FILMS BASED ON BIOPOLYMERS FROM CONVENTIONAL AND NON CONVENTIONAL SOURCES

Sobral, P.J.A.[a], Alvarado, J.D.[b], Zaritzky, N.E.[c,f], Laurindo. J.B.[d], Gómez-Guillén, C.[e], Añón, M.C.[f], Montero, P.[e], Denavi, G.[c]; Molina Ortíz, S.E.[c]; Mauri A.A.[c], Pinotti, A.[c,f], Garcia, M.A.[c] Martino, M.N.[c,f], Carvalho, R.A.[a]

a- Departamento de Engenharia de Alimentos. Faculdade de Zootecnia e Engenharia de Alimentos. Universidade de São Paulo. Av. Duque de Caxias Norte, 225. 3635-900 Pirassununga (SP). Brazil.

b- Facultad de Ciencia e Ingeniería en Alimentos. Universidad Técnica de Ambato. Calle México y El Salvador, PBox 18-01-0334, Ambato, Ecuador.

c- Centro de Investigación y Desarrollo en Criotecnologia de Alimentos CIDCA (UNLP-CONICET). Calle 47 y 116 La Plata (1900) Argentina.

d- Departamento de Engenharia Química e Engenharia de Alimentos. Universidade Federal de Santa Catarina. EQA/CTC/UFSC – Campus Universitario – Trindade – 88.040-900. PBox 476. Florianópolis, Brazil.

e- Departamento de Ciencia Y Tecnología De Carne Y Productos Cárnicos Y De Pescado Y Productos De La Pesca; Instituto Del Frío, Consejo Superior De Investigaciones Científicas. Ciudad Universitaria s/n, PBox 28040. Madrid, España.

f- Departamento de Ingenieria Quimica. Facultad de Ingenieria. Universidad Nacional de La Plata Calle 47 y 115. La Plata (1900). Argentina.

1. INTRODUCTION

Edible films are thin materials based on biopolymers. These films are also biodegradable and because that, these materials have attracted the attention of the Food Science academic community in the last decades.

The main biopolymers used in the edible films production are polysaccharides (Nisperos-Carriedo, 1994) and proteins (Gennadios et al., 1994, Torres, 1994). The polysaccharide the most used the edible film technology is the starch, because it is produced abundantly and is inexpensive. But, others polysaccharides, such as the chitosan and some cellulose derivates, have been also studied.
Normally, proteins produced industrially, such as soja and gelatin from mammalian, are largely applied in the films production. But, some proteins from less conventional sources, as muscle proteins and gelatin from fish, and feather keratins, have also been studied in the last years.
Thus, this work will present and discuss some aspects of the edible and/or biodegradable film technology based on biopolymers from conventional or less conventional resources.

2. FILMS BASED ON CHITOSAN PRODUCED IN LABORATORY SCALE

Chitosan is a polymer derived from the chitin partial o totally deacetylated. Usually it comes from the chitin, found in fungus, crustaceous, molluscs, annelids; however, in general, crab, lobster or shrimp shells are used because they are industrial wastes (Shepherd and others 1997).
Chitin ($C_8H_{13}O_5N$), is a polymer formed by units of 2-acetamide-2-deoxiglucose linked by 1,4-beta-glucosidic of the cellulose. It is insoluble in water, diluted acids and alkalis, alcohol and organic solvents; it is soluble in general with some degradation, in concentrate mineral acids. By acid hydrolysis, it is degraded to glucose amine; the alkaline hydrolysis deaceylates it extensively, with some reduction of chain length, forming chitosan (Kirk and Othmer, 1970).
Chitosan [β-(1,4)-2-amino-2-deoxy-D-glucopyranose] is an amorphous white solid, insoluble in water, soluble in acids, whose crystalline structure is substantially the same of the original purified chitin. At extreme conditions of deacetylation, the chitosan keeps the amino groups and those conferred enormous possibilities of chemical modification, because they have positive ionic links that react to substances negatively charged as fats, cholesterol, basic ions and proteins (Argukelles and others 1998).
The multiple applications of chitosan let it to be used in agriculture, medicine, effluent treatment and in foods. According to Devlieghere and others (2004), chitosan could be use in foods as antimicrobial agent,

clarifying agent for juices, antioxidant in sauces, inhibitor of enzymatic browning in apples and potatoes, additionally as films for fresh fruit and vegetables recovering, also it can act as enzyme inhibitor. Han and others (2005) established that chitosan films behave as adequate recovering material for strawberries due to their antimicotic properties and their suitableness for recovering; however the solutions of chitosane diluted in acid became bitter and astringency therefore less practical for real market.

a) Chitosan Production

For chitosan obtaining, the group N-acetyl has to be removed without the polysaccharide hydrolysis that is why, the alkaline methods are the most used. Several authors (Ramírez and others, 1998, Taboada and others, 2003) obtained chitosan by the chemical modification of the chitin, the acetyl units are eliminated with long treatments in highly concentrate alkalis. Pinelli and others (1998) showed a recompilation of methods to obtain chitosan from shrimp shells; they adapted a method to obtain chitosan from chitin and pointed out the importance of the initial control of the shells by thermal treatment for the inactivation of the hydrolytic enzymes, tunnel drying and milling.
According to the technology developed by Almeida and Arancibia (2005), chitosan was obtained form the chitin extracted from Ecuadorian shrimp shells, by a previous protein removal done with diluted solutions of sodium hydroxide at 80ºC for 30 minutes and then a second protein removal done thrice with solutions 3% (w/w) of sodium hydroxide at 80ºC. The process goes on with the demineralization using HCl 2N for 60 minutes at temperatures around 20ºC. At last, the obtained chitin was deacetylated to obtain chitosan by chemical treatment, using 50% (w/w) sodium hydroxide solution at 100ºC for 60 minutes. Alvarado and others (2006) modified the method to eliminate protein removals, obtaining chitosane directly without getting the chitin, by a severe alkaline treatment, showing the utility of the obtained product to produce eatable films.

b) Chitosan Films Production and characteristics

Films formation is based on the bio polymer dispersion or solution in a solvent and the additives use (plastificants, link agents) obtaining a dispersion or a solution. After preparing the suspension, these shall pass by processes of lamination and drying in order to get the films. In this step

the bio polymer concentration increases because of the solvent evaporation and as a consequence the molecule aggregation occurs, forming a tri dimensional network (Gontard and others 1993).
As chitosan is soluble in organic solvents, several authors have worked with organic acid solutions. Caner and others (1998) prepared chitosan films using acetic, lactic and formic acids with different concentrations of the plastificant and moulding them in glass dishes. Alvarado and others (2005) used Petri boxes where they put the chitosan suspension and let it dry at temperature of 20±5ºC for 48 hours, finally the films were removed by using NaOH 0.1% (w/w) solution. Other samples were prepared at clean and dry acrylic plaques 10x20 [cm], where the chitosane solution was pour, the films dried at the temperature of 20±5ºC for 48 hours and were removed by releasing from the acrylic without using the NaOH solution. The best results were obtained in acrylic plaques without using the NaOH.
The applications of the films depend principally of their barrier properties to water and to gases, of their mechanical characteristics, solubility, optical and thermal properties. Those characteristics depend on the polymer type, polymer obtaining process, film conditioning, drying and film thickness (Galed and others 2000, Srinivasa and others 2004). The interest on chitosan films for foods is based on their capacity as an oxygen barrier (Caner and others 1998) and carbon dioxide barrier (Choi and others 2002). The used methods to determine the film properties are derivate of the classic methods applied to synthetic material, these methods were adapted to the characteristics of bio polymers, particularly due to their great sensitiveness to the relative humidity and the temperature (Issam and others 2005).
One of the fundamental properties is the permeability to water vapour (WVP). Buonocore and others (2005) established that the chitosan films showed less permeability to water vapour in relation to the films made with casein and alginate, they pointed out this is due to their low affinity with water and less macromolecular mobility, according to this, chitosan films can act as adequate barriers to water vapour; they used a mathematical model to describe the barrier properties of these films. Caner and others (1998) worked with chitosan films done with several acid solutions and plastificants, they reported values form 0.535 to 1.320 [g/m day at] at 25ºC between 50% - 100% of relative humidity, they did not established changes on the mass transfer properties during nine weeks storage. Alvarado and others (2005) published values of permeability to water vapour (WVP) in a range of 0.097 up to 0.227 [g mm/kPa h m^2],

