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HYDROCOLLOID-BASED EDIBLE FILMS: COMPOSITION-STRUCTURE-PROPERTIES RELATIONSHIP

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PART I

1. INTRODUCTION

In the past 50 years, impressive advances have been made in the production of synthetic polymer films designed to protect foods, pharmaceuticals, and other products. With the increasing population and stress on limited resources and the environment, uses of renewable resources to produce edible and biodegradable films that can improve product quality and/or reduce waste disposal problems are being explored (Gennadios, 2002). Consequently, research edible coatings and films has been quite intense in the recent years, and there exists indication that interest will continue, as motivations for further developments in this field arose from several directions.

Consumer interest in health, food quality, convenience and safety continue to increase, facing food industry with new challenges to which edible coating and film concepts offer a potential solutions. These film, in fact, by acting as barriers to moisture or oxygen, edible coatings can reduce the complexity of packaging not improving their recyclability. In some cases, edible films may even be able to replace synthetic packaging films. In addition, food scientists and engineers have shown that there are many new materials of biological origin and that offer new opportunities in packaging. In many cases these materials are quite abundant in nature and have previously been regarded as surplus or waste and these materials can be combined in new and creative ways to enhance even more their coating and film properties.

1.1. The historical view of edible coatings

Although the use of edible films in food products may seem new, food products were first enrobed in edible films and coatings century ago. Wax was the first edible coating used on fruits and vegetables. The Chinese applied wax coatings to oranges and lemons in the twelfth and thirteenth centuries. Although the Chinese did not realize that the full function of edible coatings was to slow down respiratory gas exchange, they found that wax coated fruits

could be stored longer than non-waxed fruits. Yuba, a proteic edible film obtained from the skin of boiled soy milk, was traditionally used in Asia to improve the appearance and preservation of some foods since the fifteenth century (Park, 1999).

In England, during the sixteenth century, "larding", coating food products with fat, was used to prevent moisture loss in foods. Later in the last century, the preservation of meat and other foodstuffs by coating them with gelatin films was suggested. In the 1930s hot-melt paraffin waxes became commercially available as edible coatings for fresh fruits such as apples and pears (Han and Gennadios, 2005). The more important application of edible films and coatings until now, and particularly since the 1930s, concerns the use of an emulsion made of waxes and oil in water that was spread on fruits to improve their appearance, such as their shininess, colour, softening, carriage of fungicides and to better control their ripening and to retard the water loss. Currently, edible films and coatings find use in a variety of applications, including casings for sausages, chocolate coatings for nuts and fruits, and wax coatings for fruits and vegetables. In general, there are more applications of coatings than of films. Shellac and wax coatings on fruits and vegetables, zein coatings on candies and sugar coatings on nuts are the most common commercial practices of edible coatings (Krochta, Baldwin, Nisperos-Carriedo, 1994).

It has been established that the ripening of fruits can be retarded by a coating of SFAE (mixture of sucrose fatty acid esters) that is non-phytotoxic, tasteless, odourless. SFAE mixtures have been commercially available for coating fruits and vegetables since the 1980s, under the trade names 'TAL Pro-long' and 'Semperfresh' (Park, 1999).

A number of edible polysaccharide coatings, including alginates, carrageenans, cellulose and derivates, pectin and starch derivatives have been used to improve stored meat quality. The pharmaceutical industry uses sugar coatings on drug pills and gelatin films for soft capsules (Gennadios, 2002; Krochta 2002).

Over the last 40 years, a great number of works on the formulation, application and characterization of edible films and coatings can be found in both scientific and patent literature.

1.2. Definitions

Edible films and coatings generally can be defined as thin layers of edible material applied on foods, or between food components, by wrapping, brushing, immersing or spraying in order to control mass transfer, provide mechanical protection or add sensory appeal to food products (Gennadios and Weller, 1990). There is no clear distinction between films and coatings and often the two terms are used interchangeably.

Films are normally defined as stand-alone thin layers of materials, being formed separate of any eventual intended use. They, usually, consist of polymers able to provide mechanical strength to the stand-alone thin structure. These stand-alone films also are used as testing structures for determination of barrier, mechanical, solubility and other properties provided by a certain film material. Such films can be used as covers, wraps or separation layers. They can be potentially formed into casings, capsules, pouches and bags.

Coatings involve formation of films directly on the surface of object they are intended to protect or enhance in some manner. In this sense, coatings become part of the product and remain on the product through use and consumption. Therefore, coatings are regarded as a part of the final product (Krocta *et al.*, 1994; Gennadios, 2002;). If we consider the edible films and coatings definition, they could be classified as foods. However, in the most cases, edible packagings do not provide a significative nutritional value to the coated food and, thus, they should be considered more like an additive than an ingredient. It all depends on the application of the edible packaging. It can also be used to improve the nutritional quality of the food, and thus be qualified as a food ingredient. As food components, edible films and coatings have to be tasteless in order not to be detected during the consumption of the edible-packaged foods. When they have a significant or particular taste and flavour, their sensorial characteristics have to be compatible with those of the foods.

Because of they are both a packaging and a food component, edible films and coatings have to satisfy some requirements:

- non polluting;
- simple technologies;
- low cost of raw materials and process;
- good sensory quality;
- good barrier properties and mechanical efficiencies;
- enough biochemical, physico-chemical and microbial stability;
- free of toxics and safe for health.

Advantages for edible films over other traditional polymeric packaging materials are:

1. The films can be consumed with the packaged product. This is obviously of critical importance since it represents the environmentally ideal package.
2. Even if the films are not consumed they could still contribute to the reduction of environmental pollution. The films are produced exclusively from renewable, edible ingredients and therefore, are anticipated to degrade more readily than polymeric materials.
3. The films can enhance the organoleptic properties of packaged foods provided that various components (flavourings, colourings, sweeteners) are incorporated to them.
4. The films can supplement the nutritional value of the foods. This is particularly true for films made from proteins.
5. The films can be used for individual packaging of small portions of food, particularly products that currently are not individually packaged for practical reasons such as peas, beans, nuts, and strawberries.
6. The films can be applied inside heterogeneous foods at the interfaces between different layers of components. They can be tailored to prevent deteriorative inter-components moisture and solute migration in foods such as pizzas, pies, and candies.
7. The films can act as carriers for antimicrobial and antioxidant agents. In a similar application they also can be used at the surface of foods to control

the diffusion rate of preservative substances from the surface to the interior of the food.

8. The films can be very conveniently used for microencapsulation of food flavouring and leavening agents to efficiently control their addition and release into the interior of foods
9. Another possible application for edible films could be their use in multilayer food packaging materials together with nonedible films. In this case, the edible films would be the internal layers in direct contact with food materials (Gennadios and Weller, 1990; Debeaufort, Gallo and Voilley, 1998)

1.3. Edible ingredients

Edible films are a mixture of a high molecular weight polymer, solvent and a plasticizer. Several ingredients such as antimicrobial compounds, antioxidants, flavours, colouring and others can be incorporated into there (Krochta *et al.*, 1994). Edible films and coatings are produced from edible biopolymers and food-grade additives. Components used for the preparation of these materials are classified into three categories: hydrocolloids (such as proteins or polysaccharides), lipids (such as fatty acids, acylglycerols or waxes) and composites (made by combining substances from the two previous categories). Plasticizers and other additives are combined with the film-forming biopolymers to modify the physical properties or functionality of films. In order to maintain edibility, solvents used are restricted to water and ethanol.

1.3.1. Hydrocolloids

Hydrocolloid films can be used in applications where control of water vapour migration is not the objective. These films possess good barrier properties to oxygen, carbon dioxide and lipids. Most of these films also have desirable mechanical properties, making them useful for improving the structural integrity of fragile products. Water solubility of polysaccharide films is advantageous in situations where the film will be consumed with a product that is heated prior to consumption. During heating, the hydrocolloid film or

coating would dissolve, and ideally, would not alter the food sensory properties.

Hydrocolloids can be classified according to their composition, molecular charge, and water solubility. In terms of composition, hydrocolloids can be either carbohydrates or proteins. Film-forming carbohydrates include starches, plant gums (for example alginates, pectin, and gum arabic) and chemically modified starches. Film-forming proteins include gelatine, casein, soy protein, whey protein, wheat gluten, and zein. It should be noted, however, that great differences exist in how easily films having good integrity can be formed from these substances. The charged state of a hydrocolloid can be useful for film formation. Alginates and pectins require the addition of a polyvalent ion, usually calcium, to facilitate film formation. They, as well as proteins, are susceptible to pH changes because of their charged state. For some applications an advantage can be gained by combining hydrocolloids of opposite charge such as gelatin and gum Arabic (Krocta *et al*., 1994; Gennadios, 2002). A wide variety of edible proteins and polysaccharides have drawn attention for their film-forming ability. In particular, the *pectins* and soy proteins have been widely used as gelling agents, stabilizers, thickeners, and emulsifiers in many food products. They exhibits excellent film–forming properties.

1.3.1.1. Pectins

Pectins as defined for use in food are high molecular weight heteropolymers containing a majority (at least 65% by weight) of α-$(1 \rightarrow 4)$-linked D-galacturonic acid units. The acid group may be free (or as a simple salt with sodium, potassium, calcium or ammonium) or naturally esterified with methanol (Fig.1).

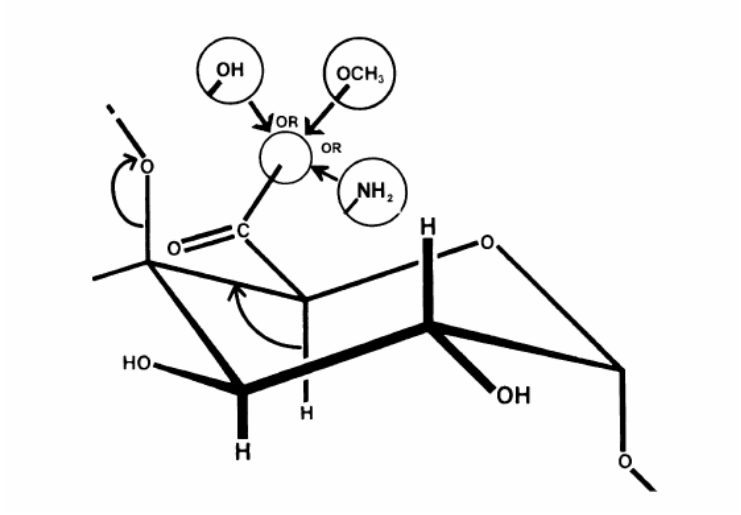

Figure 1. Galacturonic acid ester, and amide units found in pectins. Arrows indicate the potential for degradation by β-elimination in the ester form (From May C.D., 2000).

However, pectins are derived from the breakdown of more complex protopectins which are present in the plant tissue, and also contain a range of neutral sugars, including rhamnose, galactose, arabinose and lesser amounts of other sugars. These sugar units are present in a non-random structure, which consists of blocks of differing character retaining fragments of the original plant cell wall structure. The use of purified enzymes has shown that pectin extracted under very mild conditions contains both linear blocks (smooth regions) consisting of homopolygalacturonic acid, and highly branched blocks (hairy regions) which themselves contain several types of structures (Fig.2). It is not, at present, clear exactly how this complex structure and its variations influence the functional performance of commercial pectins, but the practical implications of certain basic aspects of structure are better understood. The regions of the pectin molecule which contain large galacturonic acid units consist of a mixture of methyl ester, free acid and salt derivatives of the carboxyl group of the acid.

