Biodegradable Films from Isolate of Sunflower (*Helianthus annuus*) Proteins

F. Ayhllon-Meixueiro, C. Vaca-Garcia, and F. Silvestre*

Laboratoire de Chimie Agro-Industrielle, UMR−INRA, ENSCT-INPT, 118 route de Narbonne,
31077 Toulouse Cedex 04, France

The film-forming potential of isolate of sunflower proteins (ISFP) was investigated. Homogeneous films were obtained by dissolution of ISFP in alkaline water (pH 12), addition of a plasticizer, casting, and drying. Maximum protein solubilization and unfolding led to films with the highest elasticity. The effects of five dissolving bases and five plasticizers on the mechanical properties were studied. The use of ionic bases (LiOH, NaOH) capable of interfering with the interproteic noncovalent bonds resulted in the greatest tensile strength (σ_{max}) and elongation at break (ϵ_{max}) values (3.9 MPa and 215−251%, respectively). Plasticizers conferred diverse tensile properties to the films: the use of 1,3-propanediol resulted in the highest σ_{max} (27.1 MPa), and glycerol resulted in the greatest ϵ_{max} (251%). Different mechanical properties were obtained by using mixtures of these plasticizers.

Keywords: *Sunflower; proteins; film; Helianthus annuus; tensile properties*

INTRODUCTION

In recent years, environmental concerns have increased the interest in biodegradable packaging materials. These materials are often formulated with biopolymers of agricultural origin capable of forming a cohesive and continuous matrix. In this connection, most of the efforts were originally devoted to cellulose and starch. Such polysaccharides are of prime interest as biopolymers because of their availability and rather low cost, but the low elasticity of the materials obtained therefrom is an important drawback that limits their application fields.

Increased interest in the use of commercially available proteins for the preparation of biomaterials, especially films, has been observed in the past decade (Gennadios et al., 1993; Sanchez et al., 1998). Some plant proteins show properties that are advantageous in the preparation of packaging biomaterials, for example, ability to form networks, plasticity, and elasticity. Investigations on the film-forming potential of different plant proteins have mainly focused on soy proteins (Brandenburg et al., 1993), wheat gluten (Gontard and Guilbert, 1994), cottonseed protein (Marquie et al., 1995), and proteins extracted from sorghum kafirin, rice bran, peanuts, corn zein, and peas (Guilbert et al., 1997; Cuq et al., 1998).

On the other hand, sunflower oil cake obtained from the oil industry is an inexpensive source of proteins (>30% content) that has been used mostly for animal feed purposes. It has been recently demonstrated that such proteins can be alkali-extracted to yield an isolate composed mainly of globulin and albumin (Leyris, 1998). It was therefore the objective of the present work to study the film-forming potential of sunflower protein isolate to propose an added-value outlet for the sunflower oil cake and to contribute to the development of biodegradable packaging materials. Thus, various film-forming variables, including the solubilization of pro-

teins and the choice of base and plasticizer, were examined to determine their effect on the mechanical properties of the sunflower protein-based films.

EXPERIMENTAL PROCEDURES

Materials. Isolate of sunflower proteins (ISFP) (composition is given in Table 1) was obtained by alkaline extraction of sunflower oil cake (see composition in Table 1) (1 kg) at the pilot scale with aqueous NaOH solution (20 L, pH 12) at 50 °C during 20 min according to our laboratory's method (Leyris, 1998). After centrifugation (500 rpm, 15 min), proteins were precipitated from the supernatant at their isoelectrical point (pH 4.8) by dropwise addition of concentrated H_2SO_4 in a ventilated area. The proteins were separated by centrifugation (500 rpm, 10 min), then dried overnight at 50 °C, and ground into powder with a conventional blender.

Glycerol (99%), 1,3-propanediol, D-sorbitol, triethylene glycol (TEG), tetraethylene glycol (TEEG), lithium hydroxide (LiOH), sodium hydroxide (NaOH), potassium hydroxide (KOH), aqueous ammonia (30%), and triethylamine (TEA) were of reagent grade and were purchased from Aldrich (St. Quentin Fallavier, France). They were used without further treatment.

