Selected Functional Properties of Fish Myofibrillar Protein-Based Films As Affected by Hydrophilic Plasticizers

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Myofibrillar protein-based films were developed from a film-forming solution based on fish mince. Glycerol, sorbitol, or sucrose was incorporated as plasticizer at various concentrations. The increase in film solubility in water with plasticizer content reflect plasticizer solubility in water. Solubility in water of protein network is not significantly affected by plasticizer concentration whatever the plasticizer. On the other hand, plasticization of myofibrillar protein-based films induced large decreases in film strength and elasticity and increases in deformation properties and water vapor permeability. No significant difference was observed in variations of functional properties as a function of plasticizer type when plasticizers were introduced at the same molecular contents, due to the structural similarities between glycerol, sorbitol, and sucrose.

Keywords: *Biopackaging; myofibrillar proteins; plasticizer; functional properties*

INTRODUCTION

Formation, production, and properties of hydrocolloid-based films have been comprehensively reviewed by Gontard and Guilbert (1994), Krochta *et al.* (1994), and Cuq *et al.* (1995a). Film formulation generally consists of at least two major components: a high molecular weight film-forming polymer (*e.g.* polysaccharides or proteins) and a plasticizer. Generally, plasticizers are required to be used in protein films at a minimal content to avoid brittleness; that is, so the films can be handled. For instance, soy protein films could only be handled if >17 g of glycerol/100 g of dry matter was introduced in the formulation (Stuchell and Krochta, 1994).

A plasticizer has been defined as a small, low-volatile molecule of a chemical nature similar to that of the film-forming polymer. The addition of plasticizers in polymeric materials leads to modifications in the molecular tridimensional organization, decrease in attractive intermolecular forces, and increases in free volume and chain mobility (Banker, 1966; Bakker, 1986). The addition of plasticizers also results in a decrease of the glass transition temperature of amorphous materials (Slade and Levine, 1993a,b; Donhowe and Fennema, 1993; Cherian *et al.*, 1995; Gontard and Ring, 1996).

As a result of these changes in molecular organization, the addition of plasticizers entails modifications in functional properties of films. Several authors have studied effects of type (*i.e.* shape, polarity, length of chains, and number of hydroxylic groups of plasticizers) and concentration of a few hydrophilic plasticizers on properties of various hydrocolloid-based films (Lieberman and Gilbert, 1973; Donhowe and Fennema, 1993; Gennadios *et al.*, 1993; Gontard *et al.*, 1993; McHugh and Krochta, 1994; Park *et al.*, 1994; Cherian *et al.*, 1995). The highest plasticizing effects are generally observed when the smallest and the most hydrophilic plasticizers are used. When large amounts of plasticizers are introduced in film formulation, significant changes in film properties were observed, such as increases in extensibility, distensibility and flexibility and decreases in cohesion, elasticity, mechanical resistance, and rigidity. Large amounts of plasticizers facilitate diffusion of permeant molecules and have also negative effects on the barrier properties of films.

With the exception of water molecules, which could be considered the "natural" plasticizer of most hydrocolloid-based films, the most usual plasticizers are polyols, mono-, di-, and oligosaccharides. Polyols have been found to be particularly effective to plasticize hydrophilic polymers; glycerol was thus nearly systematically incorporated in most of the hydrocolloid films. Ethylene glycol, propylene glycol, triethylene glycol, polyethylene glycol at various molecular weights (from 200 to 20 000), sorbitol, 1,4-butanediol, lactic acid and salt derivatives, triethanolamine, and sucrose were also tested as plasticizers.

In previous investigations (Cuq *et al.*, 1995b), myofibrillar protein-based films were developed. Mechanical and barrier properties were then characterized for a "standard" film containing 35 g of glycerol/100 g of dry matter. The objective of our current study was to investigate the plasticizing effect of three low molecular weight solutes, usually used as plasticizing agent in protein-based films (glycerol and sorbitol) or as cryoprotective agents in fish minces (sorbitol and sucrose), on functional properties of these films.

