.2 (0.2)	9.5 (0.5)
solubility (%, w/w, dry matter)	100	24.5 (0.2)	100

[a] Values are means ($N = 3$) and standard errors. [b] % N × 5.3 (De Rham, 1982).

Table 4. Effect of the Base Used To Prepare the Film-Forming Solution on Protein Dispersion and Mechanical Properties of the Film[a]

	bases used to adjust pH of film-forming solution to 10	
	$NH_4^+OH^-$ [b]	TEA[c]
protein dispersion in film-forming solution (%, w/w)	5.4 (0.02)	5.5 (0.02)
puncture strength (N) for a thickness of 100 μm	0.58 (0.16)	0.77 (0.08)
puncture deformation (mm)	7.7 (1.0)	6.2 (0.2)

[a] Values are means ($N = 3$) and standard errors. The films were made from glanded flour. [b] Ammonia [c] Triethylammonium. [d] % N × 5.3 (De Rham, 1982).

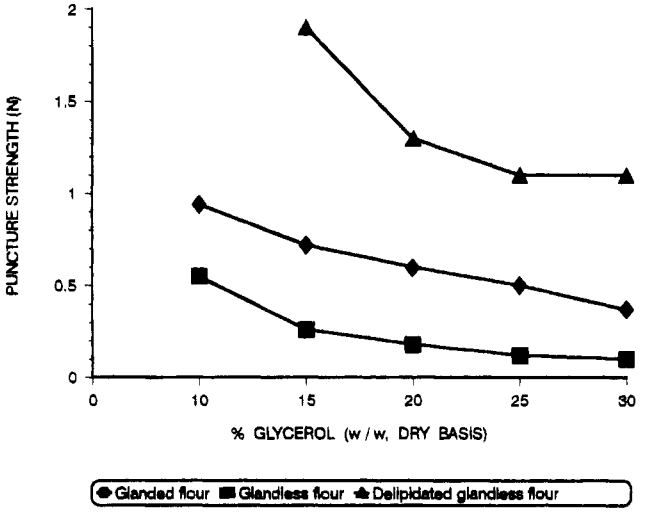

Figure 1. Effect of glycerol content (% w/w, dry matter) on the puncture strength of films made from glandless, glanded, and delipidated glandless flours.

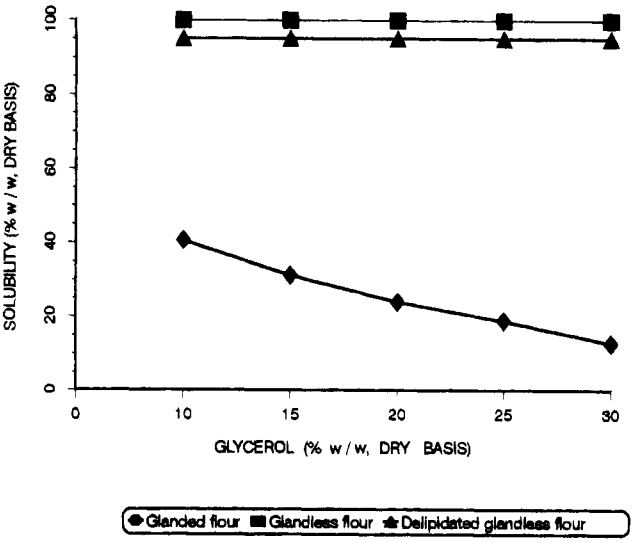

Figure 2. Effect of glycerol content (% w/w, dry matter) on the solubility (% w/w, dry matter without taking into account the glycerol content in the dry matter of the film) of films made from glandless, glanded, and delipidated glandless flours.

was dissolved at pH 12, 80% at pH 10, and only 50% at pH 1). We adjusted the pH of the film-forming solutions to 10 using volatile bases [triethylammonium (TEA) and ammonia] to limit protein desulfuration, which occurs at high pH, and to optimize protein dispersion. These bases evaporated during the film-drying step, resulting in an almost neutral final film pH (pH 6.3) with optimal protein cohesion. The results in Table 4 indicate that the mechanical parameters were not dependent on the type of base used. However, TEA seemed to provide better film homogeneity (visual observation) than ammonia. Furthermore, TEA (a tertiary amine) was not reactive with the chemical agents used in this study and was preferred over ammonia.