values increased while the concentration of chitosan increased therefore the film thickness, these values correspond to the low permeability films to the water vapour in relation to the other biodegradable films, but of high permeability in relation to the plastic inorganic films.
Tensile strength and elongation at break are among the more known mechanical properties. Miranda and others (2004) reported the following ranges: tensile strength since 7.23 to 48.3 [MPa] and elongation at break expressed as percentage since 22.9 to 167.2% for chitosan films. Tanveer and others (2003), for chitosan films, published several values of tensile strength among 59.87 to 67.11 [$N/(mm)^2$] and elongation percentage to rupture among 21.35 to 67.10%. Caner and others (1998) for chitosan films made with four organic acids suspensions published values of resistance to tension from 6.85 to 31.88 [MPa], the elongation percentage varied from 14 to 70%, decreasing while the time of storage increased.
Alvarado and others (2006) published data of chitosan films made with two organic acids suspensions, acetic and lactic. The films obtained are compact, lightly permeable to water vapour, middle strong, transparent and their thickness increases while the chitosan concentration increases in the suspension. The films made with the suspensions of chitosan in acetic acid are less hygroscopic, show less permeability to water vapour, and are more resistant to traction but less elastic; they behave as partial crystalline materials and are opaque. The films elaborated with chitosan suspensions in lactic acid are more hygroscopic, more permeable, less resistant and more elastic; they behave as crystalline materials and are transparent.

3. COMPOSITE FILMS BASED ON CHITOSAN AND METHYLCELLULOSE

Cellulose derivatives are also interesting biomaterials for film technology; they are polysaccharides composed of linear chains of β (1-4) glucosidic units with methyl, hydroxypropyl or carboxyl substituents. Methylcellulose (MC) has excellent film-making properties, high solubility and efficient oxygen and lipid barrier properties (Park, Weller, Vergano & Testin, 1993; Donhowe & Fennema a,b, 1993 and Nisperos-Carriedo, 1994).
Besides, chitin and its deacetylated product, chitosan, have received much interest for its application in agriculture, biomedicine, biotechnology and the food industry due to their biocompatibility, biodegradability and bioactivity (Muzzarelli, et al., 1988; Kumar, 2000; Tharanathan & Kittur, 2003). Among the potential applications, the use of chitin and chitosan as

food antimicrobials and biopesticides are especially attractive (Wu, Zivanovic, Draughon, Conway & Sams, 2005). Due to its antifungic, good mechanical and oxygen barrier properties (Chen, Yeh, & Chiang, 1996; Caner, Vergano & Wiles, 1998), chitosan film is a promising packaging material that can be included in the active film category, (Vermeiren, Devlieghere, van Beest, de Kruijf & Debevere, 1999). In the absence of additives, films made from these polysaccharides are brittle. Plasticizers are necessary to enhance flexibility and to improve mechanical properties. Hydrophilic compounds such as polyols are commonly used as plasticizers in hydrophilic film formulations.

a) Films Production

Commercial methylcellulose (A4M, Methocel, Dow, USA) with a substitution degree of 27.5% was purchased from Colorcon (Argentina). Commercial chitosan from crab shells with a minimum deacetylation degree of 85% was purchased from Sigma (St. Louis, USA). Solutions of 1% (w/w) of methylcellulose (MC), and 2% (w/w) of chitosan were prepared. Chitosan (CH) was solubilized in 1 % (v/v) acetic acid solution, followed by a vacuum filtration to eliminate insolubles. Mixture solutions were prepared with the following (w/w) CH: MC proportions: 25:75, 50:50 and 75:25.

In all cases, to obtain films, 40g of solutions were poured on rectangular acrylic plates (10 x 20 cm). The solutions were dried at 60°C in a ventilated oven to constant weight. Films were stored under controlled temperature and relative humidity conditions (20°C and 65% RH).

Equilibrium moisture content: water content of the films was determined measuring weight loss of films, upon drying in an oven at 110°C until constant weight (dry sample weight). Thickness of the films was determined using a digital coating thickness gauge Elcometer A 300 FNP 23 (England) for non-conductive materials on non-ferrous substrates. Film solubility in water was determined using film pieces of 2 x 3cm; samples were weighed to the nearest 0.0001g and placed into test beakers with 80ml deionizated water. The samples were maintained under constant agitation at 200 rpm for 1 hour at room temperature (25°C approximately). After soaking, the remained pieces of film were collected by filtration and dried again in an oven at 60°C to constant weight. The percentage of total soluble matter (% solubility) was calculated.

For film microstructure anlaysis, scanning electron microscopy (SEM) analysis was performed with a JEOL JSPM 100 electron microscope

(Japan). Film pieces were mounted on bronze stubs using a double-sided tape and then coated with a layer of gold (40-50 nm), allowing surface and cross-section visualization. All samples were examined using an accelerating voltage of 5 kV.

Water vapor permeability tests were conducted using a modified ASTM (1995) method E96 as described in a previous work (García, et al., 2004) using an especially designed permeation cell that was maintained at 20ºC. After steady state conditions were reached (about 2 h), eight weight measurements were made over 24 h. A driving force of 1753.55 Pa, expressed as water vapor partial pressure, was used.

Tensile tests were performed in a texturometer TA.XT2i (Stable Micro Systems, England) as described in a previous work (García, et al., 2004) using a tension grip system A/TG and probes of 6cm in length and 0.7 cm width. Puncture tests were performed using a cylindrical probe 2 mm in diameter at a constant rate of 1 mm/s. Tests were carried out with samples of 3x3 cm from each film formulation. Curves of force (N) as a function of deformation (mm) were automatically recorded by the Texture Expert Exceed software. Maximum breaking force (N), tensile strength (MPa), deformation at break (mm), percent of elongation at break (%) and elastic modulus (N/mm) were calculated according to the ASTM D882-91 method (1996).

b) Characterization of Composite Films Based on Chitosan and Methylcellulose.

Flexible, homogeneous, thin, and transparent films were obtained from MC, CH and mixture solutions. Visually, MC films were colorless and chitosan films had a slightly yellow appearance, increasing yellowness with chitosan concentration for composite films. Even though, the intensity of the yellowness is negligible when compared to values reported for whey protein based films (Trezza & Krochta, 2000 a and b). All films were easily removed from the cast plate and no pores or cracks were detected as seen by SEM (Figure 1) although film formulation did not include a plasticizer. Besides, a compact structure was observed, (Figure 1). Composite films showed multilayer structures (Figure 1c) this could be possibly attributed to the limited chain mobility even though both CH and MC are compatible polymers.

As shown in Table 1, thickness of films varied between 14.12±1.59 and 26.07±3.17 μm. CH films, obtained from a 2% solution, were thicker than MC ones, obtained from 1% solution, this was also evidenced by SEM

observations (Figure 1 a and b). Composite films exhibited intermediate thickness values within the range of those of one component film (Table 1). All films reached after drying low moisture, MC films exhibited the lowest equilibrium moisture while the highest value was observed for CH ones (Table 1).

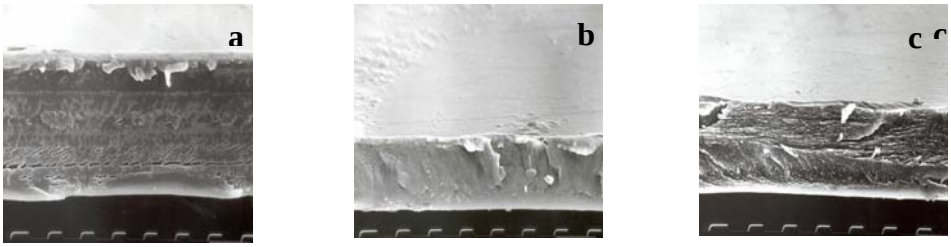


Figure 1: SEM micrographs of the cross-section of: a) CH, b) MC and c) 50:50 CH:MC films. Magnification: 10μm between marks.