Film Formation. ISFP (2 g) was dispersed in 20 mL of distilled water. Alkalinity was adjusted to pH 12 by careful addition of powdered NaOH or other bases tested. The dispersions were stirred with a homogenizer (Ultra-turrax T-25, IKA, distributed by Aldrich) at 19000 rpm for 2−30 min. NaOH pellets were avoided as they can be violently projected by the homogenizer. After addition of a plasticizer (0.0109 mol), the mixture was stirred again (19000 rpm, 1 min) and then centrifuged (5000 rpm, 5 min) to remove air bubbles and to separate the insoluble particles.

Solutions were cast in flat polystyrene Petri boxes (Poly Labo, Strasbourg, France), measuring 12 × 12 cm, on a horizontal surface to maintain constant area and uniform thickness (170−200 μm). Films were allowed to dry for 24 h at 25 ± 2 °C, then peeled off from the plates, and conditioned before testing.

Dumbbell-shaped specimens having standardized dimensions (75 × 5.5 mm, ISO 527-2, type 1BA) were cut off from the films. Thickness was measured to the nearest 0.01 mm with a hand-held micrometer (Braive Instruments, Checy, France). The average thickness was calculated from 10 random measurements.

* Corresponding author (telephone +33 5 62885725; fax +33 5 62885730; e-mail fsilvestre@ensct.fr).

Isolate of Sunflower Proteins

J. Agric. Food Chem., Vol. 48, No. 7, 2000 **3033**

Table 1. Characterization of Sunflower Oil Cake and Isolate of Sunflower Proteins[a]

	sunflower oil cake	ISFP	determination method
moisture, %	10 ± 2	6 ± 1	105 °C/24 h oven-drying
ash, %	7.6 ± 0.4	2.4 ± 0.4	incineration (525 °C/5 h)
proteins ($F = 6.25$), %	34.4 ± 1	90 ± 1	Kjeldahl
lipids, %	1 ± 0.5	0.6 ± 0.4	Soxhlet extr (hexane)
cellulose	22.3 ± 2	0	ADF−NDF (Van-
lignin, %	5.2 ± 1	1.7 ± 1	Soest, 1963;Van-
hemicellulose, %	9.8 ± 1	0	Soest and Wine, 1967)
phenolic compounds, %	5.7 ± 0.8	4.8 ± 0.8	UV spectro- photometry (Folin− Ciocalteu reagent)

[a] Average and standard deviation values of three determinations are reported (dry basis).

Plasticizer Content. The residual amount of plasticizer in films was determined by means of precise measurements of the weight loss of substances other than water during the casting of films at room temperature (25 ± 2 °C). Water content was controlled in the film-forming solution and in the cast film by Karl Fischer titration. The observed difference in the weight of dry matter was attributed to plasticizer evaporation. This assumption was successfully verified by HPLC according to the method of Sanchez et al. (1998) for two plasticizers (TEG and TEEG) before and after drying.

Film Conditioning. Before mechanical evaluations, film specimens were conditioned at 25 °C and 60% relative humidity for 24 h according to the European NF EN ISO 291 standard.

Tensile Properties. The mechanical properties were evaluated in a texture analyzer TA-XT2 (RHEO Stable Micro Systems, London, U.K.). Five specimens were used for each film. Stress−strain measurements were used to determine the tensile strength (σ_{max}) and elongation at break (ϵ_{max}) values. The initial grip separation was 55 mm, and the separation speed was 1 mm/s according to standards ISO 527-1 and ISO 527-2.

Surface Hydrophobicity. Static contact angle with water was measured as follows: The film was placed on a flat surface, and a drop of deionized water was placed on it. An image analyzer system (DigiDrop, GBX, Romans-Sur-Isère, France) measured the angle of the tangent to the basis of the drop. The average contact angle was calculated from six measurements along the film.