MATERIALS AND METHODS

Preparation of Fish Mince. Washed fish mince was prepared from Atlantic sardines (*Sardina pilchardus*) following a method proposed by Cuq *et al.* (1995b). Gutted and headed fish were passed through a meat bone separator (drum with 3 mm diameter perforations). The fish mince was washed twice with water, strained in a rotary rinser, passed through a refiner and a screw press, and chopped in a cutter. The fish

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mince was then vacuum packed in polyethylene bags (500 g) and kept at −23 °C for a maximum of 1 month. The samples were thawed for 24 h at 4 °C before experiments.

Preparation of Myofibrillar Protein-Based Films. Transparent and easily handled films were prepared from a film-forming solution based on fish mince in distilled water and acetic acid. Protein concentration (2.0 g/100 g of solution) and pH (3.0, adjusted with acetic acid) were adjusted according to previous experiments (Cuq *et al.*, 1995b). Glycerol, sorbitol, or sucrose (Merck, Darmstadt, Germany) was added as plasticizer at the following contents: 0, 0.11, 0.22, 0.33, and 0.44 mol/100 g of protein. All components were mixed at 25 °C (3000 rpm) in a vacuum thermoregulated homogenizer (Stephan UM5, Marne la Vallée, France) to obtain the film-forming solution. The film-forming solutions were stored for 6 h at 25 °C before casting on a PVC plate using a thin-layer chromatography spreader to obtain films of 2.6 mg of protein/cm^2. The thin film-forming solution layer was dried in a ventilated oven at 25 °C and 50% relative humidity for 15 h. Films were equilibrated at 59.1% relative humidity and 20 °C for 48 h before testing.

Characterization of Myofibrillar Protein-Based Films. Thickness was measured with a hand-held micrometer (Braive Instruments, Checy, France) with 7.5 mm diameter contact faces, to the nearest 1 μm. Thickness values are means of 10 measurements.

The film solubility in water was defined by the content of dry matter solubilized after a 24 h immersion in water. The initial dry matter content of each film was determined by drying to constant weight in an oven at 104 °C. Two disks of film (2 cm diameter) were cut, weighed, and immersed in 50 mL of water containing sodium azide (0.02% w/v) to prevent microorganism growth. After 24 h of immersion at 20 °C with occasional agitation, the pieces of films were taken out and dried to constant weight in an oven at 104 °C, to determine the weight of dry matter which was not solubilized in water.

Mechanical properties were determined using an SMS TAXT2 Rheometer (Champlan, France). Films were cut into 80 mm diameter disks and fixed in an annular ring clamp (34 mm diameter). A cylindrical probe (3 mm diameter) was displaced perpendicularly to the film surface at constant speed (0.5 mm s^{-1}). From the probe displacement, the film deformation was calculated as follows

$$L(t) = \sqrt{D(t)^2 + {R_0}^2} - R_0 \qquad (1)$$

where $D(t)$ is the probe displacement (mm), $L(t)$ is the film deformation (mm), R_0 is the initial film length (15.5 mm), and t is the time (s). Force−deformation curves were plotted until the probe passed through the film. Force (N) and deformation (mm) at break were determined at the break point (values are means of six measurements). The viscoelastic properties of the films were determined by fitting the experimental force−deformation data (Cuq *et al.*, 1996). Two parameters were thus calculated: K (N m^{-1}) is the apparent elastic modulus (an indicator of film stiffness and reversibility of interactions occuring in films) and η (N·s m^{-1}) is the apparent viscosity coefficient (an indicator of interaction density in films). A rheological model was proposed by Cuq *et al.* (1996) to calculate these parameters from force−deformation data and irrespective of film thickness:

$$F(L) = X\left\{\alpha KL + (1-\alpha)\eta V\left[1 - \exp\left(-\frac{KL}{\eta V}\right)\right]\right\} \qquad (2)$$

In eq 2 F is the force (N), L is the deformation (m), V is the deformation rate (m s^{-1}), X is an adimensional coefficient equal to the film thickness (expressed in μm), and α is a model parameter (from 0 to 1). The two parameters (K and η) were calculated by an optimization procedure according to the Gauss−Newton algorithm (Trigeassou, 1988). The minimized objective function was the sum of squared residues.