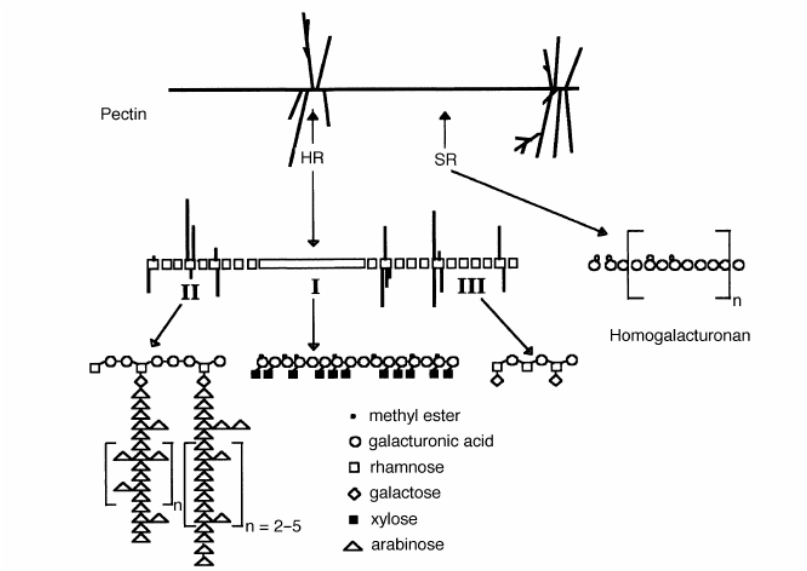

Figure 2. Hypothetical structure of apple pectin showing I xylogalacturonan region, II region with arabinan side chains, III rhamnogalacturonan region making up the 'hairy region' (From May C.D., 2000).

Because commercial pectins are extracted under hot acidic conditions, many of the regions containing a high proportion of neutral sugars are hydrolysed, leaving mostly the more acid-stable galacturonate blocks.

In certain pectins, such as those from sugar beet and potato, a proportion of the hydroxyl groups will also be acetylated. It is well known that the properties of pectin are dependent on pH, and on the percentage of acid group present in the form of ester (degree of esterification).

Commercial pectin: properties, modification and function

The pectin described above is normally from about 67–73% esterified. Apple pectin can, paying great care, be extracted up to 80% esterification. Pectin is readily degraded by a β-elimination mechanism at environment temperature or above at neutral or alkaline pH values. The ester groups can be hydrolysed under either alkaline or acidic conditions, or by pectin esterases. Commercially, acidic treatment is the most commonly used one producing pectins with about 60% of ester groups which are 'slow setting'. Under

identical conditions of 65% total sugar solids by refractometry and a pH of say 3.1, the gel will take much longer to set. The setting of these gels is both time and temperature dependent, and the setting temperature depends on the rate of cooling. Slow-setting pectins permit gels to be prepared at higher sugar contents, valuable for sugar confectionery, biscuit jams, and so on. Because of the higher charge density on the slow-set pectin molecules, there is also a change in the pH requirements for gelation towards a lower pH in gels of otherwise similar composition. Further de-esterification to below 50% esterification produces a range of 'low methoxyl' pectins. These show a markedly greater reactivity towards calcium ions, which will cause gelation under suitable conditions of soluble solids and pH. Conditions for effective gelation depend on a balance of several factors, including soluble solids content, pH, calcium and pectin concentrations, and the presence of sequestrants. Amidated pectins (mostly of the low methoxyl type) are produced by reaction of suitable high methoxyl pectins with ammonia. The reaction is normally carried out in an aqueous alcohol slurry of the pectin at ambient or lower temperature. The process requires careful control of the relative rates of de-esterification and amidation, whilst minimising the rate of polymer chain degradation.

Gelation properties of pectins

Because pectin is a charged hydrocolloid, it is sensitive to variations in pH and to a greater or lesser extent to the nature and quantity of cations present in the system. Gelation may be considered as a state between solubility and precipitation of a polymer, and therefore the nature of the solvent is also significant.

Gelation of high methoxyl pectins

High methoxyl pectins will gel only in the presence of sugars or other co-solutes, and at a sufficiently low pH, so that the acid groups in the polymer are not completely ionised. Both gel strength and setting temperature are influenced by these factors. The junction zones are stabilized by hydrogen bonding between undissociated carboxyl and secondary alcohol groups and by

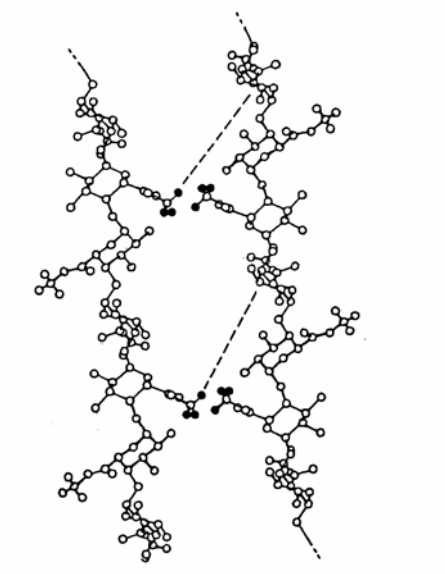

Figure 3. Gelation of HM pectins (From Voragen *et al.*, 1995).

hydrophobic interactions between methoxyl groups (Fig.3). Both types of binding forces are fortified by sucrose. In a system with sucrose as the sweetener, at around 65% soluble solids, typical of high sugar jams and preserves, high methoxyl pectins gel at up to pH3.4 (rapid set pectin) or 3.2 (slow set pectin). As the pH is reduced, gel strength and setting temperature will increase up to the point at which the setting temperature approaches the temperature at which the gel is deposited (Fig.4). Below this pH, pectin tends to pre-gel, and the resulting non homogeneous gel is weaker and more subject to syneresis. However, if the gel mixture is prepared at higher pH, and acidified immediately before or on depositing, the gel strength is maintained to low pH values.

The gelation of high methoxyl pectins is also time-dependent, and setting temperatures will therefore depend on the rate of cooling, being higher with slower cooling. Very rapid cooling under shear can be used to produce a thick heavy texture useful in some industrial applications of fruit products.

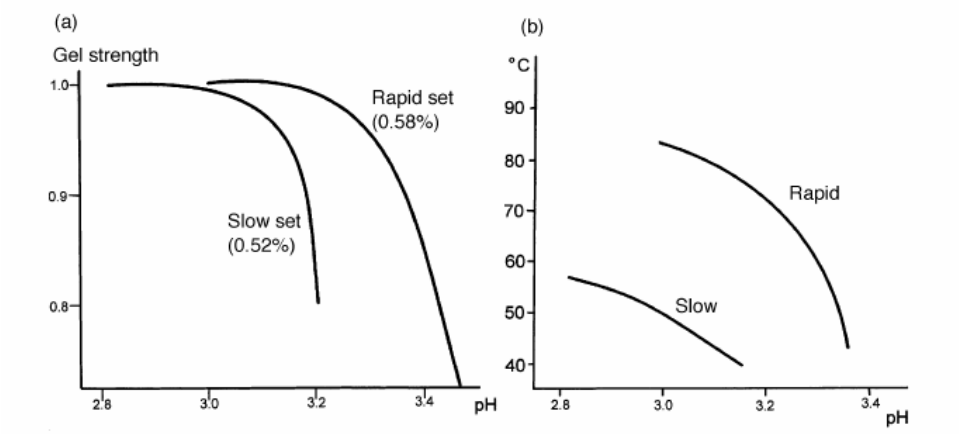

Figure 4 (a) Variation of gel strength of high methoxyl pectins with pH in a 65% sucrose gel (relative values) (b) Variation of gel setting temperature in the same system (From May C.D., 2000).

Changing the nature of the sugars present has a noticeable effect on the performance of pectin. For example, the replacement of a substantial amount of sugar by glucose syrup leads to increased setting temperature with a corresponding increase in the optimum pH for gelation, but some loss in maximum gel strength. Other sugars such as maltose may show a similar effect. Increasing the concentration of sugars increases both setting temperature and optimum pH. In making confectionery jellies, at 75–80% soluble solids, a slow set pectin would be used at a pH of 3.4–3.6 to give a suitable depositing time, enabling a batch to be deposited over 15–20 minutes or more.

Gelation of low methoxyl pectins

The gelation of low methoxyl pectins is governed mainly by the interaction between the pectin and calcium ions. For this reason, the availability of calcium ions is important, and this is commonly governed by sequestrants either naturally present (e.g. citrate and other organic acid ions from fruit or milk) or added (commonly food grade di- or polyphosphates).

Reactivity to calcium is governed by the proportion and arrangement of carboxyl groups in the pectin chain. Reactivity increases with decreasing

degree of esterification, and is greater but less controllable if the arrangement of acid groups is less random, with blocks of de-esterified galacturonate units.

Gelation is due to the formation of intermolecular junction zone between homogalacturonic (smooth) regions of different chains (egg-box model, Fig.5). Amide groups have a moderating influence, and permit gelation over a wider range of calcium concentrations . Gelation is favoured by increased soluble solids, but decreased by increasing pH, or by increasing the level of sequestrant. However, a certain level of a sequestrant such as citrate is essential to produce a practically workable gel system.

With correct formulation, low methoxyl pectins can gel over a wide range of soluble solids (10–80%), and in either acidic or less acid-tasting products, at a pH of 3.0 to above 5.0 (Voragen *et al.*, 1995; May, 2000).

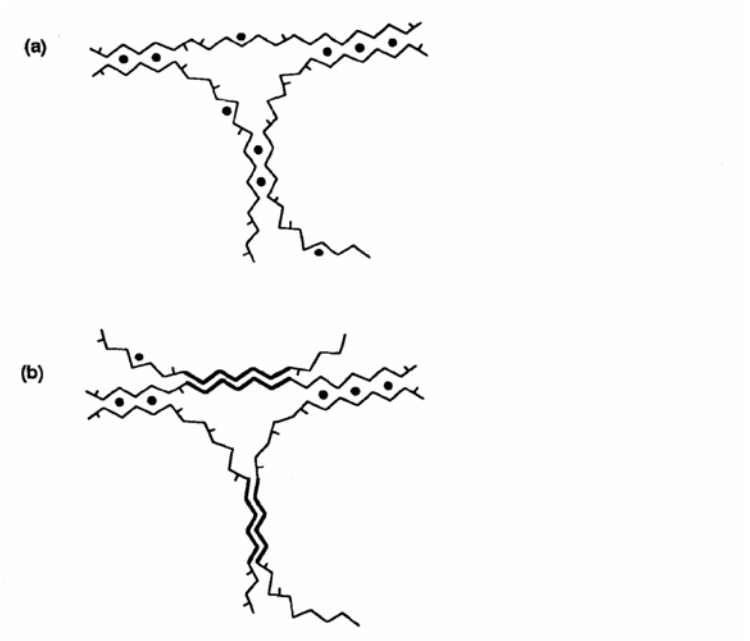

Figure 5. Models of the gelation of LM pectins: (a) model for LM pectins showing ionic interactions (•= calcium ions) between galacturonic acid residues (-); (⊥: methylated galacturonic acid); (b) model for amidated LM pectins showing ionic interaction (•= calcium ions) between galacturonic acid residues (-) and hydrogen bonding between amidated galacturonic acid residues (-);(⊥: methylated galacturonic acid)

1.3.1.2. Soy Protein

The applicability of soy proteins in foods is based on their functionality. Typical functions of soy proteins are gelation, emulsification, foaming, cohesion-adhesion, elasticity, viscosity, solubility, etc. Soy protein used in food industry is classified as soy flour, concentrate or isolate based on the protein content. Soy flour contains 50-59% protein and is obtained by grinding defatted soy flakes. Soy protein concentrate *contains* 65-72% protein and is obtained by aqueous liquid extraction or acid leaching process. Soy protein isolate contains more than the 90% protein and is obtained by aqueous or mild alkali extraction followed by isoelectric precipitations (Park *et al.*, 2002).