RESULTS AND DISCUSSION

Protein Solubility and Film Formation. It is known that the optimum pH for film formation varies with the type of protein according to their structure, configuration, and behavior in solution (Okamoto, 1978). In the case of the ISFP, the optimal solubilization of proteins occurs at pH 12 (Leyris, 1998). This value was used to prepare the film-forming solution. In the first experiments, NaOH was utilized to adjust the pH of the solution. The films directly cast from the mother solution were too brittle to be handled. The addition of a plasticizer was therefore indispensable in obtaining a flexible film. Glycerol (0.00545 mol/g of ISFP) was used in all cases except when indicated. The plasticizer/ISFP ratio was decided on the basis of preliminary experiments that were carried out to determine the feasibility range of the films. Results using glycerol as plasticizer set the pattern for other plasticizers; that is, films were excessively brittle when the glycerol content in the film was <16% (w/w, dry basis), and they became sticky when it was >50%. We therefore decided to retain a glycerol/ISFP ratio of 1:2, which corresponds to 33% of glycerol in the film.

First, the process conditions that led to the optimum solubilization of ISFP in the alkaline solvent were
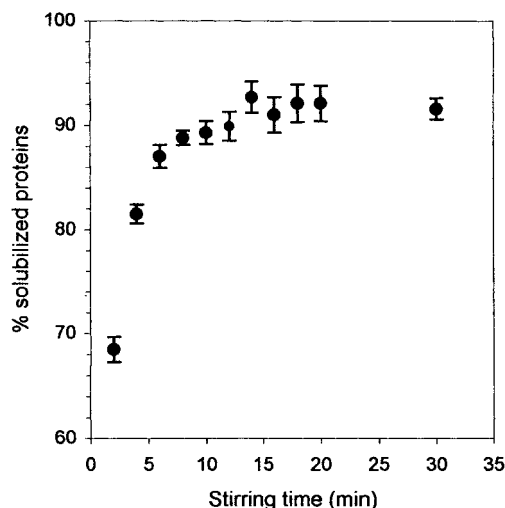
Figure 1. Solubilization of ISFP in NaOH solution (pH 12) as a function of the stirring time of 20 mL of film formation solution in a homogenizer (19000 rpm). Average and standard deviation values of three experiments were plotted.
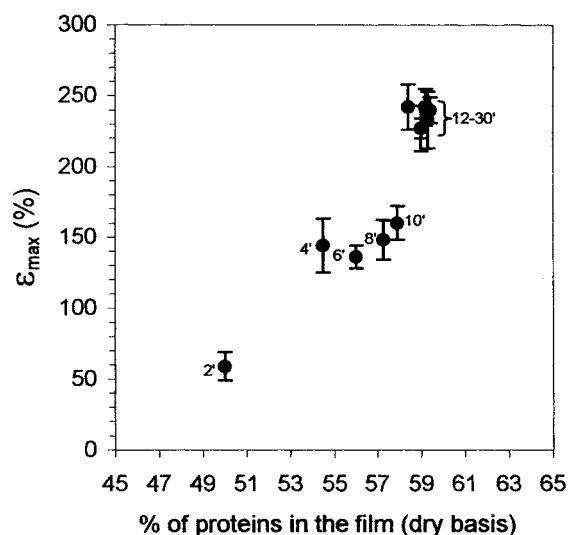
Figure 2. Variation of elongation at break (ϵ_{max}) as a function of protein content in ISFP films obtained by variation of the stirring time [numbers next to points correspond to stirring times (minutes)]. Average and standard deviation values of five experiments were plotted.

investigated. The speed of the stirrer was fixed at 19000 rpm for a volume of 20 mL, and the stirring time was varied. The solubility of ISFP was determined at different stirring times. Weighing and nitrogen content (Kjeldahl determination) analyses were carried out to quantify the total and the insoluble protein fractions. The percentage of ISFP solubilization obtained therefrom is shown in Figure 1.

The percentage of protein solubilization increased rapidly with stirring time from 68% (2 min) to 89% (10 min) and then leveled off after 12 min at ~92% (Figure 1). The films were cast after removal of the insoluble part; therefore, their final protein content on a dry basis varied from 50 to 59%. The mechanical properties of these films presented some characteristics as discussed below.