Water vapor barrier property tests were conducted using a modified version of ASTM Standard Test Method E 96-80 (ASTM, 1989). The film was sealed in a glass permeation cell containing silica gel (0% relative humidity). There was a 5 mm (±1 mm) air space between the surface of the desiccant and the underside of film samples. The cells were stored at 20 °C in desiccators with distilled water. After steady-state conditions were reached (48 h was generally sufficient), the cells were weighed at 24 h intervals (over a 5-day period). Water vapor permeability (mol·m·m^{-2} s^{-1} Pa^{-1}) was calculated as follows (values are means of six measurements):

$$\text{water vapor permeability} = \frac{wx}{At(p_2 - p_1)} \qquad (3)$$

In eq 3 A is the area of exposed film (m^2), $(p_2 - p_1)$ is the water vapor pressure differential across the film (Pa), t is the time (s), w is the water gain of the cup (mol), and x is the film thickness (m).

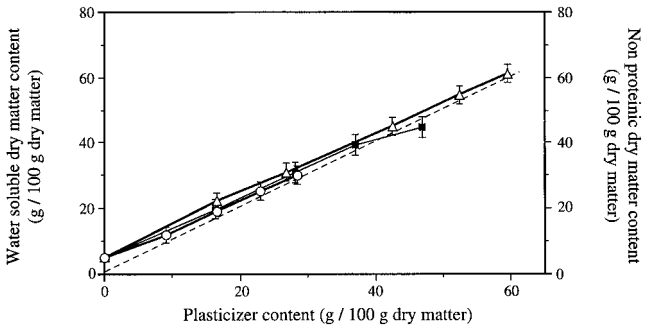

Figure 1. Effect of plasticizer content (glycerol, ○; sorbitol, ■; or sucrose, △) on the content of dry matter solubilized in water for myofibrillar protein-based films. Dashed line represents the nonproteinic dry matter contents in films (grams per 100 g of dry matter).

RESULTS AND DISCUSSION

When no plasticizer was introduced in formulation, the films were relatively brittle and needed to be handled very carefully. Irrespective of plasticizer type and content, transparent and colorless films were produced from fish myofibrillar proteins. Glycerol, sorbitol, and sucrose were compatible with myofibrillar proteins to form films within the explored concentration range. The compatibility potential limit for these plasticizers with the myofibrillar proteins is then not reached, even for sucrose and sorbitol which are known to be sensitive to crystallization in low water content conditions. Higher plasticizer contents could possibly cause phase separation or physical exclusion of such plasticizers (Aulton *et al.*, 1981; Donhowe and Fennema 1993).

Solubilities in Water. From visual observations and irrespective of plasticizer type and content, the myofibrillar protein-based films were clearly not dispersed without visual loss of integrity after a 24 h immersion in water. Irrespective of plasticizer type, an increase in its content leads to a linear increase in water-soluble dry matter content (Figure 1). First order regressions perfectly describe the solubility changes as a function of glycerol, sorbitol, or sucrose contents (respectively, $R^2 = 0.997$, 0.995, and 0.999).

It could be thus hastily concluded that hydrophilic plasticizers enhance film solubility in water. Variations of nonproteinic dry matter content in films (*i.e.* essentially plasticizers) as a function of plasticizer content appear to be close to the experimental variations of water-soluble dry matter content (Figure 1). The slight difference observed between the solubilized dry matter content and nonproteinic dry matter content in films is nearly constant and close to 3−5 g/100 g of initial dry

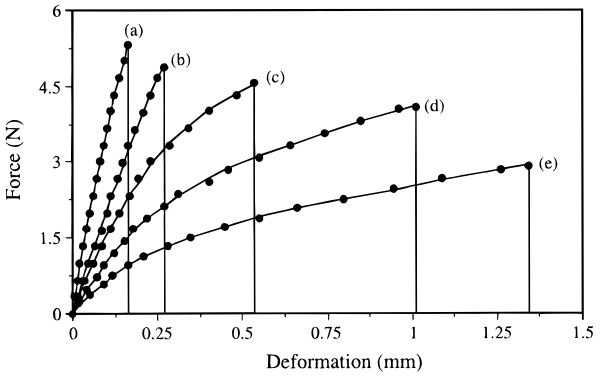

Figure 2. Typical experimental force−deformation curves for myofibrillar protein-based films (at 20 °C and 59.1% relative humidity) as a function of plasticizer content for 0 (a), 0.11 (b), 0.22 (c), 0.33 (d), and 0.44 (e) mol of sorbitol/100 g of protein. Continuous line represents the modelized curves from eq 2.

matter in films. Low molecular weight proteinic chains (*i.e.* monomers and small peptides) formed during storage of the film-forming solution and immobilized in the network (Cuq *et al.*, 1995b) could thus constitute the protein-based materials that solubilize in water.

