# COTTONSEED PROCESSING INTO BIODEGRADABLE MATERIAL FOR POTENTIAL AGRICULTURAL AND BIOMEDICAL USES

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## Abstract

A process for the preparation of biodegradable materials from cottonseed flour is described. Chemical cross-linking treatments by formaldehyde and glutaraldehyde and/or addition of cotton fibers in films were used to increase tensile strength and decrease water solubility of materials. These materials present potential uses in agricultural and biomedical fields.


## Introduction

With a world production at about 37 MT cottonseed is, after soybean, the most important source of vegetal proteins. Gererally, cottonseed are crushed to extract oil and produce cake that is chiefly used for ruminant animal feed. As they contain gossypol, these products cannot be used for human consumption. Cottonseed production corresponds only to 15% of the commercial value of the crop though it represents more than twice cotton fiber production (Raymond and Marquié, 1995). To optimize this oleoproteaginous product that is the least expensive in the world, the CIRAD Cotton Technology Laboratory (France) is currently studying the film-forming properties of cottonseed proteins, - the ability of proteins to form films- with the aim to produce biodegradable materials of economic and environmental interest.

Proteins are nonmonotonous polymers with a high potential to enhance film structure by forming intermolecular and intramolecular linkages. Functional properties were improved by either choosing low-solubility proteins such as corn zein (Aydt et. al., 1991; Guilbert and Biquet 1989; Park et. al., 1994), wheat gluten (Gennadios and Weller 1990; Gontard 1994), fish myofibrillar proteins (Cuq et. al., 1996a,b) or by chemical cross-linking treatments (Gennadios and Weller 1992; Marquié et. al., 1995,1996). Frequently, a plasticizer such as sorbitol or glycerol is added to the protein film formulation to reduce internal hydrogen bonding and decrease the intermolecular forces along polymer chains (Lieberman and Gilbert 1973), thus imparting increased film flexibility while decreasing maximum film puncture force.

Our studies were carried out with raw cottonseed flours, such as those commonly available in all cotton producing countries. These included glandless or glanded non-delipidated cottonseed flours and an industrial delipidated glandless flour whose protein, lipid and gossypol contents are given in Table 1.

## Discussion

### Techniques for forming biodegradable films from cottonseed flours

Cottonseed protein-based films can be formed from an aqueous solution by a casting process (Marquié et. al., 1995; Marquié 1996a,b) (Figure 1).The first step involves solubilizing cottonseed flour proteins under highly dispersing conditions (solvent, pH, temperature, addition of salts, plasticizers, dissociating agents, etc.) to minimize protein-protein interactions. The change in protein structure prevents the "remelting" of macromolecules and reveals potentially interactive zones. The dispersion is centrifuged to eliminate insoluble substances and to homogenize the film-forming solution. Glycerol is then added to the solution (10-40%, w/w, dry basis). This compound acts as a plasticizer, forming hydrogen bonds with lateral chains of hydrophilic amino acids; it meshes between the macromolecules, increasing the molecular mobility of the protein network and thus its flexibility.

The second step involves drying the film-forming solution to eliminate solvents — this allows the proteins to set up a 3-dimensional network through new inter- and intra-molecular linkages. Film cohesiveness is dependent upon the polymer structure, the film-forming process and various parameters such as temperature, pressure, type of solvent, solvent/solid material ratio (v/w), the film application technique and the presence of plasticizers, bulking agents and crosslinking agents (Gontard and Guilbert 1994; Marquié 1996a).

In view of the amino acid composition of cottonseed proteins, good protein dispersion can be obtained during preparation of the film-forming solution by using water at alkaline pH (adjusted with ammonia or triethylamine) and heating the solution to 20-70°C. The flour/water ratio (w/v) can range from 10% to 40%. The elimination of solvents during drying promotes linkage of protein chains and interactions, thus allowing formation of the film.

Films with different structures and compositions may be obtained with all three cottonseed flours (glandless, glanded and delipidated glandless). Films obtained with delipidated flour are transparent, while those produced from non-delipidated flour are opaque because of the presence of lipids that are spread in fine droplets throughout the protein network. The chemical characteristics of these films are given in Table 2. The lipid and protein contents of films obtained from glandless and glanded flours are almost identical. The main difference between these films is the presence of gossypol in glanded films which, as discussed below, modifies the physical and mechanical properties of

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:470-473 (1997)
National Cotton Council, Memphis TN

films. Low-energy bonds generally form between macromolecules (e.g. hydrogen bonds), along with hydrophobic and ionic interactions. These bonds are destroyed in the presence of water molecules, resulting in unresistant, highly moisture-sensitive and water-soluble films. The gossypol present in glanded flours produces films that are less soluble than those formed with glandless flours.

