

Proteins as Agricultural Polymers for Packaging Production

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ABSTRACT

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Advantages, types, formation, and properties of agricultural packaging materials based on proteins, with examples, are reviewed in detail. Proteins have long and empirically been used to make biodegradable, renewable, and edible packaging materials. Numerous cereal and vegetable proteins (such as corn zein, wheat gluten, and soy proteins) and animal proteins (such as milk proteins, collagen, gelatin, keratin, and myofibrillar proteins) are commonly used to form agricultural packaging materials. Two technological processes have been investigated to make materials

based on proteins: the "wet (or solvent) process" based on dispersion or solubilization of proteins in a solvent medium, and the "dry process" based on the thermoplastic properties of proteins under low water content conditions. 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 cross-linking agents.

For many years, various raw materials from agricultural resources have been used to produce renewable, biodegradable, and edible packaging, often called agricultural materials or agro-packaging materials. The agro-packaging concept is generally associated with use of renewable products and control of the end uses (Guilbert 1992). Among agricultural products, proteins have long and empirically been used as packaging materials (e.g., traditional lipoprotein skins in Asia and collagen sausage casings). Other examples are buttons that were made from caseins treated with formaldehyde, and soy proteins that were mixed with phenol-formaldehyde resins and used by Henry Ford to produce automobile body parts (Jane et al 1994). In addition, unlike homopolymers constituted of one repeated monomer (e.g., polysaccharides), proteins are heteropolymers characterized by specific structures that permit various interactions and bindings differing in position, type, and energy (Guilbert and Graille 1994). Generally, agro-packaging based on proteins is characterized by remarkable functional properties (Guilbert and Biquet 1989, Gennadios et al 1994, Torres 1994, Cuq et al 1995b, Guilbert et al 1996).

PROTEINS AS AGRICULTURAL POLYMERS

Until recently, the only uses and applications of proteins were in food sciences. The development of studies on nonfood uses of agricultural raw materials, and more particularly on the proteins needed, use concepts and tools classically developed in the field of polymer science, which considers relationships between macromolecular structure and function. The tools of polymer science applied to proteins attempt to: 1) study three-dimensional organization structure at different observation scales (atomic, molecular, and supramolecular organization); 2) understand the evolution as a function of temperature or functional additive incorporation; and 3) predict macromolecular network macroscopic properties (mechanical, optical, thermal, and electrical). Proteins can thus be defined as natural polymers able to form amorphous three-dimensional structures stabilized mainly by noncovalent interactions. The functional properties of these materials are highly dependent on structural heterogeneity, thermal sensitivity, and hydrophilic behavior of proteins. Numerous vegetable and animal proteins are commonly used as raw material for agro-packaging materials.

Corn Zein Proteins

Film-forming properties of corn zein have been extensively studied (Cosler 1957, Kanig and Goodman 1962, Takenaka et al 1967, Tryhnew et al 1973, Guilbert 1988, Park and Chinnan 1990, Aydt et al 1991, Herald et al 1996). The films obtained by drying of alcoholic aqueous dispersions are not water-soluble but are relatively brilliant and grease-resistant. Films and coatings based on zein are, for instance, used to preserve fresh food, to retain enriching vitamins, and for controlled release of medically active compounds. These films are used to protect dry or dried fruits and frozen or intermediate-moisture foods. Aqueous dispersions of zein are commercially available (e.g., Optaglaze from Opta Food Ingredients, Inc.). Starch and zein mixtures have been investigated for manufacturing of biodegradable plastics (Jane et al 1994). Injection-molded plastics display high water sensitivity, while cross-linked materials produce plastics with reduced water absorption and high mechanical properties.

Wheat Gluten Proteins

Numerous studies refer to the film-forming properties of wheat gluten proteins (Noznick and Tatter 1967, Wall and Beckwith 1969, Krull and Inglett 1971, Anker et al 1972, Gennadios and Weller 1990, Park and Chinnan 1990, Aydt et al 1991, Gontard et al 1992, 1993, 1994, 1995, 1996). Wheat gluten-based films are traditionally obtained by casting in a thin layer and then drying of aqueous alcoholic proteic solutions (in acidic or basic conditions) in the presence of disruptive agents such as sulfite. Wheat gluten-based films have also been produced by collecting the skin formed after boiling protein solutions (Watanabe and Okamoto 1973) or by wheat gluten extrusion in the presence of disruptive agents (Guilbert and Gontard 1995). These films based on wheat gluten are not water-soluble, and their properties and applications are similar to those of zein films. However, they could cause problems for gluten-intolerant persons (those with celiac disease). The wheat gluten films have been used to encapsulate additives, improve quality of cereal products, and retain antimicrobial or antioxidant additives on food surfaces (Redl et al 1996). Moreover, the remarkable gas barrier properties (oxygen and carbon dioxide) of these materials due to their exceptional gas selectivity could help preserve fresh and minimally processed vegetables (Gontard et al 1996; C. Barron et al, unpublished). In addition, molded biodegradable plastics were produced from wheat proteins and cereal flours (Jane et al 1994). Many nonfood uses could be expected from gluten proteins because of its thermoplastic behavior and the possibilities for chemical modifications (changes in cross-link density, modified hydrophobicity rate, or compatibility with synthetic materials). The potential applications of gluten are