The dry matter solubilized in water is likely to be constituted mainly by the plasticizer. The protein network was then not likely to solubilize or disperse in water. High interaction density and, more certainly, the presence of intermolecular covalent bonds or "physical knots" (*i.e.* chain entaglements) are responsible for partial insolubility of these films.

This water solubility behavior could not be generalized, and understanding the film solubility remains a complex subject. Plasticizer solubilization in water was already observed for films based on wheat gluten or treated soy proteins or produced by transglutaminase catalytic cross-linking of whey proteins (Gontard *et al.*, 1992; Mahmoud and Savello, 1993; Stuchell and Krochta, 1994). Mahmoud and Savello (1993) and Stuchell and Krochta (1994) have pointed out increases in the content of protein solubilized in water when the hydrophilic plasticizer content increased for treated whey protein- and soy protein-based films. A decrease in the polymer network interaction density due to the plasticizer presence was thus associated with this increase in solubility properties. On the other hand, Marquié *et al.* (1995) have displayed a large decrease in the content of dry matter solubilized in water for cottonseed protein-based reticulated films when glycerol content increased.

Mechanical Properties. Plasticizing myofibrillar protein-based films with glycerol, sorbitol, or sucrose entailed large modifications in their mechanical properties. Typical experimental force−deformation curves at various sorbitol contents are given as examples in Figure 2. It is important to note that when glycerol or sucrose was introduced in films, the force−deformation curves as a function of their content have nearly similar shape and are thus not presented here.

When unplasticized films are deformed (Figure 2, curve a), force rises almost continuously until the specimen breaks. These films thus form hard brittle materials with low distensibility (0.15 mm) and high mechanical resistance (5.1 N). When hydrophilic plasticizer is incorporated (Figure 2, curves b−e), the experimental nonlinear force−deformation curves characterize a classical viscoelastic behavior. Increasing plasticizer contents also leads to both decrease in mechanical resistance (*i.e.* force at break) and increase

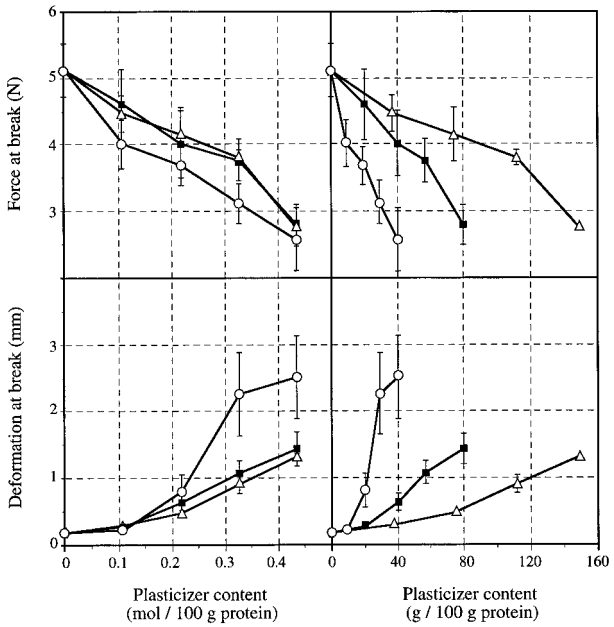

Figure 3. Effect of plasticizer content (expressed in moles per 100 g of protein or in grams per 100 g of protein; glycerol, ○; sorbitol, ■; or sucrose, △) on force and deformation at break (at 20 °C and 59.1% relative humidity) for myofibrillar protein-based films.