The materials can be strengthened by using crosslinking agents to produce a chemical modification of proteins during preparation of the film-forming solution. Crosslinking agents are natural or synthetic molecules containing at least two reactive groups that are able to form covalent inter- and/or intra-molecular links between protein chains. These agents, when used to prepare protein-based films, strengthen the material through new covalent bonds, while reducing water solubility and increasing elasticity in films (Gennadios and Weller 1992; Marquié et. al., 1995; Marquié 1996a).

We demonstrated the crosslinking effects of gossypol with cottonseed proteins. Gossypol is oxidized into quinones at alkaline pH and forms Schiff bases with $NH_2$ ϵ groups of lysine. In protein films, the presence of this compound strengthens the protein network while reducing the solubility of the film (Figure 2). We also investigated potential strengthening the protein network through the addition of formaldehyde and glutaraldehyde in the film-forming solution; these two additives are crosslinking agents that react at basic pH in aqueous solutions (Marquié et. al., 1995; Marquié 1996a; Marquié et. al., 1996). Formaldehyde is more effective than glutaraldehyde in increasing the resistance of materials made from cottonseed flours (Figure 3). Moreover, solubility (%, w/w, dry basis) is reduced from 25% to 18% in films made from glanded flour, and from 100% to about 25% in films made from glandless flours (delipidated or not).

We also demonstrated that it is possible to incorporate carded cotton fibres (mean length 20 mm) into the protein network (Marquié 1996a; Marquié et. al., 1996). The behaviour of the resulting films resembles that of bilayer films, supplemented with the properties of each polymer employed (cellulose and protein). Materials whose proteins have been crosslinked by glutaraldehyde, and which contain 8% fibres (w/w, dry basis), are 5-fold more resistant and their solubility ranges from 20% to 30% (w/w, dry basis), depending on the cottonseed flour used. Films may be strengthened with cotton fibres derived from spinning mill carding waste.

## Properties of materials made from cottonseed flours

The film color ranges from yellow (in the absence of gossypol) to dark brown (in the presence of gossypol).

Thanks to their proteic composition, films made from cottonseed flour are very hydrophilic. The water vapor adsorption isotherm is represented by an exponential curve for water activities up to 0.6 (Figure 4).

The mechanical properties of films containing formaldehyde-crosslinked proteins are affected by both temperature and relative humidity (Marquié 1996a). Water acts as a plasticizer, i.e. increasing molecular mobility in the protein network and decreasing the temperature at which the mechanical properties of the materials change (glass transition temperature). Protein crosslinking hinders complete collapse of the protein structure when the material becomes very hydrated (more than 20% w/w). With added moisture, the materials are able to remain cohesive and dilate without dissolving. The tensile strength of films made from cottonseed flour compared with that of synthetic and other biodegradable materials is given in Table 3. Films whose proteins are crosslinked by formaldehyde are 5 to 10 fold less resistant than the other materials. Films including cotton fibers are as mechanically resistant as Novon and Biopol and a little less resistant than polyethylene HD.

Water vapor permeability measured at 20°C is about $8 \times 10^{-12}$ (mole/m.s.Pa) (Marquié 1996a). These barrier properties to water are similar to other protein-based films but 100 fold less than for polyethylene or beeswax considered as impermeable.

Dynamic mechanical thermal analyses (DMTA) of the films demonstrated that these materials could be thermoformed at about room temperature (Marquié 1996a). It would thus be possible to mould them using industrial techniques commonly used for manufacturing synthetic packaging.

A modified version of Sturm's test was used to analyse the biodegradability of the materials in aerobic liquid medium. The results indicated that films made with cottonseed flours are completely biodegradable under these conditions (Marquié 1996a). Protein crosslinking, which only involves a few amino acids and does not affect peptide bonds, therefore does not hinder biodegradation.

## Potential uses of cottonseed protein-based films in agricultural and biomedical fields

Films made from cottonseed flour may be well adapted for use in some very specific applications in non-food packaging (bin tiners, supermarked bags, etc) where good mechanical resistance and insolubility in water are required.

These materials could also be used for agricultural packaging, and as film for mulching, crop protection and seed fixation — applications where the color, porosity and biodegradability of the materials are beneficial. These films, which biodegrade progressively after they have served their initial purpose, could also serve as natural fertilizers.