Glycerol Effect. Films were excessively brittle when the glycerol content was below 10% (w/w, on dry basis); they become sticky when the glycerol content was higher than 30%. From 10 to 30% (w/w, on dry basis) glycerol, puncture strength decreased rapidly (Figure 1). The presence of glycerol led to a decrease in the intermolecular forces between polymer chains, with a decrease in cohesion tensile strength and glass transition temperature (Guilbert, 1986). Films made from glandless flour (delipidated or not) completely dissolved or dispersed in water within a few minutes, regardless of the glycerol content. Films made from glanded flour were less soluble (Figure 2). Gossypol, present in glanded flour, seemed to be essential for decreasing film solubility. It reacted with available lysine, leading to protein chain cross-links (Lyman et al., 1959; Tchiegang and Bourély, 1990) with lipids entrapped inside a protein structure, thus making the films somewhat waterproof. Lipoprotein glanded films became less soluble as the glycerol content increased (only 12.7% of films were soluble at 30% glycerol content). The total nitrogen contents of lipoprotein glanded films before and after the solubility tests indicated that no proteins or peptides were lost in the solution. The observed solubility was thus due to leaching of carbohydrates, ash, pigments, etc. from the film.

Chemical Treatment of the Film-Forming Solution. *Effect of Gossypol.* In spite of the limited number of observations (Figure 3), it clearly appears that an increase in gossypol content decreased the solubility of the film with a concomitant increase in puncture strength. Puncture deformation (6.5 ± 0.5 mm) did not change. Endogenous gossypol reacts with the proteins, but its presence may be also a drawback for further

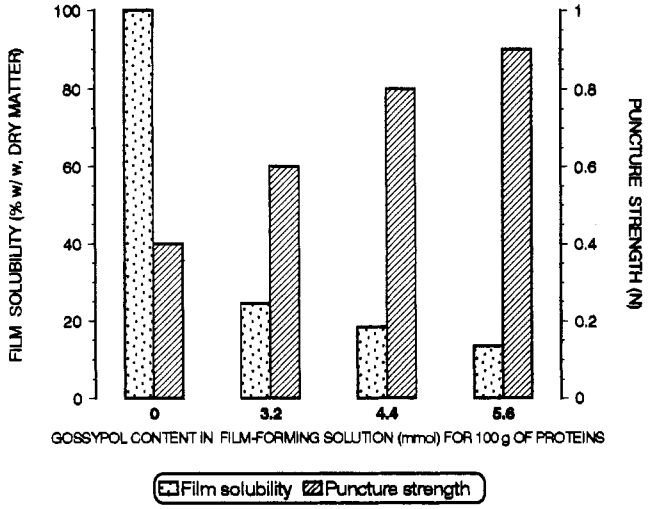

Figure 3. Influence of adding gossypol (4.4 and 6.6 mmol/100 g of proteins) to the film-forming solution on the solubility and puncture strength of films made from glandless flour. Films containing 3.2 mmol of gossypol (for 100 g of protein) were made from glanded flour which naturally contains gossypol.

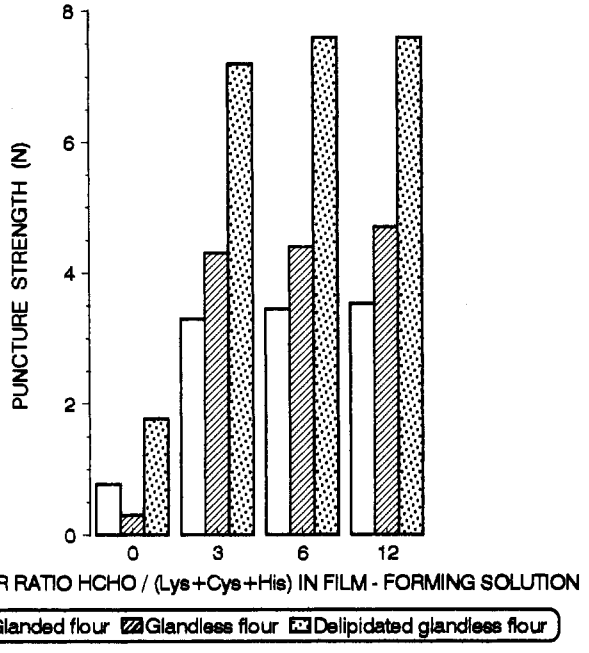

Figure 4. Chemical modifications of cottonseed proteins by formaldehyde as evidenced by the effect on the puncture strength of films made from glandless, glanded, and delipidated glandless cottonseed flours.

chemical modifications targeted to lysine. Cottonseed varieties with high gossypol content will thus be a useful raw material for the preparation of films with good mechanical properties.