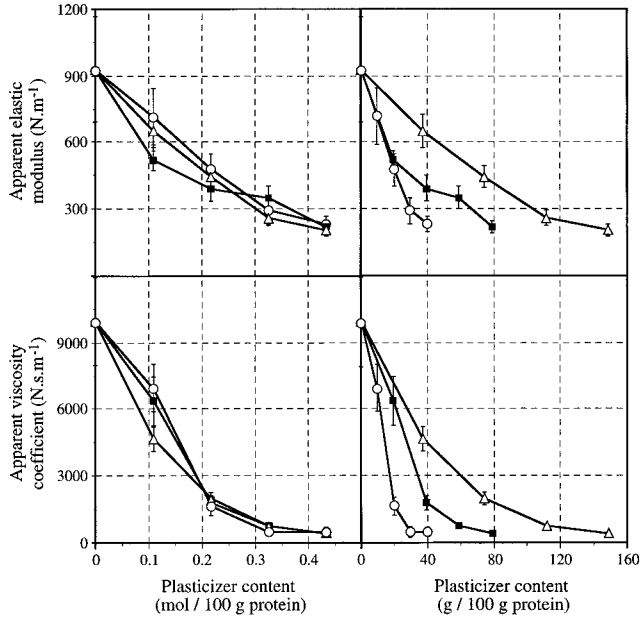

Figure 4. Effect of plasticizer content (expressed in moles per 100 g of protein or in grams per 100 g of protein; glycerol, ○; sorbitol, ■; or sucrose, △) on apparent elastic modulus and apparent viscosity coefficient (at 20 °C and 59.1% relative humidity) for myofibrillar protein-based films.

in distensibility (*i.e.* deformation at break) of films (Figure 3). Similar changes in mechanical properties as affected by hydrophilic plasticizers were previously observed for various hydrocolloid-based films (Park and Chinnan, 1990; Somanathan *et al.*, 1992; Gontard *et al.*, 1993; Cherian *et al.*, 1995).

The rheological model proposed by Cuq *et al.* (1996) was applied to describe experimental force−deformation data. Examples of modelized curves are presented in Figure 2. The rheological model perfectly describes all of the experimental data. Plasticizer effects on the two viscoelastic parameters (calculated with eq 2) are illustrated in Figure 4. An increase in plasticizer content

induces large decreases in apparent elastic modulus and viscosity coefficient.

The mechanical property changes characterize decreases in density and reversibility of intermolecular interactions occurring in the myofibrillar protein network that forms films. From their primary structure (Orban *et al.*, 1992), myofibrillar proteins are known to be relatively rich in nonionized polar amino acids (27 mol/100 mol amino acids) that contribute to formation of numerous protein−protein hydrogen bondings and, as a consequence, to cohesion and low flexibility of the unplasticized films. Glycerol, sorbitol, and sucrose are low molecular weight hydrophilic molecules that could easily fit into proteinic chains and establish hydrogen bondings with reactive groups of proteins. Bringing together plasticizers and proteins induces formation of protein−plasticizer interactions to the detriment of protein−protein interactions. As a consequence, the density of intermolecular interactions decreases in material and the free volume between polymer chains increases.

Myofibrillar proteins are also characterized by relatively high contents of glutamin and asparagin (about 10 mol/100 mol of amino acids). The replacement of "double" hydrogen bondings (85 kJ mol^{-1}) between two amide groups by "single" hydrogen bondings (25 kJ mol^{-1}) between one amide group and one plasticizer molecule could be another explanation of the decrease in molecular interaction density in these films.

When glycerol, sorbitol, and sucrose are introduced in myofibrillar protein-based films at the same *molecular contents* (Figures 3 and 4), the specific characteristics of plasticizer (number of carbons in chain = 3, 6, or 12; molecular mass = 92, 182, or 342 g mol^{-1}; number of hydroxyl groups = 3, 6, and 8) have no effect on mechanical properties of the films. However, glycerol seemed to have a slightly higher plasticizing effect than sorbitol or sucrose for the highest contents (Figure 3). This effect can be associated with the small size of glycerol, which facilitates its insertion and positioning within the tridimensional proteinic network.

The specific characteristics of glycerol, sorbitol, and sucrose do not seem to be sufficiently different to observe significant differences in the changes of mechanical properties. According to Donhowe and Fennema (1993b), the specific characteristics of plasticizers could have a significant influence on functional properties for hydrocolloid-based films when plasticizer macromolecules are quite different (*e.g.* glycerol and PEG 20000). The distance between the macromolecules and the density of molecular interactions in films could be thus sensitive to size, molecular weight, and number of hydroxyl groups of plasticizers.