The seed proteins of legumes, including soybeans are albumins and globulins. Globulins, the dominant storage proteins account for about 50-90% of seed proteins. Storage globulins are grouped into two types according to their sedimentation coefficients: 7S globulins and 11S globulins. The ratio of 11S to 7S globulins varies among cultivars. It is about 0.5-1.7 in soybean.

The *7S globulins* of soybean are classified into three major fractions with different physicochemical properties, designated β-conglycinin, γ-conglycinin and basic 7S globulin. β-Conglycinin is the most prevalent of these three and accounts for 30-50% of the total seed proteins. Basic 7S and γ-conglycinin account for less than a few percent.

Basic 7S globulin (Bg) is a glycoprotein having a higher isoelectric point (pH 9.05-9.26) than the other globulin species. It has a molecular mass of 168 kDa and is composed of four subunits consisting of a high molecular weight polypeptide and a low molecular weight polypeptide which are linked by disulfide bridges.

γ-Conglycinin is a glycoprotein and is a trimer with a molecular mass of 170kDa. It is composed by identical subunits.

β-Conglycinin is a trimer with a molecular mass of 150-200kDa. It is composed by four subunits: three major subunits α' (72kDa), α (68kDa), and β (52kDa), and one minor subunit γ similar in size to that of β subunit. Also β-conglycinin is a glycoprotein.

The 11S globulins, are widely distributed in many legume and nonlegume seeds and are generally simple proteins, although there are some exceptions. The 11S globulin of soybean, glycinin, is a hexamer with a molecular mass of 300-380kDa. Each subunit is composed of an acidic polypeptide (acidic pI) with a molecular mass of ~35kDa and a basic polypeptide (basic pI) with a molecular mass of ~20kDa. These two polypeptides are linked together by disulfide bonds.

Gelation

The gel-forming ability of soy proteins is one of the most important functional properties used in traditional foods. The two globulins, glycinin and β-conglycinin, have different structures and gel properties.

Unfolded proteins may have exposed regions with high potential to form intermolecular hydrogen bonds. Unlike hydrophobic interactions or disulfide interchange, which occur principally during heating, hydrogen bonds form mainly during and after cooling. These interactions are responsible for the thermoreversible nature of some gels. Hydrogen bonding also plays a major role in the heat-induced interactions of soy proteins.

The behaviour of soy proteins during heating is particularly interesting in a discussion on protein-protein interactions because it depends on the composition of the various subunits. The mechanisms of aggregation of soy proteins have been studied on isolated 7S and 11S proteins. Soy protein isolates or mixtures of 7S and 11S show better gelling behaviour than either of the two proteins taken in isolation. Glycinin (11S) gels are firmer than β-conglycinin (7S) gels, and the network structure formed is different, depending on the protein composition.

Heating causes soy globulins to dissociate, and the subunits interact with one another forming large molecular weight aggregates. 7S less heat stable than 11S, having an onset temperature of denaturation of about 70°C compared to 80 to 90°C for 11S. The stability to heat increases with increasing ionic strength. Although hydrogen bonding is the driving force in the formation of 7S gels, electrostatic interactions and covalent disulfide bridging are also involved in the formation of 11S protein networks. In soy protein isolates,

which are composed of a mixture of 11S and 7S, electrostatic interactions and hydrogen bonding are the main forces involved in protein gelation. The network structure of soy gels is subjected to rearrangements, and the gel stiffening is thermoreversible. Prolonged heating times cause more protein to be incorporated in the network, and the gels become stiffer. The molecular composition of the large complexes formed during heat treatment of soy isolates is heterogeneous, but there seems to be a high electrostatic affinity of the β-subunits of 7S protein for the basic subunits of 11S globulin. The formation of specific complexes between the 7S and 11S subunits may be the reason for the stronger networks formed by soy protein isolates compared to those containing only 11S. Soy proteins are another example of how by varying pH, ionic strength, or heating temperature it is possible to modulate the type of aggregation and the macroscopic appearance of the gels. At pH 7.6, fine stranded gels form with a smooth appearance and high elasticity. On the other hand, coarse, white gels are formed at pH 3.8. The gelation behaviour of the various subunits changes, depending on the pH. Whereas in the pH range from 3.0 to 5.0 all subunits seem to be included in the network, at pH>5.0 fewer acidic polypeptides take part in the reactions. During heat-induced gelation, the interactions between proteins can be modulated by controlling the balance between attractive and repulsive forces. If proteins have many exposed reactive sites and the attraction between the molecules predominates, large aggregates form, causing the gels to appear particulate and cloudy. If electrostatic repulsion is the dominating force between the proteins, no gelation occurs, or a thin strand network forms (Utsumi, Matsumura and Mori 1997).

1.3.2. Lipids

Lipid films are often used as barriers to water vapour, or as coating agents for adding gloss to confectionery products. Their use in a pure form as free-standing films is limited, because most lack sufficient structural integrity and durability.

Waxes are commonly used for coating fruits and vegetables to retard respiration and lessen moisture loss. Acetylated monoglycerides are frequently added to wax formulations to add pliability to the coating.

Many lipids exist in a crystalline form and their individual crystals are highly impervious to gases and water vapour. Since the permeate can pass between crystals, the barrier properties of crystalline lipids are highly dependent on the inter-crystalline packing arrangement.
Lipids consisting of tightly packed crystals offer greater resistance to diffusing gases than those consisting of loosely packed crystals. Also, crystals oriented with their major planes normal to permeate flow provide better barrier properties than crystals that are oriented differently.
Lipids existing in a liquid state or having a large proportion of liquid components offer less resistance to gas and vapour transmission than those in a solid state, indicating that molecular mobility of lipids detracts from their barrier properties. The barrier properties of lipids having crystalline properties can be influenced both by tempering and by polymorphic form (Han and Gennadios, 2005)

1.3.3. Blends and Composites

Composite films can be formulated to combine the advantages of the lipid and hydrocolloids components and lessen the disadvantages of each. Therefore, biopolymer composites can modify film properties and create desirable film structures for specific applications. For example, when a barrier to water vapour is desired, the lipid component can serve this function while the hydrocolloid component provides the necessary durability. Similar to multi-layered composite plastic films, biopolymer films can be produced as multiple composite layers, such as protein coatings (or film layer) on polysaccharide films, or lipid layers on protein/polysaccharide films. This multi-layered film structure optimizes the characteristics of the final film. Blended film system can be obtained by mixing two or more biopolymers, yielding one homogenous film layer (Debeaufort *et al.*, 1998).

1.3.4. Plasticizers

Hydrocolloid films are often quite stiff and brittle due to extensive interactions between polymer chains through hydrogen bonding, electrostatic forces, hydrophilic bonding and/or disulfide cross-linking. Relatively small molecular weight hydrophilic plasticizers are often added, which mainly compete for hydrogen bonding and electrostatic interactions with the biopolymer chains.

Plasticizers increase the free volume of polymer structures or the molecular mobility of polymer molecules. The result of plasticizer addition is a reduction in polymer chain-to-chain interactions, a lowering of the biopolymer glass transition temperature, and an improvement in film flexibility (lowering of film elastic modulus). Also, film elongation (stretchiness or ductility) increases, and film strength decreases.

Most plasticizers are very hydrophilic and hygroscopic so that they can attract water molecules and form a large hydrodynamic plasticizer-water complex. For protein and polysaccharide films, plasticizers disrupt inter- and intra-molecular hydrogen bonds, increase the distances between polymer molecules, and reduce the proportion of crystalline to amorphous region (Krochta, 2002).

Unfortunately, plasticizers decreases the film's ability to act as a barrier to moisture, oxygen, aroma and oils. Plasticizers generally used for edible films include glycerol, propylene glycol, sorbitol, sucrose, polyethylene glycol, fatty acids, and monoglycerides.

Water is actually a very good plasticizer, but it can easily be lost by dehydration at a low relative humidity (Guilbert and Gontard, 1995). Therefore, the addition of hydrophilic chemical plasticizers, such as glycerol, to films can reduce water loss through dehydration, increase the amount of bound water and maintain a high water activity.

There are two main types of plasticizers (Sothornvit and Krochta, 2001):

1. Agents capable of forming many hydrogen bonds, thus interacting with polymers by interrupting polymer-polymer bonding and maintaining the farther distance between polymer chains

2. Agents capable of interacting with large amounts of water to retain more water molecules, thus resulting in higher moisture content and larger hydrodynamic radius.

However, owing to the hydrophilic nature of water, biopolymers and plasticizers, and duo to the abundantly existing hydrogen bonds in their structures, it is very difficult to separate these two mechanisms. Sothornvit and Krochta (2001), suggested that several factors of plasticizers affect plasticizing efficiency including size/shape of plasticizer molecules, number of oxygen atoms and their spatial distance within the structure of the plasticizers and water-binding capacity. Moreover, the effect of hydrogen bonding, repulsive forces between molecules of the same charge or between polar/non-polar polymers can increase the distance between polymers, thus achieving the function of plasticization in the case of charged polymeric film structures. Therefore, compared to neutral polymer films (e.g. starch films), the flexibility of charged polymer films (e.g. soy protein, carboxymethyl cellulose or alginate films) may be affected more significantly by altering pH and salt addition at the same water activity level.

1.3.5. Additives

The functional, organoleptic, nutritional and mechanical properties of an edible film or coating can be altered by the addition of various chemicals in minor amounts. Edible films and coatings can carry various active agents, such as emulsifiers, antioxidants, antimicrobials, flavours and colorants thus, enhancing food quality and safety of foods (Han & Gennadios, 2005).

Emulsifiers

Emulsifiers are surface-active compounds with both polar and non-polar character, which absorb at the water-lipid interface and reduce surface tension. To produce protein-lipid or polysaccharide-lipid composite films from aqueous solution, it is often necessary to add an emulsifier to allow dispersion of the lipid material in the solution. Also, for some food-coating applications, addition of a surface-active agent to a coating formulation may be necessary to achieve satisfactory surface wetting and spreading with the coating formulation and then adhesion of the dry coating. Some protein are sufficiently

surface-active that no emulsifier is necessary to form well dispersed composite films or provide good surface wetting and adhesion.