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very diverse: windows in envelopes, surface coatings on paper, biodegradable plastic films for agricultural uses, water-soluble bags with fertilizers, detergents, cosmetics, cigarette filters and additives (Guilbert and Gontard 1995, Bietz and Lookhart 1996), and molded objects.

Soy Proteins

The film-forming properties of soy proteins have been traditionally used in Asia to obtain edible films by collecting the lipoproteic skin formed after boiling soya milk (e.g., *yuba* in Japan) (Burnett 1951, Fukushima and Van Buren 1970; Smith and Circle 1972; Wu and Bates 1972a,b; Bates and Wu 1975; Guo 1983; Snyder and Kwon 1987). Proteins are the main constituents of these films, but significant quantities of polysaccharides (sucrose, raffinose, and stachyose) and lipids (globules included in the proteic network) are also present. These films have good mechanical properties but they are generally slightly water-resistant. Films have also been obtained from soy protein isolates prepared in aqueous alcoholic solutions, either by collecting the lipoproteic skin formed after boiling soya solutions (Circle et al 1964, Wu and Bates 1973, Okamoto 1978) or by casting solutions in thin layers and drying (Jaynes and Chou 1975, Guilbert 1988, Brandenburg et al 1993, Gennadios et al 1993b, Stuchell and Krochta 1994). Soy films have often been developed as coating materials to help preserve of numerous foods (Cole 1969, Baker et al 1972). Biodegradable plastics were also produced from soy isolate and concentrate by a thermomolding process (Jane et al 1994).

Peanuts and Cottonseed Proteins

Films and water-soluble bags have been fabricated from peanut proteins by collecting the lipoproteic skin formed after boiling peanut milk, in a manner similar to film formation on soy milk surface (Wu and Bates 1973, Okamoto 1978, Aboagye and Stanley 1985). Biodegradable films based on cottonseed proteins, obtained from a film-forming solution treated with various cross-linking agents, have been studied and developed by Marquié et al (1995, 1997).

Milk Proteins

The film-forming properties of milk proteins have been indirectly demonstrated by films formed on processing equipment surfaces and at air-water interfaces during heating of nonfat milk (Leach et al 1966, Wu and Bates 1973, Mabesa et al 1979). Caseins dispersed in aqueous solutions can form transparent, flexible, and tasteless films. Covalent cross-links catalyzed by transglu-

taminases or peroxidases could be formed to improve water resistance or to allow immobilization of active enzymes (e.g., β -galactosidase, α -mannosidase) (Motoki et al 1987a,b). Film-forming properties of caseins have been used to improve the appearance of numerous foods, to produce water-soluble bags, and to produce origin or quality identification labels inserted under precut cheeses, to ensure the surface retention of additives on intermediate-moisture foods, and to encapsulate polyunsaturated lipids for animal feeds (Noznick and Bundus 1967, Cole 1969, Tryhnew et al 1973, Ashes et al 1984, Guilbert 1988, Avena-Bustillos et al 1994).

The film-forming properties of whey proteins have been used to produce transparent, flexible, colorless, and odorless films, such as those produced from caseins. Mahmoud and Savello (1992, 1993) have also produced films through enzymatic polymerization of whey proteins using transglutaminases. Films have also been obtained after collecting the lipoproteic skin formed after boiling heated whey dispersions (Wu and Bates 1973, McHugh et al 1994). Water insolubility of films based on whey proteins is partly due to the network stabilization by disulfide covalent bonds.

Collagen and Gelatin

Collagen is traditionally used in the meat industry to form edible coatings through extrusion (Courts 1977, Hood 1987). Materials based on collagen have also been developed for medical uses (Cavallaro et al 1994). Gelatin has been successfully used to form films that are transparent, flexible, water-resistant, and impermeable to oxygen (Bradbury and Martin 1952, Grouber 1983, Guilbert 1988, Hebert and Holloway 1992). These films were made by cooling and drying an aqueous film-forming solution based on gelatin. Gelatin films are commonly used in the pharmaceutical industry to fabricate tablets and capsules. Gelatin is also used as a raw material for photographic films, and to microencapsulate aromas, vitamins, and sweeteners (Balassa and Fanger 1971). Gelatin films were also tested to protect frozen meats against oxidation (Klose et al 1952), but the protective effect was limited unless antioxidants were incorporated into these films.