MC films were completely soluble in water while CH films had lower solubility values. Composite films had intermediate water solubilities, decreasing film solubility with increasing chitosan proportion (Table 1). Thus, by controlling CH concentration in film formulation, solubility can be tailored enhancing the possible applications of these materials.

Table 1. Film solubility in water, equilibrium moisture content, thickness and water vapor permeability of CH-MC films

Film composition	g CH: g MC	Film solubility in water (%)	Equilibrium moisture content (g water/ 100 g film)	Film thickness (μm)	WVP×10^{11} (g m^{-1} s^{-1} Pa^{-1})
MC	1:0	98.4±2.2[b]	0.91±0.16	16.32±2.46	7.55±0.60
25:75[a]	0.5:0.75	40.7±6.3	1.12±0.01	21.22±0.68	6.77±0.92
50:50	1:0.5	27.7±1.6	2.41±0.16	23.35±2.72	6.67±0.74
75:25	1.5:0.25	14±2.1	2.22±0.01	22.75±1.69	7.24±0.35
CH	2:0	9.3±0.9	6.33±0.58	26.07±3.17	7.24±0.81

[a] CH:MC (w/w) proportion in film formulation, [b] value ± standard deviation

Table 1 shows WVP values of individual and composite films. CH and MC films showed lower WVP values, than those of some protein films and even lower than other polysaccharide based films reported in literature (Krochta & De Mulder Johnston, 1997; García, Martino & Zaritzky, 2000; Mali, Grossman, García, Martino & Zaritzky, 2002). Our obtained results are in agreement with those of Park et al. (1993), Wong, Gastineau, Gregorski, Tillin and Pavlath, (1992) and Caner et al. (1998).

Permeability of composite films did not differ significantly from the values of one component film (Table 1). Since permeability is the contribution of diffusivity and solubility of the permeant through the solid matrix the obtained results for composite films indicate that the developed matrix should be similar to those of the individual component matrixes as seen by X-ray diffraction analysis. Chitosan films showed to be good barriers to oxygen permeation in comparison to commercial polymers. But exhibiting relatively low water vapor permeabilities.

WVP values of our composite CH-MC films are higher than other synthetic films like low density polyethylene (LDPE), with a WVP value of 9.14×10^{-13} $gm^{-1}s^{-1}Pa^{-1}$, (Smith, 1986), although CH-MC films have WVP values similar to those of cellophane as expected due to the similar chemical structure of both polymers. According to Shellhammer and Krochta, (1997) cellophane exhibited a WVP value of 8.4×10^{-11} $gm^{-1}sec^{-1}Pa^{-1}$.

MC and CH films showed different behavior patterns under tensile tests; both films showed high resistance but MC films were more flexible (Figure 2). Percent elongation values of films were 12.7% for MC, and 3.9% for CH and intermediate values were obtained with composite films (Figure 8a). Similar mechanical properties were found in literature for MC films (Donhowe & Fennema, 1993 a and b; Park et al., 1993; Debeaufort & Voilley, 1997). For CH films, data reported in literature for tensile test were higher than those obtained in the present work, differences may be due to CH composition and suppliers, plasticizer presence and film preparation (Chen & Lin, 1994; Butler, Vergano, Testin, Bunn & Wiles, 1996; Caner et al., 1998).

The main differences may be attributed to plasticizer type and content; Caner et al. (1998) reported a wide range of elongation (14-70%) depending on storage time, plasticizer and type of acid used in chitosan solubilization.

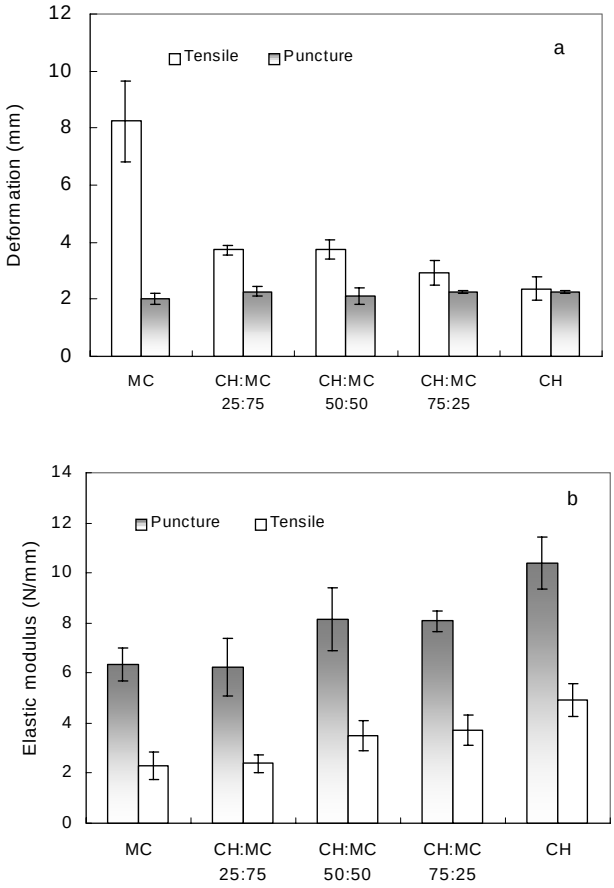


Figure 2: Mechanical properties of MC, CH and composite films with different CH:MC ratios. Tensile and puncture tests: a) deformation at break and b) elastic modulus.

In composite films, tensile strength and elastic modulus increased with CH concentration, leading to stronger films, (Figure 2b). Accordingly, a decreasing deformation trend was observed with CH concentration, indicating a reduction in film flexibility (Figure 2a).

Mechanical properties of CH-MC films were comparable to many medium strength commercial polymer films like cellophane. However, synthetic polymers like LDPE and HDPE exhibit similar tensile strength to those obtained in the present work but higher elongation (Cunningham, Ogale, Dawson & Acton, 2000).

With regard to puncture tests, elastic modulus showed the same trend as in tensile tests (Figure 2b). However, all films have a similar value of

deformation at break, about 2 mm, regardless CH:MC ratio (Figure 2a). Thus, CH may replace MC maintaining puncture resistance of films with economical benefits.

In conclusions, physical properties of composite films showed intermediate (solubility and mechanical properties) values compared to those of individual component films or did not differ significantly (WVP) reinforcing the idea of compatibility of CH and MC polymer.

These combined biodegradable films with good water vapor barrier properties have the possibility to tailor mechanical and solubility properties, within a range, based on polymer ratio composition. CH imparts rigid characteristics to composite films (high elastic modulus and small elongation) while MC contributes with a higher elongation and lower elastic modulus, increasing solubility as well. Thus, MC-CH edible films can lead to many possible applications in food, pharmaceutical and cosmetic industry considering the natural origin of both polymers.

4. FILMS BASED ON MUSCLE PROTEINS FROM CULTIVATED FISHES

Some years ago, Cuq et al. (1995) demonstrated that the myofibrillar proteins had the capacity to form transparent and resistant films, working with Atlantic sardines. In fact, innumerous works are encountered in the specialized literature about the elaboration and the characterization of films based on the myofibrillar proteins obtained from *post rigor mortis* muscles of Atlantic Sardine (*Sardina pilchardus*) (Cuq et al., 1995, 1996a,b, 1997), *anten rigor mortis* muscles of Nile Tilapia (*Oreochromis niloticus*) (Monterrey-Quintero, 1999, 2000, Sobral, 2000, Sobral et al., 2002), and bovine meat (*Bos taurus taurus*) (Sobral et al., 1998, Ocuno and Sobral, 2000, Souza et al., 2004).

The myofibrillar proteins are insoluble in water, but can be made soluble adjusting the pH of the solution. These proteins are fully stretched and closely associated with each other in parallel structures (Iwata et al., 2000). Probably in function of this, these proteins are capable of forming a continuous matrix during drying of the solution (Cuq et al., 1995, Monterrey-Quintero & Sobral, 2000, Sobral, Ocuno and Savastano, 1998).