Other additives

Antioxidants and antimicrobial agents can be incorporated into film-forming solutions to achieve active packaging and coating functions. They provide additional active functions to the edible film and coating system to protect food products from oxidation and microbial spoilage, resulting in quality improvement and safety enhancement. When nutraceutical and pharmaceutical substances are incorporated into edible films and coatings, the system can be used for drug delivery purposes (Han, 2003).

Incorporated flavours and colorants can improve the taste and the visual perception of quality, respectively. Because of the various chemical characteristics of these active additives, film composition should be modified to keep a homogeneous film structure when heterogeneous additives are incorporated into the film-forming materials (Debeaufort *et al.*, 1998).

1.4. Film and Coating formation

An edible film is essentially a interacting polymer network of a three-dimensional gel structure. Despite the film-forming process, whether it is wet casting or dry casting, film-forming materials should form a spatially rearranged gel structure with all incorpored film-forming agents, such as biopolymers, plasticizers, other additives, and solvents in the case of wet casting. Biopolymer film-forming materials are generally gelatinized to produce film-forming solutions. Sometimes drying of the hydrogels is necessary to eliminate excess solvents from the gel structure. This does not mean that the film-forming mechanism during the drying process is only the extension of the wet-gelation mechanism. The film-forming mechanism during the drying process may differ from the wet-gelation mechanism, though wet gelation is the initial stage of the film-forming process. There could be a critical stage of a transition from a wet gel to a dry film, which relates to a phase transition from a polymer-in-water (or other solvents) system to a water-in-polymer system. Unfortunately, the complete film-forming mechanisms of most biopolymers after gelation are not clearly determined yet.

Many techniques have been developed for forming films directly on food surfaces, or as separate, self-supporting films.

There are two categories of film-production process: *dry* and *wet* (Han and Gennadios, 2005).

The *dry process* of edible film production does not use liquid solvent, such as water or alcohol. Molten casting, extrusion, and heat pressing are good examples of dry process. For the dry process, heat is applied to the film-forming materials to increase the temperature to above the melting point of the film-forming materials, to cause them to flow. Therefore, the thermoplastic properties of the film-forming materials should be identified in order to design film-manufactory processes. It is necessary to determine the plasticizer effects and any other additives on the thermoplasticity of film-forming materials. Plasticizers decrease the glass transition temperature (Krochta, 2002).

The *wet process* uses solvents for the dispersion of film-forming materials, followed by drying to remove the solvent and form a film structure. For the wet process the selection of solvents is the one of the most important factors. Since the film-forming solution should be edible and biodegradable, only water, ethanol and their mixtures are appropriated as solvents (Han and Gennadios, 2005). The film-forming solution should be applied to flat surfaces using a sprayer, spreader or dipping roller, and dried to eliminate the solvent, forming a film structure. Phase separation of incompatible ingredients from the film-forming solution is not generally desirable unless the phase separation is intentionally designed for the formation of a bi-layer film structure. To produce a homogeneous film structure avoiding phase separation, various emulsifiers can be added to the film forming solution. This solvent compatibility of ingredients is very important to develop homogeneous edible film and coating systems carrying active agents. All ingredients, including active agents as well as biopolymers and plasticizers should be homogeneously dissolved in the solvent to produce film-forming solutions. Most film-forming solutions possess much higher surface tension than the surface energy of dried films, since they contain excessive amounts of water and ethanol. Therefore, it is difficult evenly to coat a flat surface that has a low surface energy with a film-forming

solution that possesses high surface energy using high-speed coating equipment in manufacturing. Nonetheless, during the solvent drying process the film-forming solution is concentred and its surface energy is decreased due to the solvent loss. Also the viscosity of film-forming solution affects the coating process. In fact, a lower viscosity accelerates the separation process (coacervation) of the film-forming solution from the flat surface and causes the uneven coating on the surface, followed by dripping down of the coating solution from the surface to the floor. Higher viscosity of the film-forming solution is desirable to reduce this coating phase separation, unless this creates an uncontrollably heavy coating thickness. If the film-forming solution has a lower surface tension and higher viscosity, the high-speed coating process is more likely to form a film layer on the flat surface. However, the lower surface energy of coated films after drying makes the peeling process of the film from the flat surfaces harder, since there is a very high adhesion between the film and the flat surface when there is a smaller difference of the surface energy between the film and the flat surface. Conversely, this is a desirable phenomenon for the direct coating process of the film-forming solution onto food surfaces to avoid the peeling problem of the coated layer (Han and Gennadios, 2005)

1.5. Film Applications

1.5.1. Dipping

This method lends itself to food products that require several applications of coating materials or require a uniform coating on a irregular surface. After dipping, excess coating material is allowed to drain from the product and it is then dried or allowed to solidify. This method has been used to apply films of acetylated monoglycerides to meats, fish and poultry and to apply coating of wax to fruits and vegetables (Krochta, 2002).

1.5.2. Spraying

Films applied by spraying can be formed in a thinner, more uniform manner than those applied by dipping. Spraying, unlike dipping, is more suitable for applying a film to only one side of a food to be covered. This is desirable when

protection is needed on only one surface, e.g., when a pizza crust is exposed to a moist sauce. Spraying can also be used to apply a thin second coating, such as the cation solution needed to cross-link alginate or pectin coatings (Krochta, 2002).

1.5.3. Casting

This technique, useful for forming free-standing films, is borrowed from methods developed for not edible films. For formation of a film the film-forming biopolymers are first dissolved in the solvent. If heating or pH adjustment enhances film formation and/or properties, this is done next. If a composite film or coating based on an emulsion is desired, a lipid material, and possibly a surfactant, is added. Next, the mixture is heated to above the lipid melting point and then homogenized. Degassing is an important step to eliminate bubble formation in the final film or coating. Finally, the edible film or coating is formed by applying the prepared formulation to the desired casting or product surface and allowing the solvent to evaporate. Providing heated air at low humidity and high velocity increases drying rates.

Coating is simple and allows film thickness to be controlled accurately on smooth, flat surfaces. Casting can be accomplished by controlled-thickness spreading or by pouring. Controlled-thickness spreading requires a spreader with a product reservoir and an adjustable gate, the height of which can be set accurately and with good reproducibility. The spreader is simply drawn over the receiving surface, depositing a layer of the film-forming solution of the desired thickness, which is subsequently dried. Alternatively, the film-forming solution can be poured into a confined area of a level receiving surface and subsequently dried (Krochta, 2002).

1.6. Functional Properties

The main properties of edible films and coatings are their barrier properties (permeability to oxygen, carbon dioxide, moisture, aroma and oil), mechanical properties and moisture sorption. Barrier properties are important to separate food from the environment, which causes food deterioration.

The barrier properties of polymer films are related to physical and chemical nature of the polymers. The chemical structure of the polymer backbone, the degree of crystallinity and orientation of molecular chains, and the nature of the plasticizer added all affect barrier properties.
The most common barrier properties of interest in edible films and coatings include: permeability to water, oxygen and grease/oil barrier.

It is known that the water can enhance the rate of several reactions (browning, lipid oxidation, vitamin degradation, enzyme activity), increase the rate of micro-organism growth and cause texture change, all of which are related to food shelf life and quality. Thus, controlling water permeability is the paramount importance in the development of an edible film and its application. The protein- and the polysaccharide-based films provide good barriers against oxygen, aromas and oil, but are poor barriers against water. Much effort has gone into decreasing film permeability to water vapour, such as combining polysaccharides or protein with lipids, cross-linking agents, irradiation, and heat curing.

Oxygen transfer from the environment to food affects the deterioration on food components (lipids, vitamins, colours and flavours) and microrganism growth, leading to sensory and nutrient changes. Most protein-based films are excellent oxygen barriers. Nuts provide an example of the use of protein films to avoid oxygen contact. Generally, films that are better water barriers are poorer oxygen barriers. Increasing the plasticizer amount always increases oxygen permeability because of the higher free volume in the film network.

Moreover, grease/oil barrier packaging is essential to limit the penetration of oil during some food preparations such as during frying, or for foods containing fats. For example, normally, synthetic polymers such as polyethylene (PE) are used to provide grease resistance for coated paperboard. Biopolymer-coated paper board is an alternative that can be used in fast-food packaging and for other forms of grease/oil barrier packaging (Lin and Krocta, 2003).

Mechanical properties are important for edible films and coatings, as they reflect the durability of films and the ability of a coating to enhance the

structural integrity and the mechanical-handling properties of a food product. The mechanical performance can be enhanced by blending polysaccharides and protein together. In addition, the introduction of new covalent bonds into protein systems chemically or enzymatically, may be expected to affect film structure and film performances such as: permeability, mechanical properties (Di Pierro, Mariniello, Giosafatto, Masi and Porta, 2005).

1.6.1. Permeability

The protection of foods from gas and vapour exchanges with the environment depends on the integrity of packages (including their seals and closures), and on the permeability of the packaging materials themselves. There are two processes by which gases and vapour may pass through polymeric materials: a pore effect, in which the gases and vapours flow through microscopic pores, pinholes, and cracks in the materials; and a solubility-diffusion effect, in which the gases and the vapours dissolve in the polymer at one surface, diffuse through the polymer by a concentration gradient, and evaporate at the other surface on the polymer. This *solution-diffusion* process (also known as *activated diffusion*) is described as true permeability.

Most polymers when sufficiently thin exhibit both forms of permeability. Porosity falls very sharply as the thickness of a polymer is increased reaching virtually zero with many of the thicker types of commercially available materials. True permeability, however, varies inversely as the thickness of the material and hence cannot be effectively eliminated merely by increasing the material thickness. In this section, we have reported only the aspects concerned the solution, diffusion and permeation of gases and vapours in effectively non-porous polymeric materials.

Under steady state conditions, a gas or vapour will diffuse through a polymer at a constant rate if a constant pressure difference is maintained across the polymer. The diffusive flux, J, of a permeant in the polymer can be defined as the amount passing through a plane (surface) of unit area normal to the direction of the flow during unit time:

$$J = Q/A \times t \qquad (1)$$

where Q is the total amount of permeant which has passed through area A during time t.

The relationship between the rate of permeation and the concentration gradient is one of direct proportionally and is embodied in Fick's first law:

$$J = -D\frac{\delta c}{\delta x} \tag{2}$$

were J, D, c and x are the flux per unit cross-section, the diffusivity, the concentration of the permeant, and the distance across wich the permeant as to travel, respectively. Fick's second law can be used to analyzed unsteady state diffusion with time t:

$$\frac{\delta c}{\delta x} = D\frac{\delta^2 c}{\delta x^2} \tag{3}$$

When the steady state of diffusion has been reached, J is constant and eq. (1) can be integrated across the total thickness of the polymer X, and between the two concentrations, assuming D to be constant and independent of c:

$$J = \frac{D(c_1 - c_2)}{X} \tag{4}$$

The above expression can be rewritten by substituting for J using eq. (1). This enables calculation of the quantity of permeant diffusing through a polymer of area A in time t:

$$Q = \frac{D(c_1 - c_2)At}{X} \tag{5}$$

When the permeant is a gas, it is more convenient to measure the vapour pressure p which is at equilibrium with the polymer, rather than measure the actual concentration. At sufficiently low concentrations Henry's law applies and c can be expressed as:

$$c = S \cdot p \tag{6}$$

where S is the solubility coefficient of the permeant in the polymer. By combining eqs. (5) and (6):

$$Q = \frac{D \cdot S(p_1 - p_2)At}{X} \qquad (7)$$

The product D S is referred to as the *permeability coefficient* or *constant* and is represented by the symbol P. Thus:

$$P = \frac{Q \cdot X}{A \cdot t \cdot (p_1 - p_2)} \qquad (8)$$

or

$$\frac{Q}{t} = \frac{P}{X} A \cdot (\Delta p) \qquad (9)$$

The term P/X is called the *permeability* or *permeance*.