On the one hand, the variation of the elongation at break increased with protein content in the film (Figure 2). A short stirring time (2 min), which solubilized a relatively low quantity of proteins, led to an ϵ_{max} of only
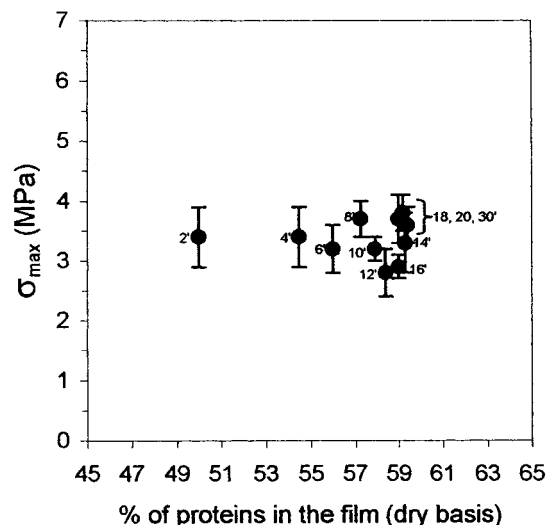
3034 *J. Agric. Food Chem.,* Vol. 48, No. 7, 2000

Ayhllon-Meixueiro et al.

Figure 3. Variation of tensile strength (σ_{max}) as a function of protein content in ISFP films obtained by variation of the stirring time [numbers next to points correspond to stirring times (minutes)]. Average and standard deviation values of five experiments were plotted.

68%. This value increased ~2-fold at a protein content ~55% (stirring time of 4−10 min) ~4-fold when the protein content was ~59% (12 min and up).

On the other hand, the tensile strength values of films showed no significant differences as they remained essentially constant (3.4 ± 0.4 MPa) when the protein content of the film was varied (Figure 3). These surprising findings suggest that the mechanical properties of the ISFP film do not depend only on the protein content but also on the protein unfolding extent. Sunflower proteins are known to possess globular structure (Linden and Lorient, 1994). The unfolding of their structure is necessary to increase the protein−protein interactions, which have a direct repercussion on elasticity. The stirring time is believed to act favorably in this process. Proteins poorly unfolded would behave like the filler of a "composite" material. If they are abundant (at low stirring times), they cause a premature breaking of the matrix (protein network). As proteins are unfolded, they incorporate into the matrix and no composite-like behavior is observed. The film shows then its actual elastic properties. From these findings, a stirring time of 14 min, which leads to 92% of protein solubilization, was chosen as appropriate for the subsequent film formation experiments.

Effect of the Base. The base utilized for the dissolution of the proteins had a significant influence on the properties of the films. The mechanical properties of materials are largely associated with the distribution and the concentrations of *intra*- and *inter*molecular interactions allowed by the chemical structure and "spatial arrangement" of proteins (secondary, tertiary, and quaternary structures). The three-dimensional structures of proteins are stabilized mainly by noncovalent interactions (van der Waals forces, hydrogen bonding, and ionic interactions). In our case, the base, which is not removed from the ISFP, can therefore interfere with such interactions as shown in Figure 4.

Five bases were tested: LiOH, NaOH, KOH, NH$_4$OH, and TEA. All of them permitted the casting of films with good mechanical properties (Table 2). In all cases, the base was added in sufficient quantity to obtain a pH value of 12. The use of strong ionic bases resulted in
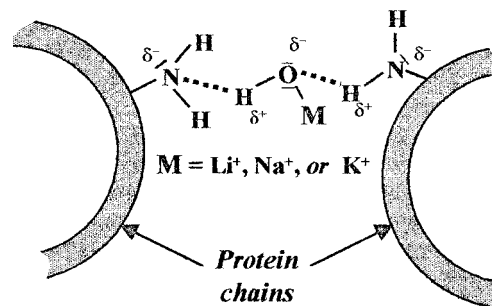

Figure 4. Schematic representation of noncovalent interaction forces between protein chains and ionic bases.