Contrarily to the most important commercial vegetal proteins, the myofibrillar proteins have to be prepared adequately before be used in the film elaboration process. In this case, after fish slaughter and evisceration, the muscles are grounded and washed conveniently to eliminate the

sarcoplasmic proteins. After that, the material is minced and passed through a screen, to separate the connective tissue (insoluble proteins) (Cuq et al., 1995; Monterrey-Quintero & Sobral, 2000). Possibly, the elimination of the connective tissue could be due to it completely insoluble character which could affect the homogeneity of films and the separation of the sarcoplasmic proteins was made due to its strong bad flavor and principally because these proteins has a low molecular weight.

Fifty years ago, Japanese researchers demonstrated that sarcoplasmic proteins are also capable of forming flexible films, in despite of its low molecular weight. Iwata et al. (2000) and Tanaka et al. (2001) developed and characterized films based on sarcoplasmic proteins extracted from Blue Marlin (*Makaira mazara*) muscle. Contrarily to the myofibrilar proteins, the sarcoplasmic proteins are globular proteins, which in general have to be thermally denatured to form a continuous matrix (Iwata et al., 2000). Heating modifies the three-dimensional structure of the globular proteins, causing exposition of the SH groups, and consequently producing S-S bonds among adjacent protein chains, promoting also the exposition of hydrophobic groups which proportionate hydrophobic interactions during the drying (Perez-Gago and Krochta, 2001).

Considering that the sarcoplasmic proteins also have the capacity to form a continuous matrix, Paschoalick et al. (2003) supposed that edible films could be produced by the mixture of these proteins with myofibrillar proteins, avoiding the washing process of the muscles. And thus, these authors developed flexible films based on Nile Tilapia muscle proteins, i.e. containing myofibrillar and sarcoplasmic proteins. After that, others work on production and characterization of films based on muscle proteins of fishes were published (Sobral et al., 2004, 2005, Garcia and Sobral, 2005).

a) Some characteristics of fish muscle proteins

According Table 2, the polar ionic amino acids (aspartic acid, glutamic acid, arginine and lysine) are in high concentration in both the muscle proteins (Paschoalick et al., 2003) and the myofibrillar (Monterrey-Quintero and Sobral, 2000) proteins from Nile Tilapia. The difference between the compositions of these proteins may be explained by the presence of sarcoplasmatic proteins in the muscle proteins.

Table 2. Amino acid composition (g amino acids/100 g of protein) for Tilapia proteins.

	Muscle proteins[1]	Myofibrilar proteins[2]
Alanine	5.50	5.00
Arginine	6.15	2.71
Aspartic acid	9.20	12.08
Glutamic acid	14.69	12.20
Phenylalanine	3.55	4.07
Cystine	0.78	0.67
Glycine	3.97	4.35
Histidine	2.05	2.57
Isoleucine	4.19	5.86
Leucine	7.35	8.36
Lysine	8.65	10.30
Methionine	2.30	3.15
Proline	3.03	8.95
Serine	3.48	4.41
Tyrosine	2.84	3.43
Threonine	4.18	4.63
Valine	4.29	6.22

1. From Paschoalick et al. (2003). 2. From Monterrey-Quintero and Sobral (2000).

These freeze-dried proteins, produced by Paschoalick et al. (2003) and Monterrey-Quintero and Sobral (2000), showed an interesting fluidity, i.e. without a characteristic of agglomeration. However, the muscle proteins were not as bright as the myofibrillar proteins of Nile Tilapia, which were almost white.

b) Some characteristics of fish muscle proteins

The control of the film thickness is very important and difficult, principally in the processes of production by casting. The fish muscle proteins, when made soluble by decreasing pH, provide extremely viscous colloidal solutions (Cuq et al., 1995). In high proteins concentration, the film forming solution behaves as fluid of Bingham, that means that it does not flow over the force of the own weight. In this in case, the solution can be spread with convenient equipment, controlling the height of the

solution in the support, that allows the knowledge of the thickness of the film after drying. This technique allows a good thickness control, but the high viscosity allows the production of bubbles and foams in the solution, decreasing the homogeneity of films after drying.
An important alternative is to work with diluted solutions. In this case, the solution is cast on a support and the control of the thickness may be made by controlling the weight of the solution applied in the support (Figure 3A).

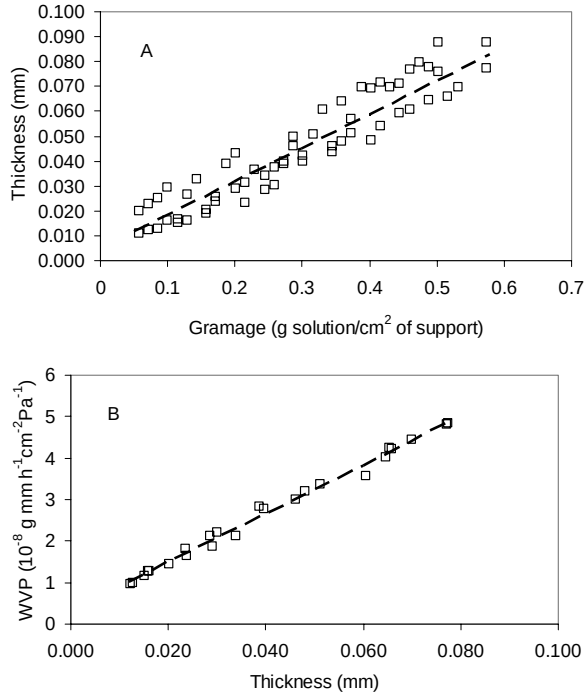


Figure 3. Average thickness (A) and Water Vapor Barrier Properties (B) of films based on myofibrilar proteins from Nile Tilapia (from Sobral, 2000).

Practically, the thickness of films affects all physical properties of films (Cuq et al., 1996, Sobral, 2000), and also the water vapor permeability (Figure 3B) contrarily to which could be expected (Sobral and Ocuno, 2000).
Among others factors, the physical properties of films based on fish muscle proteins may be affected by the plasticizer concentration and by thermal treatments of the film forming solution (Paschoalick et al., 2003).

The reduction of the puncture force with the increase of the plasticizer concentration is typical behavior of films based on proteins (Cuq et al., 1995, Gontard et al., 1993). The presence of plasticizers and water molecules, considering that the increasing of the glycerin content increased the final humidity of films, decreased the protein-protein interactions, increasing the mobility of polypeptide chains allowing the films less resistant and more elastic.

On another hand, Garcia and Sobral (2005) observed that the increase of the thermal treatment from 40ºC/30 minutes to 65ºC/30 minutes increased the resistance to the puncture of films based on muscle proteins from Tilapia. This behavior was possibly due to the formation of sulfite bonds between adjacent protein chains that proportioned more resistant films (Perez-Gago and Krochta, 2001, Choi and Han, 2002). But, the increasing of the treatment until 90ºC/30 minutes reduced the resistance of films, principally in low plasticizer concentration (Paschoalick et al., 2003). This last behavior may be occurred because the more intensive thermal treatment caused protein denaturation and consequently bury free –SH groups in the hydrophobic pockets such that they may remain unavailable for disulfide bond formation (Monahan et al., 1995).

Films produced by Tilapia muscle proteins are practically colorless. The films produced with muscle proteins from Thay and Nile Tilapia (Garcia and Sobral, 2005) presented color difference comparable to the color of soybean protein films ($\Delta E^* = 8.5\text{-}11.6$) (Kunte et al., 1997), but its were more colored than some films based on Nile Tilapia myofibrillar proteins ($\Delta E^* = 7\text{-}8$) (Sobral, 2000), egg albumins ($\Delta E^* = 1.7\text{-}2.3$) (Gennadios et al.,1996), and pigskin gelatin ($\Delta E^* < 3$) (Sobral, 1999). On another hand, these films produced by Garcia and Sobral (2005) were more opaque than the films of pigskin gelatin ($Y = 0.5$) which were extremely transparent (Sobral, 1999), and than those based on Nile Tilapia myofibrillar proteins ($Y = 3.5$) (Sobral, 2000). Monterrey-Quintero and Sobral (2000) studied the denaturation of Nile Tilapia muscle proteins by differential scanning calorimetry and determined that the sarcoplasmic proteins of these fish denatured at around 41ºC. Therefore, in some manner, the denaturation of sarcoplasmic proteins occurred during the thermal treatments (≥40ºC/30 minutes) and must have caused the increase of opacity.