There are four assumptions made in the above simple treatment of permeation. These are that diffusion is in a steady state condition, the concentration-distance relationship through the polymer is linear, diffusion takes place in one direction only (i.e. through the film with no net diffusion along or cross it), and that both D and S are independent of the concentration. Like all simplifying assumptions, there are many instances when they are not valid, and in such cases the predictions made are not subsequently borne out in practice. Thus while for many gases such as oxygen, hydrogen, nitrogen and to a certain extent carbon dioxide, D and S are independent of concentration, such is not the case where considerable interaction between polymer and permeant takes place (e.g. water and cellulose film or many solvent vapours diffusing through polymer films). The permeability coefficient is independent of thickness, since the thickness is already counted in the calculation of P. However, the total amount of protection afforded by unit area of a barrier material (i.e. P/X) approaches zero permeability only asymptotically. Consequently, as polymer thickness is increased beyond a certain value one obtain lower permeation. As a guide it is possible to assume that if the thickness of a film is doubled, the transmission rate of a permeant is halved (Robertson, 1993).

1.6.2. Mechanical Properties

The response of food materials when subjected to various forces is of the greatest importance to food scientists and engineers. An edible film or coating with very good barrier properties could be inefficient if its mechanical properties do not permit to maintain the film integrity during handling, packaging and carrying processes. Thus, the mechanical resistance and deformability of edible coatings have to be determined. The mechanical properties of films are related to structural properties and influence the handling and processing of films. Rheology is defined as the study of the deformation and flow of matter under defined conditions.

An ideal solid material will respond to an applied load by deforming finitely and recovering that deformation upon removal of load. Such a response is called "elastic". Ideal elastic material obey Hook's law which describes a direct proportionality between the stress (σ) and the strain (ε) via a proportionally constant (E):

$$\sigma = \frac{F}{A} = E\varepsilon \qquad (10)$$

where σ is the force divided by the cross-sectional area of the specimen, ε is the deformation or strain and E is the Young' modulus that represent a characteristic of each material solid.

An ideal fluid will deform and continue to deform as long as the load is applied. The material will not recover from its deformation when the load is removed. This response is called "viscous". The flow of the simple viscous materials is described by newton' law, which constitutes a direct proportionality between the shear stress and the shear rate:

$$\tau = \eta\dot{\gamma} \qquad (11)$$

where the proportionally constant η is called the shear viscosity.

From energy considerations, elastic behaviour represents complete recovery of energy expended during deformation, whereas viscous flow represents

complete loss of energy as all the energy supplied during deformation is dissipated as heat.

Ideal elastic and ideal viscous behaviours present two extreme responses of material to external stress. As the terms imply, these are only applicable for "ideal" materials. Real materials, however, exhibit a wide array of responses between viscous and elastic. Most materials exhibit some viscous and some elastic behaviour simultaneously and are called "viscoelastic". Almost all foods, both liquid and solid, belong to this group. The viscoelastic properties of materials are determined by transient or dynamic methods.

The transient methods include stress relaxation (application of constant and instantaneous strain and measuring decaying stress with respect to time) and creep (application of constant and instantaneous stress and measuring increasing stain with time).

The viscolelastic properties can be studied by transient methods and can be represented by two mechanical model: Hooknean elasticity is represented by a spring and Newtonian flow by a dashpot. The behaviour of any viscoelastic materials can be adequately described by connecting these basic elements in series or in parallel or in combination.

Though such methods are fairly easy to perform, there are several limitations. Major among them is that the material response cannot be determined as a function of frequency.

Dynamic tests are preferred for investigating viscoelastic behaviour, since they are more versatile and an cover and wider range of conditions. They are performed applying a small strain (or stress) varying sinusoidally with time (equ. 3) and measuring the resulting stress (or strain).

$$\gamma = \gamma_0 \sin \omega t \qquad (12)$$

where γ_0 represent the maximum amplitude of the strain.

It is important to empathize that the strains and the stresses used in these tests are very small, often <1%. This is to assure that the material response is in the linear range, i. e. the range within the stress is proportional to the

applied strain (linear viscoelasticity range) and the theory described below is applicable.

If the material is perfectly elastic the resulting stress, in the case of shear experiment, will be exactly in phase with the strain wave (phase angle, δ, is equal to 0):

$$\tau = G\gamma_0 \sin \omega t \qquad (13)$$

where G is the *elasticity modulus* and represent the material resistance to deformation.

On the other hand, if the material is a viscous liquid, the stress wave will be exactly 90° out of phase with the deformation:

$$\tau = \eta\gamma_0 \omega \cos \omega t \qquad (14)$$

Any viscoelastic material will lie between these two extremes, con $0° < \delta < 90°$.

The stress response of a linear viscoelastic material to a sinusoidal strain input is given as:

$$\tau(t) = \gamma_0 G'(\omega)\sin(\omega t) + \gamma_0 G''(\omega)\cos(\omega t) \qquad (15)$$

The frequency dependent functions $G'(\omega)$ and $G''(\omega)$ are shear elastic (storage) modulus and shear elastic (loss) modulus respectively. $G'(\omega)$ is a measure of the energy stored and subsequently released per cycle of deformation per unit volume. It is the property that relates to molecular events of elastic nature. $G''(\omega)$ is a measure of the energy dissipated as heat per cycle of deformation per unit volume. $G''(\omega)$ is the property that relates to molecular events of viscous nature. Another commonly used dynamic visoelastic property, the loss tangent $(\tan\delta(\omega) = G''/G')$ denotes relative effects of viscous and elastic components in a viscoelastic behaviour (Ferry, 1980).

1.7. Edible film in food application technology

The choice of an edible packaging mainly depends on the specific characteristics of the food product that requires protection and on storage conditions. Many materials have been used for film and coating formulations

as carbohydrates, proteins, lipids, or mixtures of these. Edible films and coatings have been applied on fruits, vegetables, meat, poultry, seafood, grains, candies, heterogeneous and complex foods, or fresh, cured, freezed, and processed foods.

For example, carbohydrate-based edible films and coatings have been often used to improve the quality and the stability of meat during storage and commercialization. In particular, carraghenan coatings were applied on poultry and fish to prevent superficial dehydratation. Collagen, caseins and cellulose derivates such as carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose can be used as a precoating to improve the adhesion of the batter mix onto meat and fish and significantly reduce oil adsorption during frying.

The responsible principals of fruit and vegetable spoilage are gas exchanges (respiration and transpiration) during ripening and storage, and/or microbial growth. Waxes such as mineral oils, paraffin, candellilla, shellac, carnauba, etc., are used largely as coatings on fruits such as apple, pear, orange, lemon, banana, mango, coconut, peach and on some vegetables such as carrot, pepper, asparagus, cucumber, sweet corn, tomato, potato, etc. Waxes and oils, alone or in emulsion with hydrocolloids or protein solutions are efficient barrier to water and can prevent from weight loss. However, a thicker layer of waxes strongly modify oxygen and carbon dioxide exchanges, this involves anaerobic storage conditions inducing nonhomogeneous ripening up to adulteration of fruits and vegetables. Consequently, some edible films were developed to better control the ripening by reducing oxygen penetration in the fruits and increasing carbon dioxide and ethylene evaporation. Moreover, proteins and polysaccharides whether mixed with lipids or not, present the best ratio between CO_2 and O_2 permeabilities, from 10 to 25, than plastic wrappings (CO_2/O_2 ratio is lower than 5) (Han and Gennadios, 2005). Krocta and co-workers (1994) designed milk-protein based coatings, mainly consisting of caseinate and lipids, specifically suited for minimally processed vegetables.

Nuts, almonds, hazelnuts and peanuts are well protected against oil migration, oxidation and to maintain desirable texture by low methoxy pectin, zein or

cellulose derivate coatings containing citric acid (Guilbert, Gontard and Gorris, 1996).

Many candies require an edible coating to prevent stickiness, agglomeration, moisture absorption, and oil migrations in the case of chocolate or fat containing confectioneries. The oldest example of edible coated confectioneries is the use of sugar coatings for almond or nut contained in the candy in order to reduce the lipid oxidation.

Growth of microorganisms on the surface of packaged food products is the predominant cause of spoilage, which may be counter-acted using antimicrobial compounds. Antimicrobial packaging is a form of active packaging that could extend the shelf-life of product and provides microbial safety for consumers. It acts to reduce, inhibit, or retard the growth of pathogen microorganisms in packed foods and packaging material. In order to control undesirable microorganisms on food surfaces: (1) volatile and non-volatile antimicrobial agents can be incorporated into polymers or (2) coating or adsorbing antimicrobial onto polymer surfaces can be applied. The coating can serve as a carrier for antimicrobial compounds and/or antioxidants compounds in order to maintain high concentrations of preservatives on the food surfaces.

Guilbert *et al.* (1996) discussed the use of casein or carnuba wax films with sorbic acid to protect papaya and apricot cubes from spoilage by yeast and fungi. The lamb carcasses were coated with alginates gelled with calcium chloride to limit the microbial growth. Seydim and Sarikus (2006) have proved that whey protein isolate films incorporated whit oregano and garlic essential oil exhibited good inhibitory effect on some selected food pathogens such as S. aureus, L. monocytogenes and E. coli. Presence of nisin in edible films formulated with tapioca starch and glycerol reduced L. innocua growth, producing count decrease and acting as a barrier to contamination after processing (Sanjurjo, Flores, Gerschenson, and Jagus, 2006).

The most interesting application of edible films and coatings is for heterogeneous and elaborates foods (plastic films cannot be used within the food product). Several formulations were patented to reduce moisture transfers between low water activity components (cereals, biscuits, nuts, etc.)

that have to be incorporated in high moisture content compartments such as soup, yoghurt, cream, puree, etc. (Debeaufort et al., 1998).

1.8. Structure and Properties of Proteins-Polysaccharides mixtures (blendes)

Proteins and polysaccharides are present together in many kinds of food systems, and both types of food macromolecules contribute to the structure, texture and stability of food through their thickening or gelling behaviour and surface properties.

Most structural elements present in foods at the supramolecular (or microstructural) level are thermodynamically metastable and at nonequilibrium (e.g. amorphous phase), where the nature and kinetics of interactions between them are largely unknown and uncontrolled. Knowledge of the thermodynamics of simple mixtures provides a reference point to assess the potential behaviour of the extremely complex multicomponent system that is a real food and the effect on it of variables such as temperature, pH, ionic strength, concentration, and so on (Tolstoguzov, 1997). An understanding of polymer science principles is essential for following the evolution of food materials science. The basic premise of this science is that since most food are formed by polymers, they must comply with the principles and theories that apply to synthetic polymers. It tries to interpret physical and chemical phenomena in food system through concepts such as thermodynamic incompatibility of polymer solutions, the glass transition, state diagrams, polymer rheology, etc. Material science is a well developed discipline that, building on chemistry an physics, covers such subjects as internal properties of materials, phase transitions and phase equilibria, strength and fracture of materials, and surface and transport properties.