The textural and adhesive properties of films made from glanded cottonseed flours prompted us to assess their potential use in medical areas where biodegradability is necessary, e.g. for prostheses and resorbable dressings. The results of preliminary *in vivo* studies in rats highlighted the interesting healing properties of these materials. The capacity of these films to absorb considerable amounts of moisture is a further advantage for the absorption of exudates.

Whatever the field in which these materials are used, it should be possible, during film preparation, to introduce active compounds as insecticides, fungicides, bactericides for example, able to migrate outside the material to have a specific action toward the environment. Film hydration, which is accompanied by increased water vapor permeability and diffusion of small molecules, would make these materials suitable vectors for active substances in delected environments.

## Conclusion

Our research demonstrated that it is possible to make biodegradable materials directly from cottonseed using the very easy technology described in this paper. Materials with impressive mechanical properties and low water solubility are obtained using protein crosslinking treatments and/or adding cotton fibers into the film. Their biodegradability and mechanical properties mean they have great potential in agricultural and biomedical fields. For each type of utilization (for agricultural or medical fields) our laboratory is now conducting appropriate research to optimize the properties of these films.

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Table 1. Composition of the cottonseed flours used to prepare the film-forming solution.

| Cottonseed Flours | Crude composition (g/100 g dry basis) Total Nitrogen | Lipids | Free Gossypol |
|---|---|---|---|
| Glandless | 6.5 | 38.8 | 0.002 |
| Glandless delipidated | 9.3 | 2.6 | 0.004 |
| Glanded | 6.2 | 38.7 | 0.530 |

Total nitrogen content was used to calculate the protein content of the cottonseed flour using the 5.3 conversion factors given by De Rham (1982).

Table 2. Chemical composition of films obtained from three different cottonseed flours

| Flour | Film composition (% dry weight basis without glycerol) Prot | Lipids | Carbo-Hydrates | Ash | Gossypol |
|---|---|---|---|---|---|
| Glandless | 44.5 | 31.1 | 7.6 | 1.8 | 0.00 |
| Glanded | 42.4 | 29.4 | 6.2 | 2.3 | 0.79 |
| Glandless delipidated | 60.4 | 1.8 | 6.5 | 2.2 | 0.00 |

Glycerol content in the dry matter of the film was not taken into account when calculating the nitrogen, lipid, carbohydrate, ash and gossypol contents.

Prot. = Protein content calculated using the nitrogen conversion factor of 5.3 given by De Rham (1982).

Table 3. Mechanical properties of various materials

| Material | Tensile Strength (Mpa) | Elongation (%) |
|---|---|---|
| Polypropylene (1) | 38 | 400 |
| Polyethylene HD (1) | 30 | |
| Novon (1) | 8 to 50 | 3 to 300 |
| Biopol (1) | 20 to 40 | 8 to 50 |
| Crosslinked (2) "Cotton" film | about 4 | about 90 |
| Crosslinked (3) + cotton fibers "Cotton" film | about 20 | about 10 |

(1) Fritz et. al., 1994

(2) Films are obtained from glandless delipidated flour using a cross-linking treatment with formaldehyde.

(3) Films are obtained from glandless delipidated flour using a cross-linking treatment with formaldehyde and including cotton fiber.

Mechanical properties were determined using a RHEO.TA.XT2 Rheometer (Champlan, France), operated according to the standard method NF T54-102.

Novon is a biodegradable material marketed by Warner-Lambert Company (USA).

Biopol is a biodegradable material marketed by Imperial Chemical Industries (UK).

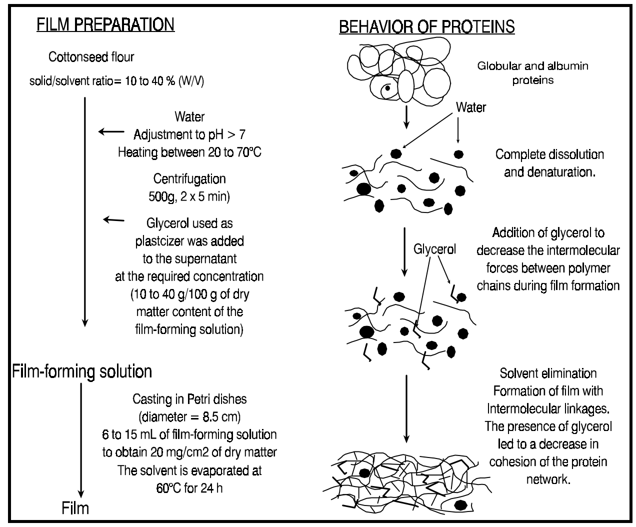


Figure 1. Experimental procedure to make films from cottonseed flours and protein behaviour during the process.