5. EDIBLE FILMS FROM FISH GELATIN

The volume of gelatin used annually by the food industry worldwide is considerable and growing. Gelatin is used not only for its functional properties but also to increase protein content. It can enhance the elasticity, consistency and stability of food products, and it may be also used as an outer film to protect against drying, light and oxygen. Its use for encapsulation and film formation makes it also of interest to the pharmaceutical and photographic industries.
Gelatin and collagen have been extensively selected for the development of edible films and coatings. Protein-based films are generally superior to polysaccharide-based films in their mechanical and barrier properties (Cuq et al., 1998). This is attributed to a wide range of potential functionalities due to specific structure. A stronger intermolecular binding potential via covalent bonds has been found in protein-based films, rather than in the films from homopolymer polysaccharides (Cuq et al., 1995).
Gelatin is a protein compound derived by hydrolysis of animal collagen contained in bones and skins. The source, age of the animal, type of collagen and extracting conditions, all influence the properties of the gelatins (Johnston-Banks, 1990). For type A gelatins, the collagen rod is extracted in acid and solubilized without altering its original triple-helix configuration. Subsequent thermal treatment cleaves hydrogen and covalent bonds; this destabilizes the triple helix by means of a helix-to-coil transition, leading to conversion into soluble gelatin (Djabourov et al., 1993). Gelatin is normally manufactured from the waste generated during the animal slaughtering and processing, especially from bovine and porcine. However due to bovine illness (ESB) and religious objections to consume food from pig origin, the production of fish gelatin is getting increase.

a) Fish gelatin

The waste from fish filleting can account for as much as 75% of the total catch weight (Shahidi 1994). This waste is an excellent raw material for the preparation of high-protein foods, besides helping to eliminate harmful environmental aspects and improve quality in fish processing. About 30% of such waste consists of skin and bone with high collagen content. A mild acid pre-treatment is normally used for collagenous material containing a low concentration of intra- and interchain nonreducible crosslinks as with fish skins (Norland, 1990). The main drawback of fish gelatins is that gels based on them tend to be less stable and to have worse rheological properties than gelatins from land mammals. This has been largely related

to the considerably lower number of Pro-rich regions of the collagen or gelatin molecule in cold water fish, than in warm blooded animals (Ledward, 1986).
Skins from tropical-fish species, such as tilapia, have been described as an optimal raw material for gelatin production (Grossman and Bergman, 1992). However, gelatin extracted from cod skin shows poor rheological properties (Gudmundsson and Hafsteinsson, 1997). Thus, the use of fish for gelatin production has to take into account the wide diversity among the fish species, which present intrinsic differences in the collagen molecules present in their skin. In addition, the nature of the collagenous material from fish skins makes them more susceptible to degradation, in contrast to the more stable collagen from mammals (Asghar and Henrickson, 1982). As reviewed by Johnston-Banks (1990), the physical properties of gelatin depends not only on the amino acid composition, but also on the relative content of α-chains, β- or γ-components and higher molecular weight aggregates, as well as on the presence of lower molecular weight protein fragments.
In recent years a considerable effort is being done in the search for different fish species and extracting conditions to improve the properties of the resulting gelatins. Gelatins with high gelling capacity were obtained from the skin of flat-fish species, such as megrim and sole (Gómez-Guillén et al., 2002). Other fish species have been also reported as a source for gelatin extraction, as for example, pollock (Zhou and Regenstein, 2005), shark (Cho et al., 2004), Nile perch (Muyonga et al., 2004), yellowfin tuna (Cho et al., 2005) or bigeye snapper (Jongjareonrak et al., 2005). Modifications in the extraction processes were introduced by the use of different organic acids in the pre-treatment of skins (Gómez-Guillén and Montero, 2001; Giménez et al., 2005a), different salts in the washing of skins (Giménez et al., 2005b), or even by the application of a high pressure treatment (Gómez-Guillén et al., 2005).

b) Gelatin based film

Numerous works in the recent literature are available concerning the use of gelatin, alone or combined with other biopolymers, to obtain edible and biodegradable films (Arvanitoyannis et al., 1998; Menegalli et al., 1999; Sobral et al., 2001; Simon-Lukasik & Ludescher, 2004; Bertan et al., 2005). An exhaustive and well documented review about properties of gelatin and gelatin films was reported by Arvanitoyannis (2002). However, all this information is related to commercial gelatin from

mammals. Although a considerable attention to the research on fish gelatin films is starting, until now the information available is very limited.
Gelatin films from skins of Nile perch, a warm water fish species, were reported to exhibit stress and elongation at break similar to that of bovine bone gelatine (Muyonga et al., 2004). On the contrary, Nile perch bone gelatin, consisting of a high proportion of low molecular weight fractions as a result of the more severe heat treatment needed for extraction, offered considerable lower film strength but higher elongation than the corresponding films from skin. Similarly, in a study comparing mechanical properties of gelatin films from tuna and halibut skins, the lower average molecular weight of halibut gelatin was related to the lower breaking force and noticeably higher elongation of the resulting films (Gómez-Guillén and Montero, 2003; Thomazine et al., 2005a). Dinamic viscoelastic studies of 6.67% solution of both type of gelatins showed a melting temperature of ≈10ºC and 25ºC for halibut and tuna gelatins, respectively (Habitante et al., 2005). The melting temperatures of both types of gelatin were a direct consequence of differences in molecular weight distribution. The corresponding films obtained with both fish gelatins, using sorbitol as plasticizer, were found to be less resistant but more elastic than with more conventional gelatins from bovine or pig skin using a comparable amount of plasticizer (Sobral et al., 2001). Studies performed with two halibut gelatins, having slightly different average molecular weight, showed that films from the higher molecular weight gelatin, which as observed by DSC studies also showed a higher glass transition temperature, were significantly more resistant, more rigid and less elastic than the film from the lower molecular weight gelatin (Thomazine et al., 2005a). Halibut skin gelatin films were also characterized by a lower water vapor permeability (WVP) than the reported for mammalian gelatin films in Sobral et al. (2001) or Vanin et al. (2005). Films from tuna skin gelatin plasticized with glycerol have been also shown to present lower WVP than the reported in pig skin gelatin (Montero and Gómez-Guillén, 2005). Sobral et al. (2001) reported WVP in pig skin gelatin films to increase linearly using sorbitol in a concentration ranging from 15 to 65 g /100 g gelatin. When using 25 g glycerol/100 g pig skin gelatin, WVP was found to be considerably higher (Thomazine et al., 2005). Glycerol is well recognized to present a higher plasticizing effect than sorbitol, causing an increase in film flexibility but a reduced resistance and WVP (Gennadios et al., 1996). Thomazine et al. (2005b) explained this behaviour in terms of molecular weight and number of molecules of plasticizers in the films. Similar findings have

been recently reported working with different concentrations and type of plasticizers in films from skin gelatin of bigeye snapper and brownstripe red snapper (Jongjareonrak et al., 2006a), showing the latter generally higher mechanical properties at any protein and glycerol concentrations tested than the former (Jongjareonrak et al., 2006b). The tendency of fish gelatin films to exhibit lower WVP than in land animals gelatin films could be explained in terms of aminoacid composition, since fish gelatins are known to be less rich in proline and hydroxyproline (Norland, 1990), thus increasing their hydrophobicity as compared to mammals gelatins. As in the case of other films of protein nature, fish gelatin films represent also a good barrier against UV light. Jongjareonrak et al. (2006b) reported that differences in light transmission between the films from bigeye snapper and brownstripe red snapper might be due to the differences in composition, density and the aggregation or alignment of gelatin molecules in the film. Tuna and halibut skin gelatin films presented also high light barrier properties, especially those from tuna gelatin, having higher molecular weight (Habitante et al., 2005).