Keratin

Anker et al (1972) developed water-insoluble films based on keratin by casting and drying alkaline dispersions. The large amount of cystine in keratin favors formation of many disulfide bonds that could stabilize the proteic network (Okamoto 1978, Gennadios et al 1993c). However, because of their unpleasant mouthfeel, edible coatings based on keratin have not found many applications (Daniels 1973).

Egg Albumin Proteins

Use of egg albumins to encapsulate organic hydrophobic compounds in cosmetics and foods has been proposed in many patents (Soloway 1964, Noznick and Bundus 1967, Kosar and Atkins 1968, Baker et al 1972). Edible coatings based on egg albumins reduced moisture loss from raisins in breakfast cereals and improved retention of additives to food surfaces (Durst 1969, Bolin 1976). Okamoto (1978) reported film formation at air-water interfaces during heating of egg albumin, which was similar to that for soy films. Mechanical and water vapor barrier properties of films based on egg albumin proteins have been studied by Gennadios et al (1996). Films appeared clearer and more transparent than films based on wheat, soy, or corn proteins. Possible applications include water-soluble bags meant for conditioning and protecting additive doses for applications in pharmaceutical or food industries.

Myofibrillar Proteins

Recent studies have shown film-forming properties of fish myofibrillar proteins (Cuq et al 1995a, 1996a-c, 1997a,b). The films obtained from an aqueous solution are water-insoluble, perfectly transparent, and have good mechanical (close to that of polyethylene films) and gas barrier properties (Gontard et al 1996). The

TABLE I
Main Proteins Used as Polymeric Materials
to Form Agro-Packaging Materials

Proteins	Tested Methods to Obtain Films			
	Film-Forming Solution ^a	Collect Skin ^b	Enzymatic Treatment ^c	Thermoplastic Extrusion
Corn zein	+			
Wheat gluten	+	+		+
Soy proteins	+	+		
Peanut proteins		+		
Cottonseed proteins	+			
Keratin	+			
Collagen				+
Gelatin	+			
Caseins	+	+	+	
Whey proteins	+	+	+	
Egg albumin proteins	+	+		
Myofibrillar proteins	+			+

^a Casting in thin layer and drying of a film-forming solution.

^b Collect skin formed after boiling protein solution.

^c Enzymatic polymerization.

thermoplastic properties of myofibrillar proteins (Cuq et al 1997c,d) suggest industrial production of these films by the traditional processes usually applied to thermoplastic synthetic polymers (e.g., extrusion, thermomolding).

Table I lists the main proteins tested as polymeric materials to form films or coatings, along with their fabrication methods. Other proteins have been also used for films, including proteins from rye, pea, barley, sorghum, rice, silk, fish, and serum albumin (Okamoto 1978, Torres 1994, Viroben et al 1994, Shih 1996).

FORMING PACKAGING MATERIAL FROM PROTEINS

Protein-based materials consist of continuous, low-moisture, and more or less ordered macromolecular networks. Interactions among proteins need to be numerous and uniform. The probability of forming intermolecular bonds depends on protein shape (i.e., chain length/diameter ratio) and on conditions during fabrication. High molecular weight proteins (e.g., glutenin) and fibrous proteins (e.g., collagen, glutenin) generally have interesting film-forming properties and form films with good mechanical properties (Guilbert and Graille 1994), while globular or pseudoglobular proteins (e.g., gliadin, glycinin, casein) usually need to unfold before network formation. At present, however, we still can not realistically predict functional properties of proteins from their primary structures (Krull and Wall 1969).

Formation of a macromolecular network from proteins requires three steps: 1) rupture of low-energy intermolecular bonds that stabilize polymers in the native state, 2) arrangement and orientation of polymer chains (shaping), and 3) formation of a three-dimensional network stabilized by new interactions and bonds after the agent that ruptured intermolecular bonds is removed. Two technological processes are used to make materials based on proteins: a wet process based on dispersion or solubilization of proteins, and a dry process based on the thermoplastic properties of proteins under low water conditions (Fig. 1).

Wet Process

Formation of materials from a protein solution or dispersion has been studied extensively (Kester and Fennema 1986, Guilbert and Biquet 1989, Donhowe and Fennema 1994). This process under controlled laboratory conditions, requires formation of a thin layer of protein solution. Thus, it is often described as a casting or continuous spreading method. Protein solubility, as defined by Osborne in 1924, varies greatly (Table II); no general procedure to solubilize proteins for these applications exists. However, it is interesting to know the types and proportions of interactions that need to be broken to solubilize the proteins (Chou and Morr 1979).