In practice, hydrocolloid-based film formulation is generally carried out on a weight basis. It is then interesting to note that if plasticizer effects are compared with similar weight contents, the lower molecular weight plasticizer shows a more important apparent plasticizing effect than those observed for the higher molecular weight plasticizers (Figures 3−5). This effect is in fact mainly due to higher molecular contents.

Water Vapor Barrier Properties. The effects of plasticizer content and type on water vapor barrier properties for myofibrillar protein-based films are presented in Figure 5. As expected, an increase in plasticizer content involves a large increase in water vapor permeabilities.

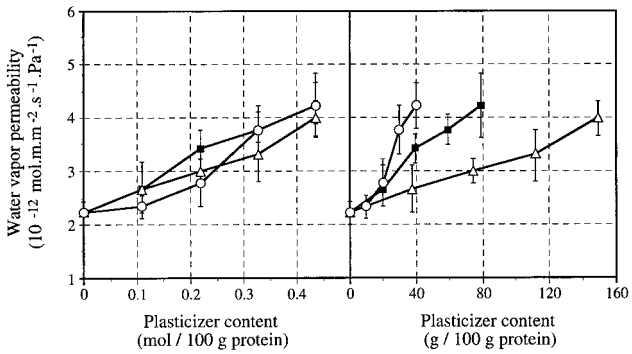

Figure 5. Effect of plasticizer content (expressed in moles per 100 g of protein or in grams per 100 g of protein; glycerol, ○; sorbitol, ■; or sucrose, △) on water vapor permeability (at 20 °C and 0−100% relative humidity gradient) for myofibrillar protein-based films.

The incorporation of plasticizers modifies the molecular organization of the proteinic network, with an increase in free volume. The network becomes less dense and as a consequence more permeable (Banker *et al.*, 1966; Ashley, 1985). Permeability increase with plasticizer content could be related to hydrophilicity of plasticizer molecules. Introducing hydrophilic plasticizers, favorable to adsorption and desorption of water molecules, is known to enhance the water vapor permeability of hydrocolloid-based films (Gontard *et al.*, 1993; McHugh *et al.*, 1994; Cherian *et al.*, 1995). As previously observed with the mechanical properties, the specific characteristics of plasticizers (size, molecular weight, and number of hydroxyl groups) have no effect on the water vapor barrier properties when similar molecular contents are introduced in myofibrillar protein-based films (Figure 5).

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LITERATURE CITED

Ashley, R. J. Permeability and plastics packaging. In *Polymer Permeability*; Comyn, J., Ed.; Elsevier Applied Science Publishers: London, 1985; pp 269−308.

ASTM. *Annual Book of ASTM Standards*; American Society for Testing and Material: Philadelphia, PA, 1989.

Aulton, M. E.; Abdul-Razzak, M. H.; Hogan, J. E. The mechanical properties of HPMC films derived from aqueous systems. Presented at the 2nd Congress International: Technologie and Pharmacy, Paris, June 10−12, 1981.

Bakker, M. *The Wiley Encyclopedia of Packaging Technology*; Wiley: New York, 1986.

Banker, G. S.; Gore, A. Y.; Swarbrick, J. Water vapour transmission properties of free polymer films. *J. Pharm. Pharmacol.* **1966**, *18*, 457−466.

Cherian, G.; Gennadios, A.; Weller, C.; Chinachoti, P. Thermomechanical behavior of wheat gluten films: effect of sucrose, glycerin, and sorbitol. *Cereal Chem.* **1995**, *72*, 1−6.

Cuq, B.; Gontard, N.; Guilbert, S. Edible films and coatings as active layers. In *Active Food Packagings*; Rooney, M. L. Ed.; Blackie Academic & Professional: Glasgow, 1995a; pp 111−142.

Cuq, B.; Aymard, C.; Cuq, J. L.; Guilbert, S. Edible packaging film based on fish myofibrillar proteins: formulation and functional properties. *J. Food Sci.* **1995b**, *60*, 1369−1374.

Cuq, B.; Gontard, N.; Cuq, J. L.; Guilbert, S. Rheological model for the mechanical properties of myofibrillar protein-based films. *J. Agric. Food. Chem.* **1996**, *44*, 1116−1122.