The properties of fish skin gelatin-based films, especially water vapor permeability have been shown to improve by the addition of fatty acids or their sucrose esters (Jongjareonrak et al., 2006c). These authors also observed that light transmission and tensile strength of films decreased with increasing the amount of fatty acids. However, incorporation of the sucrose esters generally increased tensile strength and reduced elongation at break. Tuna skin gelatin has been also used to produce transparent and flexible films with added aqueous plant extracts, without modifying significantly mechanical and vapor barrier properties (Montero and Gómez-Guillén, 2005). The resulting films were characterized by an increased antioxidant capacity as a result of the high content of polyphenolic compounds. In this connection, gelatin films enriched with oregano or rosemary aqueous extracts have been successfully applied to improve the conservation of cold-smoked fish by reducing significantly the lipid oxidation during chilled storage (Gómez-Estaca et al., 2006). On the other hand, the unique property of gelatin to produce thermoreversible gels upon cooling make it also an adequate material to produce coatings for preservation of foodstuffs under refrigerated conditions. In this sense, fish hamburgers coated with a blend of fish gelatin and chitosan were reported to show a noticeable reduction in microbial growth during chilled stored, without altering rheological properties (Lopez-Caballero et al., 2005).

6. FEATHER KERATIN FILMS

Feathers are an important waste disposal of poultry industry and are composed basically by structural proteins, the keratins, which have a great ability to form films. It has been known that the stability of the keratin in the solid state is due to cross-linking produced by the formation of cystine disulphide bonds, hydrogen bonds and to salt linkages (Schrooyen et al., 2001b). It is possible to dissolve keratin using solvents that break the –S–S– bonds and the hydrogen bonds (Schrooyen et al., 2000, 2001a). The sodium dodecyl sulfate (SDS) can be used to obtain a stable dispersion of keratins (Yamauchi et al., 1996; Schrooyen et al. 2000, 2001a).
The regeneration of disulfide bonds in these materials during the drying of film forming dispersion may be used as a method to impart water insolubility and good mechanical properties to the films. The aim of this study was to prepare and characterize some properties of feather keratin films.

a) Preparation and characterization of feather keratin films

Keratin was extracted from white chicken feathers. Freshly plucked wet feathers were cleaned according to ASTM (1997), dried in a ventilated oven at 40ºC for 72 h and cut into small filaments (75-700 μm). This material was treated in a Soxhlet device for 12 h with petroleum ether to remove grease. The keratin extraction was performed according to the method proposed by Schrooyen et al. (2000) and Yamauchi et al. (1996), with some modifications (Martelli et al., 2006). Fifty ml of the aqueous dispersion of the reduced keratins (7 g keratin/100 ml dispersion) were mixed with glycerol in the following concentrations: 0.01, 0.03, 0.05, 0.07 and 0.09 g/g of keratin, which were used to prepare the films by the casting procedure. Prior to properties determinations, films were conditioned at 35ºC and 75% relative humidity (RH) for 48 hours. The films solubility (%) in water and the films swelling (SW) were determined according to the methods proposed by Turhan and Sahbaz (2004) and Lee et al. (2004), respectively. The mechanical properties of the films were determined by the tensile tests, with a texturometer TA.XT2i (SMS, Surrey, UK). Thermal properties of films were studied using a DSC analyses.

b) Some characteristics of feather keratin films

The extracted keratin presented a high content of sulfured amino acids, namely cysteine and methionine (Moore et al, 2006). These results agree with those found in the literature for wool keratin (Yamauchi et al., 1996) and for feather keratin from fowl (Arai et al., 1983). On another hand, it can be observed that the polar amino acids, ionizable (4.7% aspartic acid, 7.7% glutamic acid and 5.4% arginine) or not ionizable (9.3% serine, 8.8 proline and 3.5% threonine), represents almost 40% of total content of amino acid. Films properties are shown in Table 3. Water solubility increased from 30% for films without plasticizer to about 51% for films prepared with 0.09 g glycerol/g keratin. The relatively low solubility (30%) of keratin films without plasticizer allow to suggest that some -S-S- bridges were formed during drying of films, although others interactions such as hydrogen bonds and electrostatic and hydrophobic interactions could also be improved. The results indicated also that the addition of glycerol avoided the formation of these bridges, increasing the films solubility. Swelling was 155 %, for films without glycerol, increasing to 207% for films with 0.07 g glycerol/g keratin. Yamauchi and Yamauchi (2002) reported swelling data of 140% for films of wool keratin plasticized with 50 g glycerol/100g keratin.

Table 3 - Films Solubility, films swelling index in water, glass transition temperature and mechanical properties of keratin films.

Glycerol concentration (g glycerol/g keratin)	*Film solubility in water (%)*	*Swelling index (%)*	*Glass transition temperatures of dry films (°C)*	*Mechanical properties*		
				Tensile strength (MPa)	Elongation at break (%)	Young module (MPa/%)
0.00	30.7 ± 1.5	155±7	70	16.6±5.5	1.7 ± 0.2	10.2 ± 7.1
0.01	--	--	--	6.3 ± 0.7	11.9 ± 2.6	2.0 ± 0.8
0.03	37.0 ± 1.3	182±2	67.5	7.6 ± 0.6	13.8 ± 2.4	2.1 ± 0.6
0.05	40.6 ± 3.8	191±35	64.5	5.3 ± 0.7	19.8 ± 4.1	1.2 ± 0.2
0.07	48.3 ± 2.7	207±15	56.3	5.4 ± 0.5	30.5 ± 7.7	0.9 ± 0.6
0.09	50.7 ± 1.0	110±13	--	2,0 ± 0.2	31.9 ± 4.5	0.2 ± 0.0

Films without plasticizers showed brittle characteristics. The addition of 0.01 g glycerol/g keratin decreased the tensile strength in about 38% when compared with the value found for the films without glycerol, while the addition of 0.09 g glycerol/g keratin decreased 8.3 times this property, which represents about 12% of the tensile strength for the non-plasticized films. Similar results were observed with the Young Modulus, which decreased from 10.18 to 0.21 MPa when 0.09 g glycerol/g keratin was added to the films. On the other hand, glycerol had a positive effect on the films elongation that increased about 7 and 18.8 times for films prepared

with 0.01 and 0.09 g glycerol/ g keratin, respectively. Yamauchi and Yamauchi (2002) found values ranging between 5 MPa (films conditioned at 85% RH) and 11 MPa (films conditioned at 65% RH), for films plasticized with 0.5 g glycerol/g keratin. As expected, the glass transition temperatures (Tg) decreased with the increasing of the glycerol. The values of Tg obtained in this work implies that the dry films were in the glass state when conditioned at room temperature (T<Tg), but these films with plasticizer constituted flexible materials. This behavior was also reported for others protein-based films (Sobral et al., 2001, 2005).
In conclusion, it is possible to prepare films with good properties from keratin extracted of chicken feather. Low glycerol concentrations are enough to modify significantly the films mechanical properties. These films showed relatively low water solubility when compared with the values of other protein-based films present at literature.

7. EDIBLE FILMS BASED ON SOY PROTEINS

a) Soybean Proteins

Protein accounts for approximately 38 – 44 % of soybean composition, and 7S and 11S globulins constitute the 90% of the total proteins (Fukushima, 1991). The 7S globulin or β-conglycinin is a trimeric glycoprotein of 150 to 200 kDa while the 11S globulin or glycinin is a heterogeneous oligomeric protein of 360 kDa (Lawrence et al., 1994). Based on the protein content, soy proteins used in the food industry are classified as soy flours (50-59%), soy concentrates (65-72%) or soy isolates (more than 90%).
Physicochemical and functional properties of soy globulins have been widely studied. Both globulins (7S and 11S) are soluble in water and neutral saline solutions and develop an extensive range of functional properties. From all of these properties, those that are dependent on the molecular superficial characteristics (capability to form foams and emulsions) and on the protein–protein interactions (capability to form networks, particularly gels and films) are the ones that present bigger perspectives to their development.
The development of edible films from plant proteins represents an interesting way to increase the utilization of these proteins as well as to reduce the environmental problems generated by the accumulation of synthetic polymers (Gennadios 2002).

b) Soy Protein Isolate (SPI) Films

A native SPI (SPIn), which was obtained in the laboratory from soybean defatted flour by alkaline extraction and isoelectric precipitation, was used as raw material (Petruccelli and Añón 1995).
Relationship among structural, mechanical and physical properties of soy protein isolate films was studied using the pH of initial film forming solutions as a variable, because pH affects the protein charge and the degree of protein denaturation.
Films were obtained by casting from aqueous dispersions of 5% w/v SPI and 2.5% w/v of glycerol (used as plasticizer) at pH 2, 8 and 11, and dried at 60ºC.
DSC studies showed that proteins retained their native conformation in films at pH 8, but were partially or extensively denatured at pH 11 and 2. Once unfolded, protein chains could interact strongly, mainly through disulfide bonds, SEM verified producing denser protein networks. These films at pH 2 and 11, showed a greater tensile strength – by about 1.05 MPa, and a higher Young´s modulus – by at least 0.15 MPa, than the film made from the pH 8-SPI solution. However, films formed at alkaline pHs (8 and 11) exhibited about 70% greater deformation than films at pH 2. It seems that the native proteins may unfold during the mechanical test providing a greater deformation before breaking (Table 4).