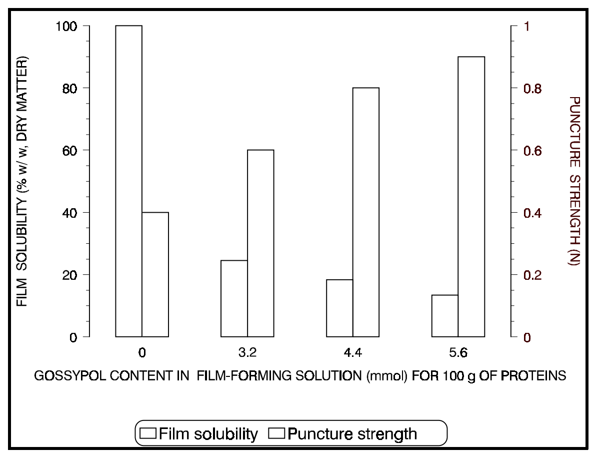


Figure 2. Influence of adding gossypol (4.4 and 6.6 mmol/100 g of proteins) to the film-forming solution on the solubility and puncture strength of films made from glandless flour. Films containing 3.2 mmol of gossypol (for 100 g of proteins) were made from glanded flour which naturally contains gossypol. Puncture strength was evaluated with a RHEO TA.XT2 texturimeter and expressed in Newtons (N) per 100 µm of thickness. Films for measurement were equilibrated in a 56% relative humidity atmosphere. Solubility was measured according to the method described by Marquié et. al., 1995.

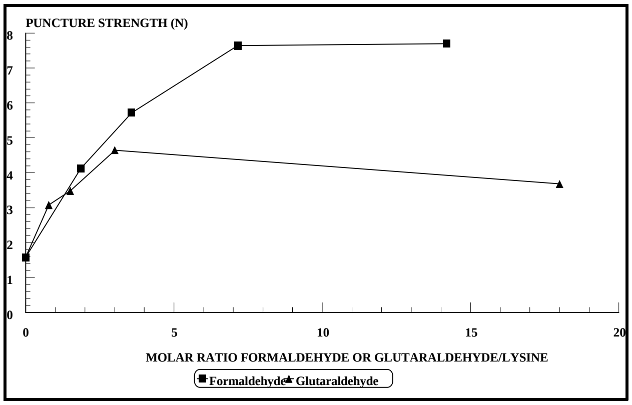


Figure 3. Chemical modifications of cottonseed proteins by formaldehyde and glutaraldehyde as evidenced by the effect on the puncture strength of films made from delipidated glandless flour. Protein cross-linking treatments with formaldehyde (40%, w/v) or 5.6M glutaraldehyde were conducted at room temperature for 1h, up to 20 : 1 molar ratios with reference to reactant lysine in the film-forming solution. Puncture strength was evaluated with a RHEO TA.XT2 texturimeter and expressed in Newtons (N) per 100 µm of thickness. Films for measurement were equilibrated in a 56% relative humidity atmosphere.

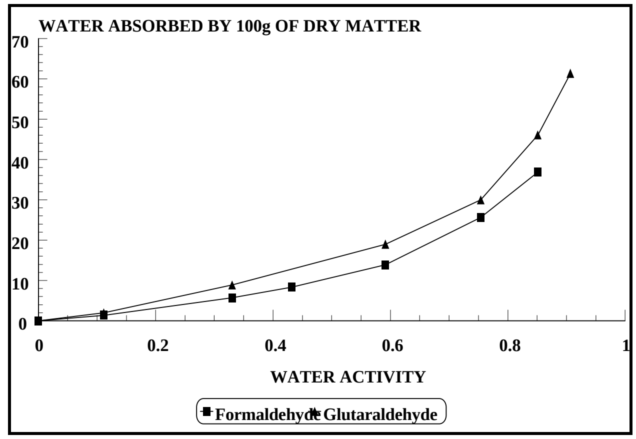


Figure 4. Isotherm for the moisture adsorption of cottonseed protein based films made from a glandless flour at 20 °C. Cottonseed proteins have been crosslinked by formaldehyde and glutaraldehyde.