Table 2. Mechanical Properties of ISFP Films Formed with Different Bases at pH 12[a]

base	tensile strength σ_{max}, MPa	elongation at break ϵ_{max}, %
LiOH	3.9 ± 0.1	215 ± 14
NaOH	3.9 ± 0.1	251 ± 12
KOH	2.6 ± 0.1	229 ± 16
TEA	1.8 ± 0.1	137 ± 9
NH$_4$OH	1.3 ± 0.05	86 ± 3

[a] Glycerol/ISFP = 0.00545 mol/g. Average and standard deviation values of five experiments.

higher σ_{max} and ϵ_{max} values than ammonia or TEA. Among the former, those with the smaller cation (LiOH and NaOH) offered the best results. It is supposed that such alkalis may interpose easily between protein chains, creating bridges that stabilize and strengthen the network (Figure 4). On the contrary, a bulky cation finds steric hindrance; ionic or van der Waals interactions cannot be readily formed. This hypothesis seems invalid for ammonia as it might form H-bond bridges, too; however, its mechanical properties were the lowest. This could be accounted for by the fact that this base is too volatile to remain in the final network after the drying of the film. In the case of TEA, this base is able to form H-bonding only with one NH$_2$ group of the protein chain; therefore, bridges cannot be formed. Moreover, its volatility is also high, and as a consequence, it showed poor mechanical properties.

Effect of Plasticizer. The addition of a plasticizer in the film-forming solution was indispensable. Without plasticizer, the protein network was too brittle to be handled. The protein−protein interactions, which determine the properties of the film, can be varied by the presence of a plasticizer. The lubricity theory of the plasticization mechanism accounts for this fact: the plasticizer acts as a lubricant to facilitate the movements of the protein chains over each other, avoiding thus brittleness. In the previous experiments, the plasticizer used was glycerol (0.50 g/g of ISFP). We investigated the effect of other plasticizers of polyol type on the mechanical properties and hydrophobicity of films. All of the plasticizers were added to ISFP on the same molar basis (0.00545 mol/g of ISFP). After removal of the insoluble proteins, the plasticizer content in the film-forming solution was controlled again as described under Experimental Procedures. It was verified that films contained comparable amounts of proteins (weight basis) and residual plasticizer (molar basis). The eventual loss of plasticizer in films during the drying step (25 °C) was also controlled. As shown in Table 3, the loss of plasticizer can be considered to be negligible. It is therefore possible to compare the mechanical properties of these films.

Isolate of Sunflower Proteins

J. Agric. Food Chem., Vol. 48, No. 7, 2000 **3035**

Table 3. Composition and Surface Hydrophobicity of ISFP Films Cast with Different Plasticizers[a]

plasticizer	protein content (dry basis), g	plasticizer content before film drying, mmol	residual plasticizer after drying, mmol	loss of plasticizer over film drying at ±25 °C, %	contact angle with water, deg
glycerol	1.671 ± 0.003	9.9 ± 0.4	9.7 ± 0.1	2.2 ± 0.3	29 ± 2
1,3-propanediol	1.669 ± 0.004	9.7 ± 0.5	9.2 ± 0.4	5.5 ± 0.2	12 ± 2
D-sorbitol	1.662 ± 0.005	10.0 ± 0.2	9.4 ± 0.3	6.5 ± 0.1	13 ± 1
triethylene glycol	1.662 ± 0.005	9.9 ± 0.3	9.5 ± 0.2	4.9 ± 0.3	18 ± 3
tetraethylene glycol	1.667 ± 0.004	10.0 ± 0.4	9.4 ± 0.2	6.4 ± 0.1	30 ± 3

[a] Average and standard deviation values of six experiments.
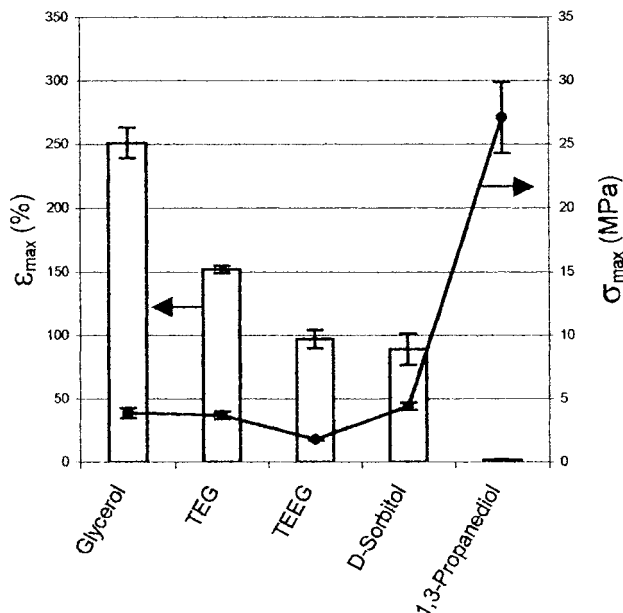