For instance, intermolecular disulfide bonds in keratin must be disrupted to obtain homogeneous solutions (Fraser et al 1972). Similarly, the low solubility in water of wheat gluten has been attributed to the low content of ionized polar amino acids (14.0%), to numerous hydrophobic interactions between nonpolar amino acids (39.6%), and to the presence of covalent disulfide bonds (Reiners et al 1973). The water-insolubility of zein could also be due to its high content of nonpolar amino acids (46.6%) (Wall and Paulis 1978, Shewry and Mifflin 1985). Moreover, physicochemical properties and characteristics of proteins in aqueous solvent systems are dependent on pH. Sensitivity of proteins to pH change is usually associated with a high content of ionized polar amino acids (Table II). For instance, zein and keratin films form over a wide pH range because they have low ionized amino acids contents (10 and 10.7%, respectively), and thus are not sensitive to pH changes (Okamoto 1978). On the other hand, high contents of ionized polar amino acids in soy proteins (25.4%) limit film formation at low pH (Sian and Ishak 1990). Film formation is based on separation of proteins from the solvent phase by precipitation or phase changes due to: 1) changes in solvent conditions (polarity or pH changes, electrolyte additions); 2) thermal treatments

(heating); or 3) solvent removal (drying). Film formation by solvent removal is due to increased polymer concentration in the medium, inducing bonds and forming a three-dimensional network. Film formation by collecting the lipoprotein skin formed after boiling heated protein dispersions is due to polymerization of unfolded proteins by heating and solvent removal (Fukushima and Van Buren 1970, Gennadios et al 1994). This process is called simple coacervation when only one macromolecule is involved. Most protein-based agro-packaging materials have been fabricated by this process. In complex coacervation, however, at least two oppositely charged macromolecules are combined to yield an insoluble mixed polymer by the mechanism of charge neutralization. Association of proteins and chitosans is one example of a potentially interesting method of film formation.

The solvents used to prepare protein film-forming solutions are generally based on water and ethanol, and occasionally acetone. However, dispersing proteins in solvents may require addition of disruptive agents (mercaptoethanol, sodium sulfite, cysteine, sodium borohydride, *N*-ethylmaleimide), pH adjustment by addition of acids (lactic, hypochloric, or acetic) or bases (ammonium, sodium, or potassium hydroxides, triethylamine), or ionic strength control by electrolyte addition. The functional properties of agro-packaging materials obtained by the wet process depend on protein concentration in solution, pH, additives, solvent polarity, drying rate, and temperature (Gontard et al 1992, Donhowe and Fennema 1993a, Gennadios et al 1993a).

Dry Process

The thermoplastic behavior of proteins has been studied and used to make agro-packaging materials by thermal or thermomechanical processes under low-moisture conditions (i.e., the dry process), similar to those for thermoplastic starch-based materials (Savary et al 1993, Cuq et al 1997d). Thermoplastic properties of proteins were defined in relation to the glass transition theory, which explains textural changes during thermoplastic polymer processing (Van Krevelan 1976). The glass transition characterizes changes from a metastable glassy state to an unsteady rubbery state, at a specific glass transition temperature. Glass transition induces variations of material physical properties, and more particularly of thermal and mechanical properties. The molecular response of a glassy material as a system transforming from a metastable glassy state to an unsteady rubbery state corresponds to a general increase in disorder, free volume, and mobility of macromolecules (Ferry 1980, Cherian and Chinachoti 1996). The glass transition phenomenon is affected by macromolecule characteristics, such as flexibility, size, length of chains, size and polarity of lateral groups,

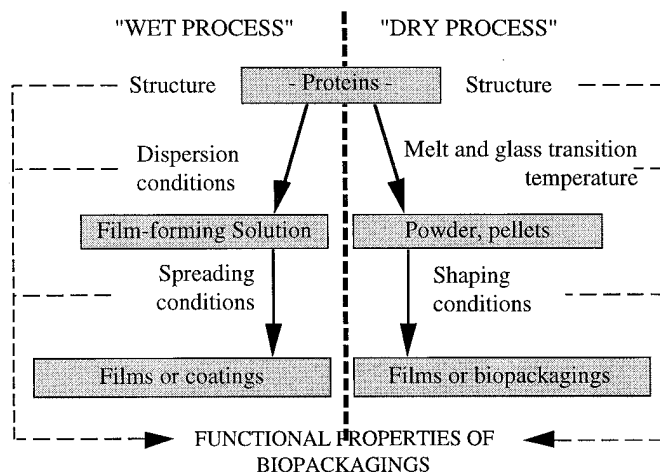


Fig. 1. Schematic representation of the two technological processes used to form agro-packaging materials based on proteins.

molecular weight, presence of intermolecular covalent bonds or crystal organizations, and by presence and content of plasticizers (Slade and Levine 1993a, Cherian et al 1995). For instance, the glass transition temperature of wheat gluten is greatly affected by water content as illustrated in Fig. 2.