Table 4. Mechanical properties, WVP and thickness of SPIn films at pH 2, 8 and 11, SPIc at pH 10.5, and bilayers films

Film	**TS (MPa)**	**E (%)**	**WVP.10^{10} $gm^{-1}s^{-1}Pa^{-1}$**	**Thickness (μm)**
SPIn pH 2	1.50±0.09	92.64±13.72	3.52±0.21	114.6±6.2
SPIn pH 8	0.45±0.05	163.59±14.91	2.86±0.58	94.6±8.0
SPIn pH 11	1.90±0.28	205.41±25.67	2.84±0.25	105.1±9.3
SPIc pH 10.5	1.86±0.32	144.9±18.3	2.28*±0.66	62.8±10.3
SPIc-Beeswax	2.70±0.19	197.9±23.4	0.12±0.08	87.9±13.9
SPIc-Sunflower	2.28±0.21	173.9±9.9	1.23*±0.08	80.2±13.0
SPIc-Carnauba	2.18±0.34	177.6±33.6	0.76±0.38	94.0±9.62

Solubility assays in buffers with different chemical activity (water, phosphate, with urea, SDS and urea-SDS-mercaptoethanol) and the analysis of the electrophoretic profiles of the soluble protein fractions, showed that, at pH 2, the protein networks were mainly stabilized by

hydrogen and disulfide bonds, and that when the pH of the initial solution was increased, the hydrophobic interactions also become important. Films at acid pH had a higher water vapor permeability (WVP) and lower Tg than the films obtained at alkaline pH (Table 4), confirming than the polymers linked by hydrogen bonds are more susceptible to moisture.
The effect of storage on the films properties was studied. Films kept stable for long periods of time (at least up to 120 days at 20ºC and 59% RH), as no variations in their mechanical and barrier properties, and in their color parameters were observed.

c) Soy Globulins Films

Films formation from the 7S and 11S pure globulins was studied in order to know the role of each fraction in SPI films. These proteins were obtained in the laboratory from defatted soy flour as was described by Nagano et al. (1992).
It was observed that the 7S film differs from 11S one especially in their mechanical properties but not in their WVP, as also was observed by other authors (Cho and Rhee, 2004; Kunte et al. 1997). Figure 4 shows the stress-strain curves obtained by tensile tests for 7S, 11S and SPIn films at pH 2 and 10.5.

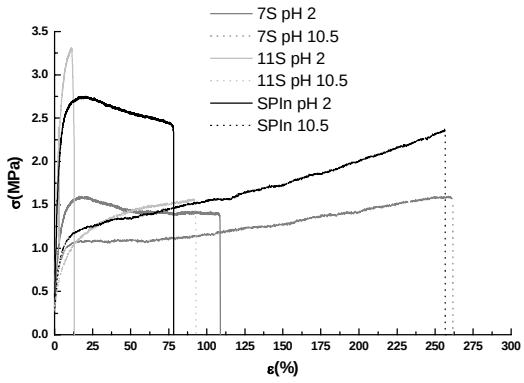


Figure 4. Stress-strain curves of SPIn, 7S and 11S films at pH 2 and 10.5 obtained by tensile test

In films at acidic pH, proteins were totally denatured and could interact chain to chain easily and strongly, producing denser films with higher

tensile strength and lower elongation than basic films. 7S films were more elastic, while the 11S were more resistant due to their higher tendency to form disulfide bonds. At alkaline pH, this effect was not so evident because of the 11S lower capacity to be deformed and the stress-strain behavior. The high tensile strength of 7S could be in part attributed to the presence of 11S as impurity in 7S-enriched fraction when it is obtained by Nagano procedure, as demonstrated by DSC and electrophoretic assays. The mix of both globulins in SPIn improved the film mechanical properties, showing a synergic effect of both globulins. SPI films presented similar elongations than the 7S ones and alike tensile strength than 11S films, indicating the presence of interactions between both fractions in SPI films.

d) Soy Protein Isolates- Waxes Bilayers Films

In order to improve WVP of SPI films, lipids were included in their formulation. Waxes of different origin (bees, carnauba and sunflower) were used as lipid material (Gontard et al. 1995), and a commercial SPI (SPIc, Samprosoy 90 EG, Bunge Alimentos) as protein source.
Bilayer films were obtained spreading a thin layer of the molten waxes on a protein film, which was previously made by casting from soy protein isolates dispersions, using glycerol as plasticizer. All composite films showed a higher yellowness color and a higher opacity (as was demonstrated by the increased in the Hunter Parameter b and the decreased of L), lower water vapor permeabilities and higher tensile strengths than soy protein films, without modifying their elongation at break. Beeswax films presented the best properties (WVP 19 times lower and tensile strength 45 % higher than protein films) probably due to they contained a higher amount of lipid compounds in its formulation and its lipid layer was more homogeneous than the ones formed by the other waxes (as was seen by SEM) (Table 4).
Beeswax-SPI film showed a great stability during storage at 20°C and 75% of relative humidity. Till 120 days, WVP and tensile strength did not change.
As plasticizer concentration play an important role in protein films behavior, they were used as a variable to evaluate the role of each component (proteins and lipids) in the functionality of these bilayers films. So bilayer films were formed applying the melted beeswax on SPIc films containing sorbitol or glycerol as plasticizers, in different concentrations

(1, 1.5, 2, 2.5 and 5%) and were compared with the corresponding protein film.
As was expected, the increment in plasticizer concentration increased the elongation, WVP and moisture content of protein films, while decreased their puncture and tensile strength, being these effect greater for glycerol than for sorbitol. The increased of plasticizer concentration show the same tendency on mechanical properties for bilayer films. And no differences in strength and elongation were found between the bilayer film and its corresponding protein films (both with the same type of plasticizer and concentration). WVP did not show differences among bilayer films with different plasticizer concentrations and between films with glycerol and sorbitol, but it was significantly lower in bilayers than in SPI film.
These studies suggest that the protein film dominates mechanical properties, as differences were not seen neither in deformation nor in strength between films with or without the wax lay. And the beeswax layer determines the WVP and the color of the bilayer films, since both parameters do not vary with the type and concentration of plasticizer.

8. EDIBLE FILMS FROM AMARANTH

Protein films are made of raw materials of high molecular weight such as gelatin, myobrilar proteins, gluten, and whey protein, among others. These films are characterized by good mechanical properties, although they are usually quite permeable to water vapor and gases (McHugh & Krochta, 1994).
Starch films have also received a lot of interest since they are very low cost materials, the most abundant and important polysaccharide in nature. Numerous studies have been reported on its film forming capabilities and industrial applications. Also, various studies have attempted to improve the functional properties of these films, by using mixtures of protein and starch as the raw material for the formation of edible films (Parris et al., 1997, Arvanitoyannis et al., 1996,1997).
However, when the raw material cereals, it is not necessary to extract the starch nor the proteins for film production. Rayas and Hernández (1997) prepared edible films from three types of wheat flours, and more recently, Mariniello et al. (2003) used whole soy flour and apple pectin as the raw materials for producing edible films.
It is interesting to investigate other new renewable resources for the production of edible and biodegradable materials. The use of natural blends of protein, polysaccharides and lipids directly obtained from

agricultural sources, take advantage of each components in the original system, appears as a new opportunity of material in the area of edible films. The *Amaranthus cruentus* flour can be considered such natural mixture and consequently an interesting source of raw material for the edible film technology.