Figure 5. Effect of plasticizer type on tensile strength (σ_{max}) and elongation at break (ϵ_{max}) of ISFP films. Plasticizer/ISFP = 0.00545 mol/g. Average and standard deviation values of five experiments were plotted.

Figure 6. Mechanical properties of ISFP films cast with mixtures of glycerol and 1,3-propanediol. Total plasticizer/ISFP = 0.00545 mol/g. Average and standard deviation values of five experiments were plotted.

Protein films are known to possess hydrophilic character, and the ISFP films were not excluded from this attribute. The surface properties of films prepared with different plasticizers are shown in Table 3. The contact angle values were essentially the same as those obtained for pea protein films (Gueguen et al., 1998).

The tensile strength and the elongation of films cast with different plasticizers are shown in Figure 5. The σ_{max} values varied between 1.8 and 27.1 MPa, and the ϵ_{max} values ranged between 1.6 and 251%. Films cast with 1,3-propanediol presented the highest σ_{max}, which is at least 14 times that of the film cast with TEEG (the smallest value). The σ_{max} values of the other plasticizers (glycerol, D-sorbitol, and TEG) were comparable among them.

In the case of glycerol, ISFP films were more elastic and resistant (σ_{max} = 3.9 MPa and ϵ_{max} = 251%) than films obtained from isolate of soy proteins (3.3 MPa and 100%, respectively; Krochta, 1997) at comparable contents of plasticizer [37% w/w versus 35% (dry basis) in our case].

It was observed that 1,3-propanediol yielded the film with the highest tensile strength but with the poorest elasticity. Conversely, glycerol conferred the highest elasticity associated with moderate strength. It was therefore interesting to perform combinations of these two plasticizers, in different percentage blends, to seek a compromise between tensile strength and elasticity (Figure 6).

When the percentage of 1,3-propanediol in the mixture of plasticizers was increased from 0 to 75%, the
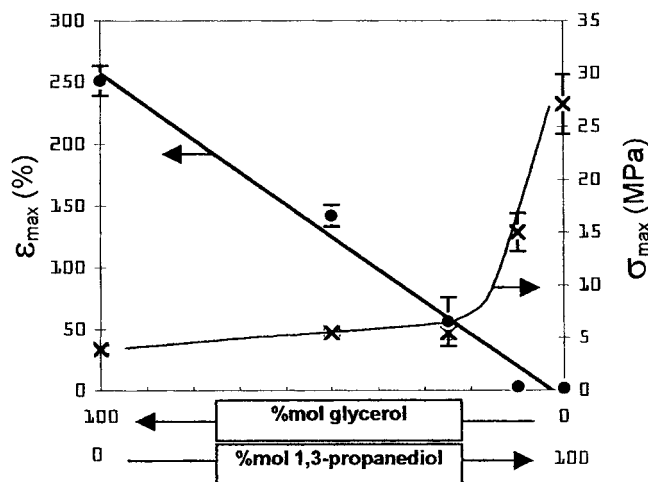
σ_{max} values increased only slightly, but in the range 75–100%, the total increase was remarkable (~600%). On the other hand, ϵ_{max} values decreased linearly in the whole range. In an equimolar mixture, an increase of ~40% in tensile strength (σ_{max} = 5.5 MPa) was obtained with a loss of about half of the elasticity (ϵ_{max} = 142%).