According to Oudet (1994), thermoplastic polymers are macromolecules where lateral cohesion is only ensured by low-energy interactions sensitive to plasticizers and temperature changes. The glass transition behavior of proteins depends on type and density of intermolecular interactions. Proteins could thus be considered as partially thermoplastic polymers that could be changed in a reversible way from a rigid state to a soft state through a temperature increase or plasticizer addition. Plasticization (by water or polyhydroxy compounds) is critical for the interaction of proteins to form a continuous network from powdered raw materials. For instance, thermoplastic properties of collagen applied for film fabrication by extrusion are due to the lack of covalent intermolecular bonds in molecules.

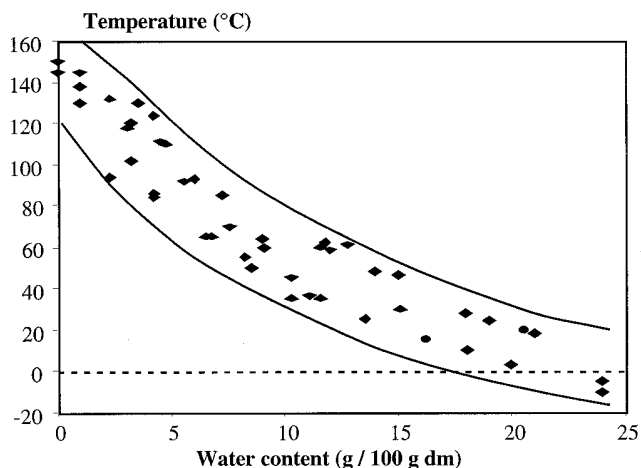


Fig. 2. Effect of water content on the glass transition temperature of wheat gluten proteins. Measurements were obtained using differential scanning calorimetry (DSC) and DMTA.

Protein complexity is partially responsible for large differences in properties of materials formed by thermal or thermomechanical treatments under low-water conditions. Molecular organization and structural characteristics of proteins are probably responsible of the differences noted in thermomechanical properties when compared with common thermoplastic synthetic polymers. Polydispersity, heterogeneity of intermolecular interactions within proteins, and probable presence of chain entanglements in proteins and formation of some covalent cross-links could be responsible for the high rubbery storage modulus, the lack of flow region, and the glass transition breadth observed for various proteins (Kokini et al 1995, Noel et al 1995, Madeka and Kokini 1996). Understanding the physicochemical factors that are involved remains difficult because we lack knowledge of modifications in proteins above the glass transition at low-water content (Mitchell et al 1994).

Heating amorphous thermoplastic agro-polymers above the glass transition temperature produces soft and rubbery materials, and may permit their incorporation into specific products such as packaging materials (Fig. 3). Cooling to room temperature can reconvert rubbery products to glassy materials, giving more or less rigid forms with the desired structure (Savary et al 1993, Oudet 1994, Cuq et al 1997d).

Protein-based material can be shaped by extrusion, roller milling, or thermomolding (traditionally called thermoplastic processing technologies). These methods are adapted to synthetic materials for which shaping properties are fully known and fabrication parameters are optimized. To transform proteins, we must consider typical behavior, including relative thermal, mechanical, and chemical sensitivity, and high viscosities of the soft materials above the glass transition temperature. Process parameters such as temperature, plasticizer concentration, and residence time must thus be optimized for these natural raw materials.

Polymers able to form intermolecular covalent bonds could, however, give insoluble and cross-linked networks with thermomechanical behaviors close to those of elastomers or thermosetting polymers (Oudet 1994). Elastomers are polymers with low-energy intermolecular secondary interactions and some covalent bonds ($\approx 1/100$ repeated units), giving a stable three-dimensional structure. Thermosetting polymers are highly cross-linked polymers (10–100 times more so than elastomers), formed through thermal