1.8.1. Protein-polysaccharide interactions

Much is now known at the molecular level about the functional properties of individual biopolymers, except the fact that molecular weight polydispersity is rarely taken into account. Nevertheless, our knowledge of the role of protein-polysaccharide interactions, in relation to their functionality in complex

multiphasic systems, such as food mixed solutions, biopolymeric films or coatings, emulsions or gels, is still rather limited.

Functional properties of food proteins, such as solubility, surface activity, conformational stability, gel-forming ability, emulsifying and foaming properties, are affected by their interactions with polysaccharides. Interactions of these biopolymers with each other and their competitive interactions with other system components (water, lipids, surfactant, metal ions, etc.) determine structure-property relationships in a food system such as biopolymeric packaging that differ strongly from those of the macromolecular reactants.

Thermodynamics provides valuable information as to the direction in witch a system (such as polysaccharide-protein mixture) will move, what condition will be reached at equilibrium, and what would be the effect of variables such as temperature, concentration, pH, ionic force, etc. The Gibbs free energy G is the key thermodynamic parameter for studying phases at equilibrium (Aghilera and Stanley, 1999). A necessary (but insufficient) condition for a homogeneous solution to be formed after mixing is given by this expression:

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S < 0 \qquad (16)$$

where ΔG_{mix} or (G_{mixture}- $G_{\text{pure components}}$) is the free energy of mixing, ΔH_{mix} is the enthalpy of mixing, T is temperature and ΔS_{mix} is the entropy of mixing. Thus, mixing generally involves changes in enthalpy and entropy.

An ideal solution is a fictitious model for mixtures of identical molecules in which molecular interactions are the same (or none) and the change in volume after the mixing is zero. For an ideal solution of small molecules (e.g. those that follow Raoult's law), ΔH_{mix}=0 (athermal mixing), so the sing of ΔG_{mix} depends only on the entropic term. For the so-called *regular solutions*, ΔH_{mix} is finite, and the free energy of mixing takes this form:

$$\frac{\Delta G_{mix}}{N} = \Delta H_{mix} + RT(x_1 \ln x_1 + \ln x_2) \qquad (17)$$

where x_1 and x_2 are the molar fractions of solvent and solute, respectively, and N is the total number of moles. Since $\ln x_1$ and $\ln x_2$ are always negative, components 1 and 2 will always if they behave as an ideal solution (ΔH_{mix}=0).

A polymer solution behaves differently than a solution of small molecules, obviously because of the large size of the polymers in comparison to the solvent molecules. However, the theoretical treatment of conditions for polymer-solvent miscibility is not very different from that used for dilute ideal solutions of small molecules. In fact, it involves calculating entropic and then enthalpic effects and determining their contribution to ΔG_{mix}. In the Flory-Huggins theory, the polymer solution is modelled as a lattice, where each lattice site is occupied by either a solvent molecule or a polymer segment. The change in free energy of mixing for a polymer solution is given by the Flory-Huggins equation:

$$\frac{\Delta G_{mix}}{N} = RT\left(\chi_{12}\phi_1\phi_2 + \phi_1 \ln \phi_1 + \frac{\phi_2}{x} \ln \phi_2 \right) \qquad (18)$$

The last two terms in this equation contain the entropic contribution arising from the different placements that polymer (component 2) and solvent (component 1) may have in the lattice; x represents the relative length (number of segments per molecule). These terms are similar to the last two terms of equation (2), but the molar fractions of solvent and solute have been replaced by the volume fractions of solvent (ϕ_1) and polymer (ϕ_2).

The first term represents the enthalpic contribution or interaction energy between the solvent molecules and the polymer segments. The coefficient χ_{12} is called the Flory-Huggins interaction parameter and is equal to:

$$\chi_{12} = \frac{\Delta H_{mix}}{RTN_1\phi_2} \qquad (19)$$

where ΔH_{mix} is the excess energy involved in neighbouring interaction, N_1 in the number of moles of solvent, and R is the gas constant. RT is a sort of "thermal energy" that at normal temperatures is of the order of magnitude of the energies involved in intermolecular bonds such as hydrogen bonds or van

der Waals' forces. Thus, χ_{12} is a kind of ratio between the energy involved in the interaction of neighbouring molecules and the thermal energy, and it is positive for endothermic mixing and negative for exothermic mixing. Negative values for χ_{12} indicate miscibility, while positive values indicate repulsion. According to the Flory-Huggins theory, the critical value of the interaction parameter for phase separation of a polymer-solvent mixture is given by:

$$\chi_{12c} = \frac{1}{2} + \frac{1}{2x} + \frac{1}{\sqrt{x}} \qquad (20)$$

The critical interaction parameter is a measure of the amount of effective segment-segment repulsion that a mixture can tolerate before phase separation occurs. This parameter depends only on the relative lengths (x) of components. For monomeric mixture ($x = 1$), $\chi_{12c} = 2$, whereas for large polymer ($x \to \infty$) in solution, it approaches $1/2$. So if $\chi_{12} < 1/2$ the polymer should be soluble if amorphous and linear. For a mixture of two long polymers, it can be shown that χ_{12c} approaches zero, which explains why binary polymer blends almost always phase separate.

Therefore, using the Flory-Huggins theory it is possible to account for equilibrium thermodynamic properties of polymer solutions such as deviations from Raoult's law, phase separation, melting point depression in crystalline polymers and swelling of networks (, Tolstoguzov, 1997; Aghilera and Stanley, 1999).

1.8.2. Phase separation

Phase separation in mixed polymer solution is quite common and as important technological applications in foods and biotechnology. Almost all foods contain complex mixtures of different proteins or proteins in combination with polysaccharides that can also form gels. In these mixtures, molecular interactions occur which powerfully influence the gelation characteristics of the individual components.

Interaction between proteins and polysaccharides can result in one of three consequences:

- Co-solubility, in which there is no significant interaction between the two classes of polymer molecule and both coexist in solution;
- Incompatibility, where repulsion between the two types of polymer causes them to form separate phase.
- Complexing, where attraction between the two polymers causes them to form a single phase or precipitate.

The mixing process is spontaneous when changes in Gibbs free energy ($\Delta G=\Delta H-T\Delta S$) is negative. The mixing process can only give rise to complete compatibility when the entropy difference ($T\Delta S$) between the two-phase and single-phase states is larger than the mixing enthalpy (ΔH). This is true for low molecular weight compounds, but not for polymers. Then, the entropy of mixing significantly decreases when monomers are replaced with biopolymers. Because of the large size and the rigidity of macromolecules typical of biopolymers, biopolymers solutions contain less independently moving particles. Since the entropy of mixing is a function of the number of individual particles being mixed, the value of the entropy of mixing (ΔS) of biopolymers is several orders of magnitude smaller than that corresponding monomers. Therefore, molecularly homogeneous mixtures of biopolymers could be prepared if ΔH is negative. This means that the attractive forces between different macromolecules are equal to or greater than those between the same type of macromolecules. Therefore, the biopolymer compatibility is related to the ability to form soluble interbiopolymer complexes.

When the energies of interaction between the chains of two polymers are favourable, for example, in polyanion-polycation systems, the two polymers may associate into a single gel-like phase or form a precipitate. More commonly, the interactions between the two polymers are less favourable than between like segments of each type. There is therefore a tendency for each to exclude the other from its polymer domain, so that the effective concentration of both is raised in their respective domains. At sufficiently high concentrations, the system can separate into two liquid phases, or one component may be driven out of solution by the other. In particular, two types

of phase separation are commonly encountered I mixtures of two polymers: segregative and associative (Fig. 6).

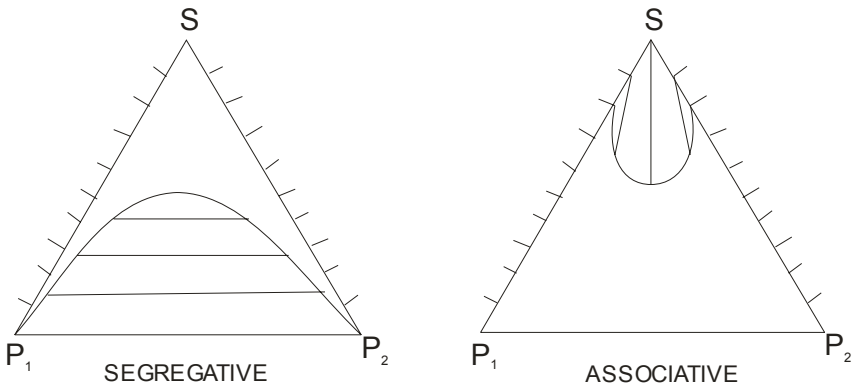

Figure 6. Phase diagrams representing phase separation in ternary polymer systems. S is the solvent and P_1 and P_2 the polymers. Segregative (thermodynamic incompatibility) phase separation and associative (complex coacervation) phase separation are shown.

Segregative phase separation, also known as thermodynamic incompatibility, is typical of ternary systems (protein-polysaccharide-water) and results in one phase containing most of the protein and the other phase nearly all of the polysaccharide.

In contrast, associative separation, or complex coacervation results in one of the two phases being enriched in both of the polymeric components. This latter behaviour is typical of oppositely charged polyelectrolytes. The main difference between these two mechanisms that thermodynamic incompatibility is predominantly entropically driven, whereas complex coacervation is both entropically and enthalpically driven.

Incompatibility of mixed polymers in solution depends on interactions between the two polymers measured by the Flory-Huggins interaction parameter $\chi_{P_1-P_2}$ and interactions of each polymer with the solvent, measured by χ_{P_1-S} and by χ_{P_2-S}. A positive value for $\chi_{P_1-P_2}$ is indicative of the exclusion of one polymer from the neighbourhood of the other (net repulsion between the polymers).

Clearly, solvent-biopolymer1 (biopolymer2) interactions are favoured to the detriment of biopolymer1-biopolymer2 and solvent-solvent interactions, so that the system finally demixes into two phases, each being enriched with one of the two biopolymers 1.The second phase separation phenomenon, the complex coacervation, occurs when the interactions between the two biopolymers are favoured ($\chi_{P_1-P_2} < 0$). This occurs when both polymers carry an opposite charge, for instance at a pH slightly lower than the isoelectric point of the protein, while the polysaccharide still carries a negative charge. Complexation takes place, which can yield either the formation of soluble complexes or an aggregative phase separation. In an associative phase separation, the two coexisting phases have the following composition: a rich solvent phase with very small amounts of biopolymer(s) and a rich biopolymer(s) phase forming the so-called coacervate (Doublier, Garnier, Renard and Sanchez 2000).

Molecular characteristics of biopolymers (molecular weight, conformation, charge density, etc.), factors affecting them (pH, ionic strength, solvent quality, etc.), mixing conditions (ratio, total concentration, etc.) and mixing procedures (heat treatment, pressure, shearing, etc.) must be considered as determining factors in separation phase.