Effect of Formaldehyde. The reaction with formaldehyde (HCHO) was conducted with a molar excess of HCHO ranging from 3 to 12 in consideration of the 4 mmol of potentially reactive amino acids (Lys, Cys, and His) contained in 100 g of cottonseed film-forming solution at pH 10. According to Saroso (1989), Bourély (1990), and Marquié (1994), the Lys, Cys, and His contents in 100 g of cottonseed protein are 30, 15, and 20 mmol, respectively. Figures 4 and 5 give the physical properties of films obtained from glandless and glanded cottonseed flours after HCHO treatment. At low modifying agent concentrations, an HCHO/potentially

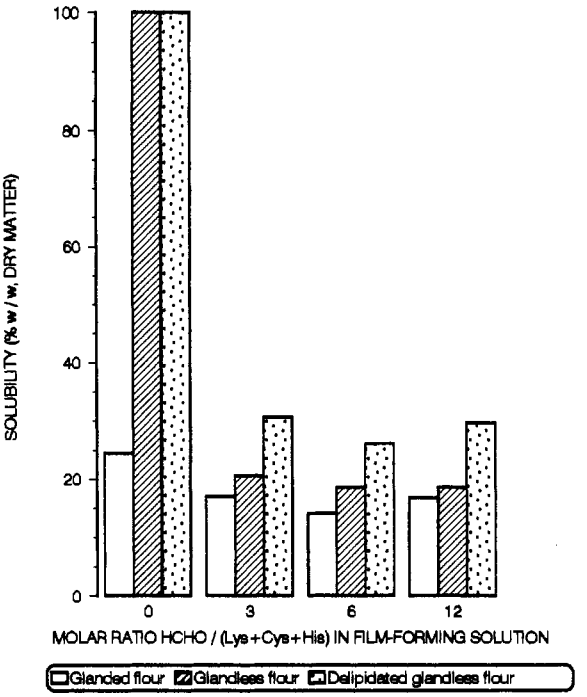

Figure 5. Chemical modifications of cottonseed proteins by formaldehyde as evidenced by the effect on the solubility of films made from glandless, glanded, and delipidated glandless cottonseed flours.

reactive amino acids (Lys, Cys, and His) ratio of 6 seemed to be optimal for enhancing puncture strength and decreasing film solubility. At higher ratio values, the effect was maintained regardless of the type of raw material. After chemical modification, the puncture strength and solubility performances varied for the three different raw materials. The glandless delipidated flour with the highest protein content had the highest score (with a puncture strength of 7.65 N). The puncture deformation of films made with delipidated cottonseed flour increased from 9.5 ± 0.5 to 13 ± 0.4 mm with an HCHO/(Lys, Cys, and His) molar ratio of 3, while it was not changed for films made from glandless and glanded flour (10 ± 1 mm).

Effect of Glutaraldehyde. The predominant reaction product was the conjugated Schiff base with ϵ-amino groups of lysine (Peter and Richard, 1977). As noted for the HCHO-induced chemical modifications, we observed variations in the performances for the three raw materials (Figures 6 and 7). With regard to puncture strength, the optimal glutaraldehyde/lysine molar ratio was close to 6. In contrast, solubility seemed to be minimal at a molar ratio of 3. Puncture deformations of films made from glanded and glandless flours were the same as the results obtained with HCHO treatments.

The effects of glutaraldehyde and formaldehyde on film solubility and puncture strength depended on the raw material source. Chemical treatment by glutaraldehyde produced more resistant films (5.98 ± 0.32 N) with glandless flour than with glanded and delipidated glandless flour (3.78 ± 0.13 N and 3.68 ± 0.10 N, respectively). This could have resulted from a higher available lysine content in the film-forming solution made from glandless flour than in that made from glanded flour (due to the presence of gossypol) and in that made from glandless delipidated flour, in spite of a higher protein content (the oil extraction process decreased the available lysine content, through the