TABLE II
Main Physicochemical Characteristics of Proteins^a

Proteins	Amino Acid Ratios (mol/100 mol) ^b			Name	Main Subunits			Citation
	A	B	C		W_R	Mol. Wt. (kDa)	S^c	
Corn zein	36.3	10.0	46.6	α -Zein	80	21–25	IV	Sosulski and Imafidon (1990)
Wheat gluten	39.4	14.0	39.6	Gliadin	40	30–80	IV	Sosulski and Imafidon (1990)
				Glutenin	46	200–2,000	III	Sosulski and Imafidon (1990)
Soy proteins	30.8	25.4	36.4	β -Conglycinin	35	185	II	Boldwell and Hopkins (1985)
				Glycinin	40	363	II	Boldwell and Hopkins (1985)
Peanut proteins	30.5	26.7	32.5	Arachin	75	330	II	Boldwell and Hopkins (1985)
Cottonseed proteins	41.0	23.2	31.8	Albumin	30	10–25	I	Defromnt (1985)
				Globulin	60	113–180	II	Defromnt (1985)
Keratin	33.9	10.7	41.7	10	III	Fraser et al (1972)
Collagen	12.6	13.3	40.2	Tropocollagen	...	300	III	Veis (1964)
Gelatin (type A)	12.0	14.1	41.3	3–200	III	Rose (1987)
Caseins	31.5	20.3	44.5	α_{S1} , α_{S2} , β , κ , g	...	19–25	...	Sosulski and Imafidon (1990)
Whey proteins	30.2	26.3	40.4	β -Lactoglobulin	60	18	I	Vanaman et al (1970), Braunitzer et al (1972)
				α -Lactalbumin	20	14	I	Vanaman et al (1970), Braunitzer et al (1972)
Myofibrillar proteins								
Sardine	27.5	31.3	35.5	Myosin	50	16–200	II	Orban et al (1992)
Beef meat	26.6	26.8	38.7	Actin	50	42	II	Sosulski and Imafidon (1990)

^a Mol. Wt. = molecular weight and W_R = rate of subunit weight (%) in raw materials. Amidation rates of 50% for aspartic and glutamic acids are supposed for peanut proteins, keratin, collagen, and gelatin.

^b Amino acid ratios: A, nonionized polar (Asn, Cys, Gln, His, Ser, Thr, Tyr); B, ionized polar (Arg, Asp, Glu, Lys); and C, nonpolar (Ala, Ile, Leu, Met, Phe, Pro, Trp, Val).

^c S = solubility of proteins according to Osborne (1924): I, in water; II, in diluted salt solutions; III, in diluted acidic or basic solutions; and IV in ethanol (80%) solutions.

^d Miscellaneous associations.

treatments, cross-linking agents, or radiation (UV, gamma). For instance, collagen films made by extrusion could be cross-linked by chemical processes and then behave as insoluble materials, meaning they are incapable of being melted, such as elastomers or thermosetting polymers. In this way, fabrication of wheat gluten films by thermoplastic extrusion could be expected only in the presence of agents that break intermolecular disulfide bonds that stabilize native proteins. After extrusion and removal of disruptive agents, covalent bonds reform and materials behave as elastomers or thermosetting polymers.

Shaping Agents

Plasticizer agents. In general, material formulation requires addition of plasticizer agents. A plasticizer is a small molecule of low volatility which, when added to polymeric materials, modifies the three-dimensional organization, decreases attractive intermolecular forces, and increases free volume and chain mobility (Banker 1966, Bakker 1986). As a result of these changes in molecular organization, addition of plasticizers modifies the functional properties of films by increasing extensibility, distensibility, and flexibility, and by decreasing cohesion, elasticity, mechanical resistance, and rigidity (Lieberman and Gilbert 1973, Donhowe and Fennema 1993b, Gennadios et al 1993d, Gontard et al 1993, McHugh and Krochta 1994b, Park et al 1994, Cuq et al 1997b). With the exception of water molecules that could be considered as the natural plasticizer of most hydrocolloid-based films, the most usual plasticizers are polyols and mono-, di-, and oligosaccharides. It is also interesting to note that the addition of plasticizers results in a decrease of the glass transition temperature of amorphous materials, often necessary for forming materials using a thermomolding process (Donhowe and Fennema 1993b; Slade and Levine 1993a,b; Cherian et al 1995; Gontard and Ring 1996).

Cross-linking agents. These are traditionally used to improve water resistance, cohesion, rigidity, mechanical strength, and barrier properties of materials, although they reduce transparency (Szyperski and Gibbons 1963; Kumins 1965; Lieberman and Gilbert 1973; Guilbert 1986, 1988; Osawa and Walsh 1993; Marquié et al 1995). Functional properties of films based on caseins have thus been highly improved by calcium addition (Guilbert 1986). Commonly used covalent cross-linking agents are glutaraldehyde, glyceraldehyde, formaldehyde, gossypol, tannic acid, and lactic acid. Enzymatic cross-linking by transglutaminases or peroxidases can also stabilize films based on proteins (Motoki et al 1987a). However, food use of films made with such cross-linking agents is highly questionable.