Moreover, when both polymers can independently from a network, three extremes of structure may be formed:

- interpenetrating network, where the two types of polymer are mutually entangled;
- a phase-separated network, in which domains of pure polymer are interspersed with domains of the other pure polymer;
- a coupled network, in which at least some junction zones involve both polymers;

In particular, when two polymers form a complex, they may gel as a coupled network; when they are incompatible they may gel as two separate but interpenetrating networks or phase-separate networks, with isolated domains of each gel type. Protein-polysaccharide complexes might be regarded as a

new type of gelling agent where the formation conditions, as well as the rheological and other physicochemical properties could be controlled to give a product of the desired textural characteristics.

1.8.2.1. Mechanisms

By nature, phase separation in protein–polysaccharide systems is a kinetic process. It arises from local fluctuations of the biopolymer concentration within the entire volume of the mixture. These fluctuations can result from inefficient mixing of the components, temperature fluctuations or specific/non-specific interactions. The general models conveniently used to describe phase separation kinetics are nucleation and growth (NG) or spinodal decomposition (SD). The former is characterized by initial short-range/high-amplitude concentration fluctuations and is associated with metastability, implying the existence of an energy barrier, whereas SD proceeds through long-range/small-amplitude fluctuations. Spinodal decomposition is a process by which a mixture separates, having no nucleation free energy barrier. NG generally ends up with spherical droplets dispersed in a continuous phase, whereas SD exhibited a 3D interconnected network (Turgeon *et al.*, 2003).

When proteins and polysaccharides are blended together they may form mixed solutions of biopolymers that can exist either in stable or phase-separated states. In particular, interbiopolymer complex formation occurs at pH value below the isoelectric point (IEP) of proteins and at low ionic strengths. At pH values below the IEP, protein molecules have a net positive charge and behave as polycations. At mild acid and neutral pH values, carboxyl containing polysaccharides behave as polyanions. Electrostatic complex formation between proteins and anionic polysaccharides generally occurs in the pH range between the pK value of the anionic groups (carboxyl groups) on the polysaccharide and the protein's IEP. The formation of an electrostatic complex is usually a reversible process depending on such variables as pH and ionic strength. Generally, electrostatic complexes dissociate when the ionic strength exceeds 0.2-0.3. Interbiopolymer complexes can be regarded as a new type of food biopolymer whose functional properties differ strongly from those of the macromolecular reactants.

Protein-polysaccharide incompatibility in solution may result in flocculation of a protein suspension or an emulsion. *Depletion flocculation* occurs when a disperse system contains two incompatible biopolymers, one of which is adsorbed on the dispersed particles and the other of which is dissolved in the dispersion medium. Because of biopolymer incompatibility, the concentration of the dissolved biopolymer is strongly reduced near the surface of dispersed particles covered by the other incompatible biopolymer. When two particles surrounded by this depletion layer approach due to Brownian motion, a microvolume with a very much reduced concentration of the dissolved biopolymer between the two particle is formed. This results in formation of a solvent chemical potential gradient. The solvent is transferred by diffusion from the gap between the particles to the dispersion medium, which in turn causes aggregation of the particles (Tolstoguzov, 1997).

1.8.2.2. Composition-structure-property relationship of electrostatic complexes

Macromolecular interactions responsible for complex formation are divided into three type: 1) between macroions, 2) between oppositely charged side groups, and 3) between other available side groups of macroions. In the first case, the net opposite charge, the shape, size and flexibility of macroions are important factor. In the other two cases, reactivity of side chain groups of amino acid residues available on the exterior surface of the protein molecules and on the sugar units of polysaccharide backbone are important. The spacing, geometrical arrangements of the side chains, and the ability of side chain groups to mutually orient themselves also contribute to the efficiency of interbiopolymer complex formation. During formation of electrostatic complexes, the overall net charge of anionic polysaccharides decreases with gradual attachment of each successive protein macroion. Diminishing net opposite charges on macromolecular reactants reduces both the hydrophilicity and the solubility of the resultant complex. It also leads to a decrease in the IEP of the complex compared to that of the initial protein. The higher the relative content of polysaccharide, the lower the pH at which the complex precipites. This process leads to an electrostatically neutral insoluble complex.

Electrostatically associated insoluble complexes can aggregate by ionic bonds, hydrophobic interactions and hydrogen bonds.

When biopolymers form the complexes their also form the junction zones. The junction zones of a complex are regions where segments of two or more molecules of different biopolymers are joined together. The heterogeneity of junction zone in terms of number, size and stability is duo to differences in chemical structure, size, and shape of interacting chains. Because of rigid and compact conformation of proteins the number of active sites (oppositely charged groups) are restricted. Thus, linear flexible macromolecules are able to form more extended interchain junction zones than globular macromolecules (such as globular protein molecules).

The composition-property relationships of complexes are affected by conformations of the interacting macromolecules. Proteins with an unfolded structure, such as gelatin and casein, are able to form a maximum number of contacts with an oppositely charged polysaccharide. Moreover, the composition and the properties of an electrostatic interpolymer complex depend on the pH, ionic strength and the nature and the ratio of the biopolymers.

The stability of protein-anionic polysaccharide complexes against high ionic strength and pH values above the IEP of protein may be increased by thermal denaturation of the bound globular protein and by adding divalent cations (such as Ca, Fe, Cu, etc.). The increase in stability caused by thermal denaturation is attributable to an increase in the number of hydrophobic interactions, hydrogen bonding and coordinate bonds within the complex duo to unfolded structure formation of proteins as consequence of denaturation. The stabilizing effect of divalent cations may be attributable to their ability to cross-link proteins and anionic polysaccharides.

The formation of junction zones affects both particle-solvent and particle-particle interactions. The solubility of proteins may be increased by electrostatically complexing them with anionic polysaccharides. Moreover, in this way, it is possible to avoid the protein precipitation at the IEP. Anionic polysaccharides can act as protective hydrocolloids inhibiting aggregation and

precipitation of like-charged dispersed protein particles, e.g., of denatured proteins. This protective action also can increase the stability of protein suspensions and oil-water emulsions stabilized by soluble protein-anionic polysaccharide complexes.

Moreover, an increase in junction zone size decreases the solubility and causes precipitation of complex. The resulting insoluble complex may be dissociated to recover and reuse the polysaccharide. Thus, protein-polysaccharide interactions can be used as a method to fractionate proteins.

Interbiopolymer complexes can be used to encapsulate liquid and solid materials. Generally, for encapsulation, the protein and polysaccharide solutions are mixed at a pH above the protein's IEP. Then, the pH is readjusted to a value below the protein's IEP; this causes phase separation and coating of each dispersed particle by a layer of the complex phase formed.

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3. Aim of the thesis

The concept of using edible films or coatings to extend the shelf life of foods and protect them from harmful environmental effects has been emphasized in recent years. Increased consciousness of environmental conservation and protection, the need for higher quality foods, the demand for new food processing and storage technologies, and the scientific discovery of the functionality of new materials have renewed researchers and industrial interests in edible films and coatings.

The success of an edible films or coatings in extending the shelf life and enhancing the quality of food strongly depends on its barrier properties to moisture, oxygen, and carbon dioxide which in turn depends on the chemical composition and structure of the film-forming polymer.

Therefore, the aim of work was to investigated the effect of composition on network formation and functional properties of hydrocolloid-based films. The results of the work will help the designing of coating materials obtained by natural biopolymers with specific properties, such as solubility, barrier and mechanical properties, to control physiological, microbiological and physicochemical changes in food products, with an improvement of the food quality and an increasing of their shelf-life.

To reach this objective the research was articulated in the following study cases:

I Study case: Protein-polysaccharide interactions: study of phase behaviour of pectin-soy protein mixture.

II Study Case: Effect of composition on hydrocolloid edible film network formation: use of Friedrich-Heymann model.

III Study Case: Performance of pectin-based edible films.

IV Study Case: Performances of soy protein-pectin films cross-linked by TGase enzyme.

V Study Case: Food application of pectin-based edible film: oil absorption reduction on French fried potatoes.

PART II

1. I STUDY CASE

Protein-polysaccharide interactions: study of phase behaviour of pectin-soy protein mixture.

ABSTRACT

Numerous investigation on protein-polysaccharides systems have recently been undertaken and are leading to a better understanding of the key parameters implied in protein-polysaccharide interactions. Scattering methods are being developed to describe the structure formation in the mixed systems in combination with rheological characterization. In this work the phase behaviour of pectin-soy protein mixtures was investigated. In order to obtain information on pectin-soy protein interaction, in electrostatic compatibility condition, turbidity and viscosity measurements were performed. The results indicated that pectin-soy protein mixtures were characterized by associative interaction that leads to a stabilisation of soy flour components from precipitation.

1.1. Introduction

Biopolymers have important technological applications as thickeners, gelling agents and coatings. When two different biopolymers are mixed together, it is unusual for the behaviour of the individual components to be unaffected by the presence of the other polymer. A proper understanding and control of these different interactions should enable food scientists to design products with desired structure and texture. Future efforts should be focused on the study of the relationships between the structure and the molecular interactions, as well as on the effect of the interaction on the molecular structure. Normally, the enthalpic interactions between unlike chains will be either more favourable or less favourable than interactions between like chains of each type. When the heterotypic interactions are enthalpically unfavourable, there will be a tendency for the system to segregate into regions where the individual chains are surrounded by others of the same type, whereas enthalpically favourable heterotypic interactions will promote association between the two polymers.

In a few systems, association appears to occur by formation of specific heterotypic junctions analogous to the homotypic junctions in single-component polysaccharide gels. Normally, however, segregative interactions are far more common, and occur in virtually all biopolymer mixtures where there is no over-riding drive to heterotypic association. Associative interactions involve electrostatic attraction between polyanions (such as negatively charged polysaccharides) and polycations (such as proteins below their isoelectric point). Basically, associative phase separation implies the formation of primary soluble macromolecular complexes that interact to form electrically neutralised aggregates, then unstable liquid droplets and/or precipitates that ultimately sediment to form the coacervated phase containing both biopolymers.

Attractive and repulsive interactions forces compete to give associative or segregative phase separation. *Attractive interactions* are the usual van der Waals attraction (with depends mainly on colloid size), the depletion force (attraction between colloidal particles induced by osmotic pressure differences between polymer and colloid solutions), and the adsorption force caused by the collapse of the charged polymer onto the surface of the charged colloid of opposite sign. The *repulsive interactions* are the electrostatic repulsion between similarly charged macromolecules and the steric repulsion in the case where adsorbed polymer layers are formed at the surface of the colloidal particles (Renard, Boué and Lefebvre, 1997).

The phase behaviour of protein-polysaccharide-water mixtures has been the subject of a considerable number of investigations.

In protein-polysaccharide systems, many parameters such as solvent conditions (pH, ionic strength, etc.), the chemical structure of the polysaccharide, its molecular weight and conformation, the type of protein, etc., are known to be involved in the separation process (Doublier, Castelain, Llamas and Lefebvre, 1995).