Conclusion. This study demonstrates that it is possible to prepare films from sunflower proteins by an alkaline casting process. The base utilized for the dissolution of the proteins had a large influence on the properties of the films. Particularly, ionic bases capable of interfering with the interproteic noncovalent bonds increased tensile strength and elasticity. The drying of films at room temperature permitted comparable amounts of residual plasticizer on the films formed to be obtained. Plasticizers conferred diverse tensile properties to the films: the use of 1,3-propanediol resulted in the highest σ_{max}, and glycerol resulted in the greatest ϵ_{max}. Mixtures of these plasticizers do not improve both mechanical properties at the same time. Nevertheless, different mechanical properties were obtained by these means. These results open new outlets for the exploitation of sunflower residues.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; ISFP, isolate of sunflower proteins; TEA, triethylamine; TEG, triethylene glycol; TEEG, tetraethylene glycol; σ_{max}, tensile strength; ϵ_{max}, elongation at break.

LITERATURE CITED

Brandenburg, A. H.; Weller, C. L.; Testin, R. F. Edible films and coatings from soy proteins. *J. Food Sci.* **1993**, *58*, 1086–1089.

Cuq, B.; Gontard, N.; Guilbert, S. Proteins as agricultural polymers for packaging production. *Cereal Chem.* **1998**, *75*, 1−9.

Gennadios, A.; Brandenburg, A. H.; Weller, C. L.; Testin, R. F. Effect of pH on properties of wheat gluten and soy protein isolate films. *J. Agric. Food Chem.* **1993**, *41*, 1835−1839.

Gontard, N.; Guilbert, S. Edible wheat gluten films: Optimization of the main process variables and improvement of water vapor barrier properties by combining gluten proteins with lipids. *Dev. Food Eng. Proc.* **1994**, *2*, 790−792.

Gueguen, J.; Viroben, G.; Noireaux, P.; Subirade, M. Influence of plasticizers on the properties of films from pea proteins. *Ind. Crops Prod.* **1998**, *7*, 149−157.

Guilbert, S.; Cuq, B.; Gontard, N. Recent innovations in edible and/or biodegradable packaging materials. *Food Addit. Contam.* **1997**, *14*, 741−751.

ISO Standard. Plastics—Determination of tensile properties—Part 2: Test conditions for moulding and extrusion plastics. Reference ISO 527-2, 1993(F).

ISO Standard. Plastics—Determination of tensile properties—Part 1: General principles. Reference EN ISO 527-1, 1996(F).

ISO Standard. Plastics—Standards atmospheres for conditioning and testing. Reference EN ISO 291, 1997(F).

Krochta, J. M. Edible protein films and coatings. In *Food Proteins and their Applications*; Damodaran, Paraf, Eds.; Dekker: Paris, France, 1997; pp 529−550.

Leyris, J. Valorisation du tourteau de tournesol: Etude, procedé et modélisation de l'extraction des protéines—Exploitation de leur propriétés fonctionnelles en vue de l'obtention d'agromateriaux. Ph.D. Thesis, INP Toulouse, France, 1998.

Linden, G.; Lorient, D. Propriétés Technofonctionnelles. In *Biochimie Agro-Industrielle*; Masson: Paris, France, 1994; pp 67−75.

Marquie, C.; Aymard, C.; Cuq, J. L.; Guilbert, S. Biodegradable packaging made from cottonseed flour: formation and improvement by chemical treatments with gossypol, formaldehyde, and glutaraldehyde. *J. Agric. Food Chem.* **1995**, *43*, 2762−2767.

Okamoto, S. Factors affecting protein film formation. *Cereal Foods World* **1978**, *23*, 256−262.

Sanchez, A. C.; Popineau, Y.; Mangavel, C.; Larré, C.; Gueguen, J. Effect of different plasticizers on the mechanical and surface properties of wheat gliadin films. *J. Agric. Food Chem.* **1998**, *46*, 4539−4544.

Van-Soest, P. J. Use of detergents in the analysis of fibrous feeds II. A rapid method for the determination of fiber and lignin. *J. Assoc. Off. Anal. Chem.* **1963**, *46*, 829−835.

Van-Soest, P. J.; Wine, R. H. Use of detergents in the analysis of fibrous feeds IV. Determination of plant cell-wall constituents. *J. Assoc. Off. Anal. Chem.* **1967**, *50*, 50−55.