TABLE III
Mechanical Properties of Various Films

Film	Tensile Strength (MPa)	Elongation (%)	Thickness (mm)	Temp. (°C)	rh (%)
Cellulose acetate ^a	65.6	30
Low-density polyethylene ^a	12.9	500
Methylcellulose ^b	56.1	18.5	...	25	50
Myofibrillar proteins ^c	17.1	22.7	0.034	25	57
Whey protein isolate ^d	13.9	30.8	...	23	50
Soy protein, pH 3 ^e	1.9	35.6	0.088	25	50
Wheat gluten proteins, pH 3 ^e	0.9	260	0.088	25	50
Corn zein proteins ^f	0.4	...	0.081	26	50

^a Briston (1988).

^b Park et al (1993).

^c Cuq et al (1995a).

^d McHugh and Krochta (1994).

^e Gennadios et al (1993a).

^f Aydt et al (1991).

PROPERTIES OF AGRO-PACKAGING MATERIALS BASED ON PROTEINS

The macroscopic properties of agro-packaging materials based on proteins and macromolecular three-dimensional networks are largely dependent on interactions between polymers. Water-solubility of protein-based materials depends on type and density of intermolecular interactions in the network. Intermolecular bonds in water-soluble materials are probably of lower energy than interactions between free water and polar groups not involved in a network. The presence of physical knots (i.e., chain entanglements), covalent intermolecular bonds, or numerous interactions can lead to films that have little or no water solubility (Fukushima and Van Buren 1970). For instance, intermolecular covalent cross-links in films based on wheat gluten or keratin make them insoluble.

The mechanical properties of materials are largely associated with distribution and concentration of inter- and intramolecular interactions allowed by the primary and spatial structures. Cohesion of protein-based materials depends mainly on type and density of intra- and intermolecular interactions, but also results from interactions with other constituents. For instance in soy films, hydrophobic interactions between proteins and lipids play a major role in network stability (Farnum et al 1976). Cooperative phenomena generally allowed optimal thermodynamic stability of systems. The effect of interactions depends on interaction probabilities and interaction energies. The mechanical properties of films depend on major interactions that may stabilize networks. From a simplistic point of view, when covalent bonds stabilize a network, or when density of bond energy is high, films are very resistant and relatively elastic (e.g., films based on keratin). On the other hand, when most interprotein interactions are of low energy, films are easily distended (Table III).

Substantial gas- and moisture-barrier properties of protein-based films are required for many applications, such as controlling gas exchange for fresh or oxidizable foods or reducing moisture exchange with the external atmosphere (Cuq et al 1995b; C. Barron et al, unpublished). Water vapor permeability of some protein-based films are given in Table IV. Due to their relatively low water vapor barrier properties, protein-based films can only be used as protective barrier layers to limit moisture exchange for short-term applications or in low-moisture foods.

The O₂ and CO₂ permeability of some biopolymer-based and synthetic films are given in Table V. Protein-based films have impressive gas barrier properties when they are not moist, especially against O₂. For instance, O₂ permeability of wheat gluten film was 800× lower than low-density polyethylene and 2× lower

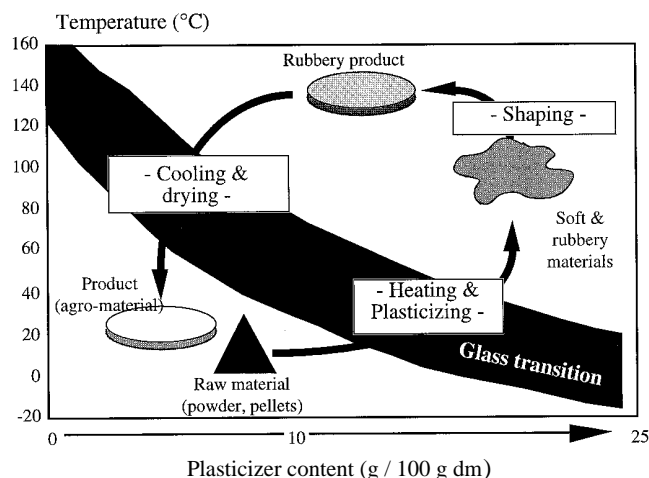


Fig. 3. Schematic representation of the thermoplastic process applied to shaping agro-packaging materials based on wheat gluten proteins in relation to the glass transition temperature.