Donhowe, I. G.; Fennema, O. The effects of plasticizers on crystallinity, permeability, and mechanical properties of methylcellulose films. *J. Food Process. Preserv.* **1993**, *17*, 247−257.

Gennadios, A.; Weller, C. L.; Testin, R. F. Property modification of edible wheat, gluten-based films. *Trans. ASAE* **1993**, *36*, 465−470.

Gontard, N.; Guilbert, S. Bio-packaging: technology and properties of edible and/or biodegradable material of agricultural origin. In *Food Packaging and Preservation*; Mathlouthi, M., Ed.; Blackie Academic & Professional: Glasgow, 1994; pp 159−181.

Gontard, N.; Ring, S. Edible wheat gluten film: influence of water content on glass transition temperature. *J. Agric Food Chem.* **1996**, *44*, 3474−3478.

Gontard, N.; Guilbert, S.; Cuq, J. L. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *J. Food Sci.* **1992**, *57*, 190−195.

Gontard, N.; Guilbert, S.; Cuq, J. L. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *J. Food Sci.* **1993**, *58*, 206−211.

Krochta, J. M.; Baldwin, E. A.; Nisperos-Carriedo, M. *Edible Films and Coatings to Improve Food Quality*; Technomic Publishing: Lancaster, PA, 1994.

Lieberman, E. R.; Gilbert, S. G. Gas permeation of collagen films as affected by cross-linkage, moisture, and plasticizer content. *J. Polym. Sci.* **1973**, *41*, 33−43.

Mahmoud, R.; Savello, P. A. Solubility and hydrolyzability of films produced by transglutaminase catalytic crosslinking of whey protein. *J. Dairy Sci.* **1993**, *76*, 29−35.

Marquié, C.; Aymard, C.; Cuq, J. L.; Guilbert, S. Biodegradable packaging made from cottonseed flour: formation and improvement by chemical treatment with gossypol, formaldehyde and glutaraldehyde. *J. Agric. Food Chem.* **1995**, *43*, 2762−2766.

McHugh, T. H.; Krochta, J. M. Sorbitol- vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *J. Agric. Food Chem.* **1994**, *42*, 841−845.

McHugh, T. H.; Aujard, J. F.; Krochta, J. M. Plasticized whey protein edible films: water vapor permeability properties. *J. Food Sci.* **1994**, *59*, 416−419, 423.

Orban, E.; Quaglia, G. B.; Casini, I.; Caproni, I.; Moneta, E. Composition and functionality of sardine native proteins. *Lebens. Wiss. Technol.* **1992**, *25*, 371−373.

Park, H. J.; Chinnan, M. S. Properties of edible coatings for fruits and vegetables. Presented at the International Winter Meeting, American Society of Agricultural Engineers, Chicago, IL, Dec 18−21, 1990; Paper 90-6510.

Park, H. J.; Bunn, J. M.; Weller, C. L.; Vergano, P. J.; Testin, R. F. Water vapor permeability and mechanical properties of grain protein-based films as affected by mixtures of polyethylene glycol and glycerin plasticizers. *Trans. ASAE* **1994**, *37*, 1281−1285.

Slade, L.; Levine, H. The glassy state phenomenon in food molecules. In *The Glassy State in Foods*; Blanshard, J. M., Lillford, P. J., Ed.; Nottingham University Press: Loughborough, U.K., 1993a; pp 35−102.

Slade, L.; Levine, H. Water relationships in starch transitions. *Carbohydr. Polym.* **1993b**, *21*, 105−131.

Somanathan, N.; Naresh, M. D.; Arumugan, V.; Ranganathan, T. S.; Sanjeevi, R. Mechanical properties of alkali treated casein films. *Polym. J.* **1992**, *24*, 603−611.

Stuchell, Y. M.; Krochta, J. M. Enzymatic treatments and thermal effects on edible soy protein films. *J. Food Sci.* **1994**, *59*, 1332−1337.

Trigeassou, J. C. *Recherche de Modèles Expérimentaux Assistée par Ordinateur*; Technique et Documentation−Langage Informatique, Lavoisier: Paris, 1988.

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