Amaranth is a pseudocereal of rapid growth, with a high tolerance to arid conditions and poor soils where traditional cereals cannot be grown, so many countries have been adapting certain varieties to their soils. The main cultivars used are *Amaranthus hipochondriacus, Amaranthus cruentus* and *Amaranthus caudatus*. The grain composition is a function of the cultivars and of the environmental conditions of culture (Saunders & Becker, 1984). Amaranth (*Amaranthus* spp.) is a tiny grain (~1 mm diameter) typical from South America, which the specie (*Amaranthus cruentus*) is composed of 15-22% protein (rich in lysine), 3.0-11.5% fat and 9-16% dietary fiber, depending on cultivation technique and environmental effects. The Amaranth main constituent is the starch, 48-62%, with small granule size (< 1μm), characteristic that allows easier dispersion and hence it may yield good properties of resultant films.

a) Films based on amaranth flours

Tapia-Blácido et al. (2005,2006) obtained films using amaranth flour of two varieties, *A.caudatus* and *A.cruentus.* The amaranth flours were produced by the alkaline wet milling method. The chemical compositions of both materials are shown at Table 5. *Cruentus* variety has higher amylose content and lower lipid content than the *caudatus* one. The analysis of fatty acids content revealed a major fraction, up to 92% of linoleic acid (C18:2), oleic acid (C18:1), palmitic acid (C16:0), with composition depending on variety (Tapia-Blácido,2006).

Table 5- Composition of Amaranth varieties.

Component (g/100g dried mass)	*Amaranth flour (Amaranthus cruentus)*	*Amaranth flour (Amaranthus caudatus)*
Ashes	2.10 ± 0.03	2.11±0.03
Lipids	7.96 ± 0.19	8.93±0.03
Protein	14.08 ± 0.	14.21±0.32
Starch	75.7 ± 0.25	74.75±0.25
Amylose	11.89 ± 0.26	7.58±0.77

These authors obtained films using the casting process using glycerol (*caudatus*) and glycerol and sorbitol (*Cruentus*) as plasticizers. The influence of the most relevant variables of the process of film production on film properties, were studied by a full factor design. The influence of plasticizer content, pH, temperature of heating process, and temperature and relative humidity (RH) of the air during the film drying on mechanical and barrier properties were evaluated and the optimal process conditions were found in each case.

The best mechanical properties and the lower solubility were found, for films made with *A. caudatus* flour, at a process temperature of 82ºC, dried at 40ºC and 55% RH, glycerol content 22.5% and pH of 10.7 (Tapia-Blácido et. al, 2005). Meanwhile, the optimal process conditions for the *A. cruentus* films were obtained at 75ºC and 20.02% of glycerol content. The Table 6 shows the properties of films measured at those conditions (Tapia-Blacido, 2006).

Table 6- Properties of amaranth flour films.

Properties	Flour film (A.caudatus)*	Flour film (A.cruentus)**
Solubility (%)	42.25 ± 1.82	40.64±2.36
Puncture test		
Force (N)	2.34 ± 0.09	4.09 ± 0.37
Deformation (%)	15.62 ± 0.65	4.91 ± 0.79
Tensile test:		
Tensile strength (MPa)	1.455 ± 0.045	5.35 ± 0.85
Elongation at break (%)	83.736 ± 5.107	12.93 ± 5.23
Young Modulus (MPa)	21.50 ± 1.40	252.00±12.40
Water permeability ($g\ m^{-1}\ s\text{-}^{1}Pa^{-1}$)	(8.20±0.15) $x10^{-11}$	(3.76±0.22) $x10^{-10}$
Oxygen permeability ($cm^3.\mu m/m^2.dia.kPa$)	5.63±3.68	22 ± 0.71
Optical properties		
a*	-1.16 ± 0.01	-1.16±0.01
b*	8.09 ± 0.49	12.44±0.24
L*	89.97 ± 0.28	87.28±0.15
ΔE*	8.89 ± 0.62	13.35 ± 0.28
Opacity	15.24±0.90	17.04 ± 0.84

*Films made with 22.5% glycerol (g glycerol/100 g flour). **Films made with 20.02% glycerol (g glycerol/100 g flour)

Films are not very tough and their properties are function on variety. Nevertheless, these films furnish better barrier properties than other edible films. This was unexpected fact as the major constituent of Amaranth is a waxy starch. The presence of native lipids and proteins of its formulation may explain the lower permeabilities of these materials. However, some noticeable differences between both varieties are verified that are not completely defined by differences in composition of both materials. Therefore, the interaction between the components may differ in both materials resulting in diverse structures.

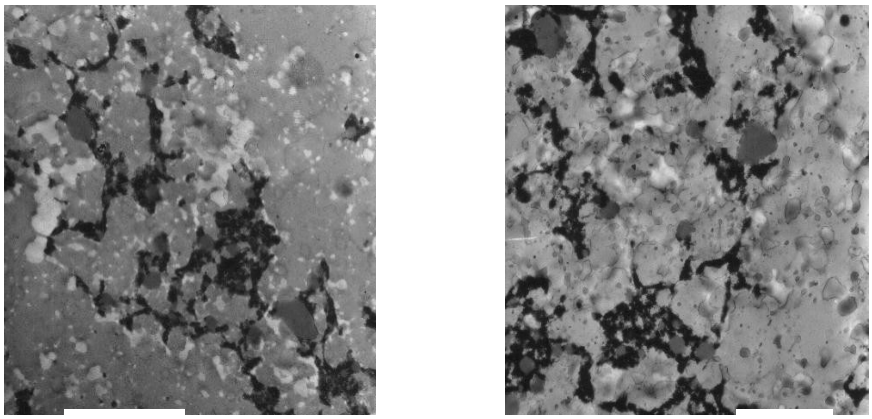
The changes in plasticizer also result in differences in process conditions and properties. Tapia-Blácido (2006) made films with Amaranth flour (*A. cuentus*), plasticized with glycerol and sorbitol. The optimal conditions with glycerol and sorbitol were: 20.02 and 29.6% for plasticizer content and 75ºC of process temperature. The drying condition also varied with the change of plasticizer. The optimal drying conditions were: 46.8ºC and 74.7% of relative humidity when glycerol was used and 28.6ºC e 72.5% for sorbitol. The Table 7 show properties of the films as a function of the plasticizer used. Better water vapor and oxygen barrier and mechanical properties were verified using sorbitol. Although, the solubility was higher when using sorbitol as plasticizer.

Table 7- Film properties with glycerol and sorbitol as plasticizer.

Properties	Films of flour with glycerol	Film of flour with sorbitol
Solubility	40.64±2,36	50.79±4,35
Tensile strength (Mpa)*	4.09±0,37	6.72±0,43
Elongation (%)*	4.91±0,79	2.82±0,32
Water Permeability ($g.m.s^{-1}m^{-2}.Pa^{-1}$)	3.761×10^{-10} $\pm 2.25 \times 10^{-11}$	3.10×10^{-10} $\pm 1.95 \times 10^{-11}$
Oxygen permeabilidade ($cm^3.\mu m/m^2.24h.kPa$)	22 ± 0.71	13.5 ± 1.77

*Tensile test at break, measured at 57% relative humidity

Figure 5 shows the structure of both films, formed by a protein network (dark zones), linked to lipid globules, and dispersed through the starch phase (gray surfaces).



(a) (b)

Figure 5 – TEM micrographs of films plasticized with glycerol (a) and sorbitol (b).

The lipid phase reduced permeabilities and also acts as plasticizer. Some improvements of properties can be obtained if additional lipids are mixtured to the flour. The feasibility of producing composite films of amaranth flour and stearic acid with improved water vapour barrier, low oxygen permeability and acceptable mechanical properties was demonstrated by Colla et al. (2005). The addition of stearic acid to films of amaranth flour (*Amaranth cruentus*) decreases solubility up to 15.2% and water permeability to 8.9×10^{-11} g/m·s·Pa. The optimal conditions of process were: stearic acid concentration: 10 g/100 g flour; glycerol concentration: 26 g/100 g flour and stirring speed: 12000 rpm.
It can be conclude that Amaranth flour seems to be an interesting material for the formulation of coatings and edible films.

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