TABLE IV
Water Vapor Permeability of Various Films

Film	Water Vapor Permeability (10^{-12} mol·m·m ⁻² ·sec ⁻¹ ·Pa ⁻¹)	Temp. (°C)	Thickness (mm)	rh (%)	Citation
Starch	142	38	1.190	100–30	Allen et al (1963)
Sodium caseinate	24.7	25	...	100–0	Avena-Bustillos and Krochta (1993)
Corn zein	6.45	21	0.200	85–0	Park and Chinnan (1990)
Hydroxypropyl methylcellulose	5.96	27	0.019	85–0	Hagenmaier and Shaw (1990)
Glycerol monostearate	5.85	21	1.750	100–75	Landman et al (1960)
Wheat gluten and glycerol	5.08	30	0.050	100–0	Gontard et al (1993)
Wheat gluten and oleic acid	4.15	30	0.050	100–0	Gontard et al (1994)
Fish myofibrillar proteins	3.91	25	0.060	100–0	Cuq et al (1995a)
Wheat gluten and carnauba wax	3.90	30	0.050	11–0	Gontard et al (1994)
Low density polyethylene	0.0482	38	0.025	95–0	Myers et al (1961)
Wheat gluten and beeswax bilayer	0.0230	30	0.090	100–0	Gontard et al (1995)
Aluminium foil	0.000289	38	0.025	95–0	Myers et al (1961)

TABLE V
Oxygen and Carbon Dioxide Permeability of Various Films

Film	Permeability (10^{-18} mol·m·m ⁻² ·sec ⁻¹ ·Pa ⁻¹)		Temp. (°C)	<i>a_w</i>	Citation
	O ₂	CO ₂			
Low-density polyethylene	1,003	4,220	23	0	Bakker (1986)
Ethylene-vinyl alcohol	0.2	...	23	0	Poyet (1993)
Methylcellulose	522	29,900	30	0	Park and Chinnan (1990)
Beeswax	480	...	25	0	Donhowe and Fennema (1993a)
Corn zein	35	216	38	0	Aydt et al (1991)
Soy protein	2	...	23	0	Gennadios et al (1990)
Wheat gluten	1	7	25	0	Gontard et al (1995)
Fish myofibrillar proteins	1	9	25	0	Gontard et al (1995)
High-density polyethylene	224	...	23	1	Ashley (1985)
Ethylene-vinyl alcohol	6	...	23	0.95	Salame (1986)
Pectin	1,340	21,300	25	0.96	Gontard et al (1995)
Wheat gluten	1,290	36,700	25	0.95	Gontard et al (1995)
Starch	1,085	--	25	1	Allen et al (1963)
Fish myofibrillar proteins	873	11,100	25	0.93	Gontard et al (1995)

than polyamide 6, a well-known high O₂ barrier polymer. When moisture is present, the macromolecule chains become more mobile, which leads to a substantial increase in O₂ and CO₂ permeability. The development of packaging or edible films with selective gas permeability could be very promising for controlling respiratory exchange and improving the conservation of fresh or minimally processed fruits and vegetables (C. Barron et al, unpublished).

The barrier properties of materials based on proteins are dependent on network type and density, and more particularly on the ratio and distribution of nonpolar to polar amino acids (Guilbert and Graille 1994). If protein composition and network structural organization allow some groups to be free in films, these may permit interactions with permeating molecules. In general, most free hydrophilic groups of proteins favor sorption and water vapor transfer, rather than hydrophobic gas transfer (e.g., nitrogen, oxygen).

It seems possible that edible films based on proteins might enhance the nutritional quality of foods (Krochta 1992). However, the amount of protein in such films is not significant. Also, although edible films and coatings are eaten at the same time as the foods they protect, no extensive study has been done of their digestibility. In fact, film fabrication could induce large changes in conformation, sensitivity to enzymatic hydrolysis, and digestibility of proteins (Swaigood and Catignani 1991). Bates and Wu (1975) found a protein efficiency ratio of 1.26 for soy protein in lipoproteic films, in contrast to 1.8–2.6 for native proteins. On the other hand, Garcia-Rodenas et al (1994) showed that sensitivity of films based on casein or wheat gluten to *in vitro* enzymatic proteolysis was not significantly different than that of the native proteins.

CONCLUSIONS

Use of proteins as agro-polymers for packaging, coating, and film fabrication suggests many applications in the food, agricultural, pharmaceutical, and medical industries. Indeed, the specific functional properties (including mechanical, barrier, and optical properties) of these agro-packaging materials could be exploited for many applications. Products could be formed from these proteins using traditional shaping methods, such as thermoplastic processing technologies (extrusion, roller milling, or thermomolding) and solvent processes (such as spreading, bed fluidizing, or dipping with subsequent drying). In addition, proteins (e.g., wheat gluten and corn gluten meal) are inexpensive, widely available, and relatively easy to handle.

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