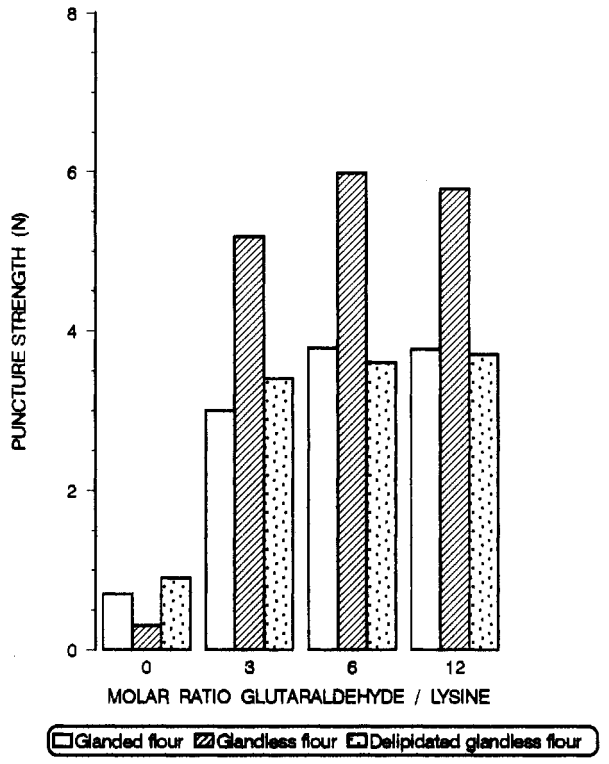

Figure 6. Chemical modifications of cottonseed proteins by glutaraldehyde as evidenced by the effect on the puncture strength of films made from glandless, glanded, and delipidated glandless cottonseed flours.

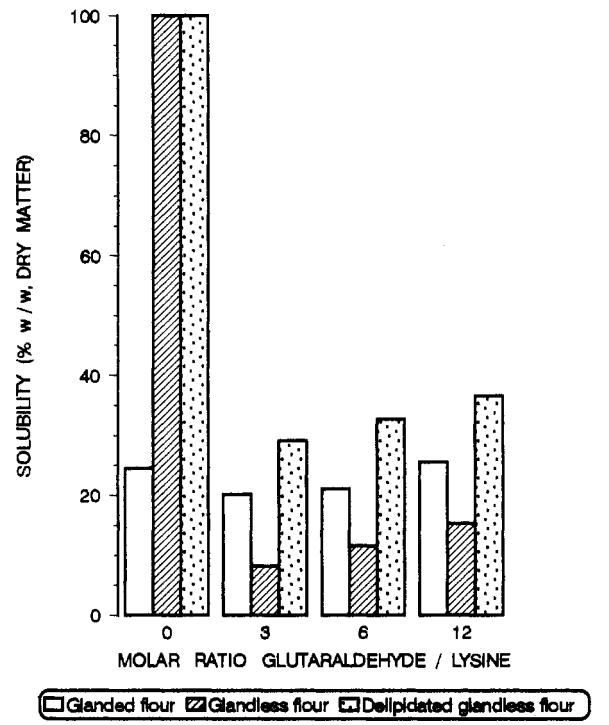

Figure 7. Chemical modifications of cottonseed proteins by glutaraldehyde as evidenced by the effect on the solubility of films made from glandless, glanded, and delipidated glandless cottonseed flours.

Maillard reaction). At pH 10, HCHO reacted with lysine and also with cysteine and histidine (Fraenkel-Conrat and Olcott, 1948; Hopwood, 1969; Myers and Hardman, 1971; Dunlop et al., 1973; Bizzini and Raynaud, 1974; Martin and Lam, 1975; Galembeck et al., 1977). Chemical treatment with HCHO was more effective with glandless delipidated flour (richer in protein than the other raw materials) than with non-delipidated glanded and glandless flours.

In both cases, the worst results were obtained with glanded flour following glutaraldehyde and formaldehyde chemical treatments. We suppose that cross-linking between gossypol and proteins occurred during preparation of the film-forming solution, further decreasing the extent of the reaction between HCHO or glutaraldehyde and the amino groups (by decreasing the mobility and thus the reactivity of the protein chains).

The complex composition of cottonseed is a major difficulty that must be overcome to obtain films from this raw material and to understand the mechanisms of film formation. In this study, we have demonstrated that it is possible to make films from cottonseed flours according to the well-defined process described here. Some of them (made from glanded flour) have low solubility, which is an advantage over most biopackagings made from starch for example. Films made from cottonseed flour (delipidated or not) are not strong enough to be used as packagings, and chemical modification of proteins is required to enhance the mechanical properties. Biodegradability of protein could be affected by chemical treatments, so biodegradability tests are now in progress to characterize the effect of cross-linking.

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