The phase behaviour of bovine serum albumin-hydroxyethylcellulose (BSA-HEC) systems in aqueous media at neutral pH was investigated by Doublier and coworkers (1997). The results obtained in this work demonstrate that the phase behaviour in BSA-HEC systems is strongly dependent upon the

molecular weight of the polymer. The rheological properties of the system which, in the absence of interactions, should reflect those of the polysaccharide solution, are strongly modified by the presence of the protein even in the single phase region and even though phase separation does not occur at the macroscopic scale. Moreover, the addition of BSA to the polysaccharide solution involve a drastic increase of the elasticity of the medium. Both phenomena obviously indicate solid-like properties suggesting the formation of a weak and labile network would originate from flocculated BSA-rich droplets.

Renard *et al.*, 1997, were studied the structure of the systems bovine serum albumin (BSA)+hydroxyethylcellulose (HEC) and BSA+carboxymethylcellulose (CMC) mixtures in the one-phase region, and to show how it is affected by shear. They have performed small-angle neutron scattering (SANS) and rheological measurements. The results were proved that according to combined SANS and rheological measurements, the structures of protein/polysaccharide mixtures located in the one-phase region of the phase diagram are heterogeneous and can be compared to colloidal suspensions where the beginning of flocculation is evident. In agreement with the distances between neighbouring particles, the structure of these systems appear to fall into two classes: a) in the situation where no attraction were establish between the polysaccharide and the protein, flocculation of BSA particles via depletion forces or electrostatic repulsions is important; b) in the case where attraction exists between the protein and segments of the polysaccharide coils, the extent of flocculation via these mechanisms is considerably reduced.

Michon, Cuvelier, Lauay and Parker (1997), were investigated the effect of protein net charged on phase diagrams, the type of interactions and the rheological properties of gelatin + ι-carrageenan systems. They were found that the viscoelastic behaviour of the mixed transparent system during melting provides evidence for associative interactions between gelatin PS (from pig skin) and ι-carrageenan. The interaction between gelatin PS and ι-carrageenan seems to always be associative even in transparent one-phase mixtures. The effect of these associations is more obvious when the gelatin chains are in a helical conformation, but are unable to form a separate

continuous network. They conclude that mixed gelatin PS/ι-carrageenan gels are probably structured by interpenetrating, coupled gelatin and ι-carrageenan networks according to Morris' classification (Oakenfull, Pearce and Burley, 1997). Phase separation may some-times occur at low total concentration, when gelation does not prevent the precipitation of the complexes.

Moreover, for gelatin LH (from limed hide)+ι-carrageenan systems, this study suggests that associative interactions are either completely absent or only very local, which is reasonable since the net charge of both polymers is negative. Gelatin LH and ι-carrageenan are compatible over a wide range of compositions.

This research was focused on binary mixtures of two different types of biopolymer. The purpose of the present investigation was to explore the phase behaviour of pectin-soy protein mixtures in order to identify conditions of thermodynamic compatibility.

1.2. Materials and Methods

1.2.1. Materials

Pectin from citrus fruits and soy flour were purchased from Sigma (Milano, Italy). *Pectin characteristics*: Galacturonic acid content 93.5% (as is); methoxy content 9.4%; loss on drying 7.3% (pKa= 3.0-4.5). *Soybean flour characteristics*: 52% protein (85+% dispersible) and 1% fat (pI=4.75-8.50).

1.2.2. Sample preparation

Two pectin suspensions at 16mg/ml and 8mg/ml concentrations and two soy flour suspensions at 13mg/ml and 6.5mg/ml concentrations were prepared by dispersing pectin and soy flour in deionized water and stirring for five hours at 80°C and for one hour at 25°C respectively. Then, in order to eliminate the no solubilized fractions, suspensions were centrifuged (Centrifuge-Sigma 3-18K, Alemagne) at 3800G for 30 minutes at 20°C.

Pectin (P) and soy flour (SF) suspensions were mixed together in different ratios in order to obtain four mixture groups:

A) [16mg/ml]P / [13mg/ml]SF: 10/90, 25/75, 50/50, 75/25, 90/10;

B) [8mg/ml]P / [13mg/ml]SF: 10/90, 25/75, 50/50, 75/25, 90/10;
C) [16mg/ml]P/ [6.5mg/ml]SF: 10/90, 25/75, 50/50, 75/25, 90/10;
D) [8mg/ml]P/ [6.5mg/ml]SF: 10/90, 25/75, 50/50, 75/25, 90/10.

Then, in order to involve the formation of electrostatic complexes between biopolymers, the pH of samples was adjusted to 4.6 (pK_a of pectin carboxyl group<pH<soy protein pI). Finally, the mixtures were storage for ten day at 25°C and 70°C.

1.2.3. Determination of soluble proteins

The protein content of soy flour suspension at concentration of 13mg/ml were evaluated by means of a colorimetric assay, using the Micro BCA™ Protein Assay Kit (Pierce, USA). In particular, aliquots of 8ml of soy flour suspension were stored in glassy tubes until 10 days, in order to monitor an evolution of soluble soy protein content versus aging time. Then, the supernatant was taken regularly and assayed.
The dry matter content of the supernatant was determined too.

1.2.4. Dry matter content

Dry matter for all samples was determined measuring weight loss upon drying in an oven at 105 °C until constant weight and expressed as dry matter content /total weight %.

1.2.5. Turbidity measurements

Turbidity of mixtures was evaluated by measuring the backscattering intensity of incident light (wavelength 860 nm) along the height of an optical glass tube, using the Turbiscan MA 2000 apparatus (Ramonville St. Agne, France). The obtained profile gives a qualitative indication of distribution of the aggregates along the height of the tube since the backscattering intensity depends on particle number and distribution. The turbidity measurements were carried out at two different temperatures (25°C and 70°C) and at two times (0 day and 10 days).

1.2.6. Turbidimetric titrations

Mixtures of soy flour [13mg/ml] and pectins [16mg/ml] suspensions in different ratios (10:90, 50:50, 90:10) were prepared in presence and in absence of 0.5M NaCl. The initial pH was adjusted to 4.6.
Changes in turbidity were monitored at 600 nm (Biorad, Smartpec 2000) and at temperature of 25°C.

1.2.7. Rheological measurements

Flow curves were obtained using of a strain controlled rheometer (RFS II, Rheometrics Inc. Piscataway, NY), fitted with coaxial cylinders (34mm o.d. and 32mm i.d.). The stress (σ) versus shear rate ($\dot{\gamma}$) was recorded at constant temperature. The shear rate ranged between 10^{-2}-10^{2} s^{-1}. To study the effects of time and temperature on complex formation, tests were performed at two different aging times (0 day and 10 days) and at two different temperatures (25°C and 70°C). Water evaporation was prevented by sealing the sample with low viscosity immiscible silicon oil.

1.3. Results and discussion

1.3.5. Soluble protein content

The concentration of suspensions after centrifugation changed and become: 15.7mg/ml and 7.4mg/ml for pectin suspensions, 10.4mg/ml and 4.6 for two soy flour suspensions.
The Figure 1 shows the evolution, during 10 days, of the soluble protein concentration in a soy flour solution containing 10.4mg/ml of dry matter just after centrifugation . The dry matter evolution during the same time was reported too.
The soluble protein content in soy flour suspension was about 7 mg/mg in the solution just after preparation plus centrifugation (t= 0 day) representing 66% of the dry matter. Then, the soluble protein concentration decreased to reach a plateau after 7 days: about 2 mg/mg after 10 day of storage.
The same decrease was also observed for the dry matter. After 10 days, the protein concentration corresponds to about 50% of the dry matter. Thus, a

precipitation occurs, involving proteins but probably also polysaccharides that are present in soy flour. Soy flour solution is a non stable systems in which complexes may be formed during aging, become insoluble and settle to the bottom of the suspension.

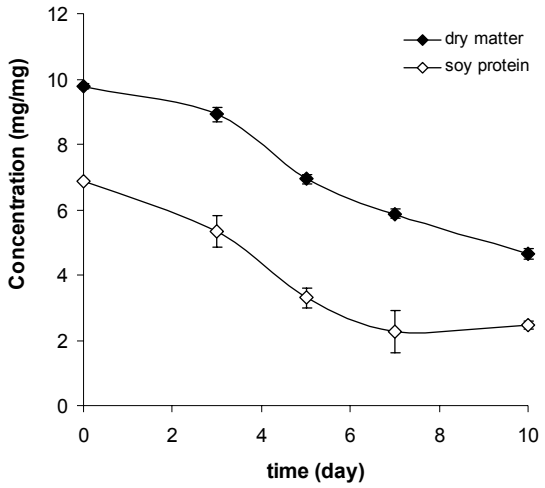


Figure 1. Soy protein content (◊) and dry matter (◆) evolution versus aging time.

1.3.6. Turbidity results

Figure 2a shows the transmission percentage versus pectin/soy flour ratio (%) for the two mixtures (A and B groups) evaluated just after dispersion and centrifugation (t=0 day).

The soy flour in water solution is cloudy (20% of transmission) event just after centrifugation. For the 10/90 P/SF ratio the cloudiness increases a lot, such as the % transmission decreases to almost 0. For higher P/SF ratio, the % transmission increases regularly but not linearly to reach a level of 90 and 70 for 100% P systems containing respectively 7.4 and 15.7 mg/ml of pectin. Soy flour contributes more to the cloudyness then the pectin. The straight line joining the 100% SF and the 100% P points corresponds to the level of transmission we could expect in case of additivity of the turbidity. All the % transmission level of the ratio in between 100% SF and 100% P are below the additivity line. Thus all the mixed systems are more turbid then predicted by a

simple additivity law. This could be due to the formation of associative aggregates between pectin and soy flour macromolecules (protein and/or polysaccharides). In order to verify this hypothesis salt was added to mixed system. In every case 0.5 M NaCl addition leads to a decrease of the turbidity (Table 1) which is in good agreement with a screening effect of the macromolecules charges leading to a decrease of the complex formation.

Table1. Turbidity titration

[15.7mg/ml]P: [10.4mg/ml]FS ratios	Absorbance	
	without 0.5NaCl	with 0.5NaCl
10:90	1.238±0.006	1.135±0.005
50:50	0.531±0.001	0.359±0.004
90:10	0.273±0.003	0.268±0.002

After 10 days (figure 2b), the evolution of the transmission versus P/SF ratio is the same as for 0 days excepted the 100% soy flour system which is initially very turbid (only 20% transmission) and becomes clear after 10 days of aging. A sediment is observed in that case at the bottom of the tube corresponding to the precipitation of polysaccharide-protein insoluble complexes. All the other studied systems showing no evolution of their transmission level are rather stable. Associative phase separation implies the formation of primary soluble macromolecular complexes that interact to form electrically neutralised aggregates (Doublier *et al.*, 2000). Even if complexes are formed they are, at least, partially soluble. It seems that the system containing 50/50 P/SF shows a % transmission nearer to the additivity line than all the other mixed system. The complexes solubility depends on the proportion of pectin and soy flours macromolecules.

When decreasing the initial concentration of pectin, the transmission is systematically higher whatever the P/SF ratio. This may be due only to the decrease of pectin concentration. However for the 50/50 and 75/25 ratios the difference is much higher than for the 100/0 ratio. Thus the larger increase of %transmission for 50/50 and 75/25 ratios is probably due to the modification of the stechiometry and in consequence the composition of complexes that seem to give less turbidity to these mixed systems.

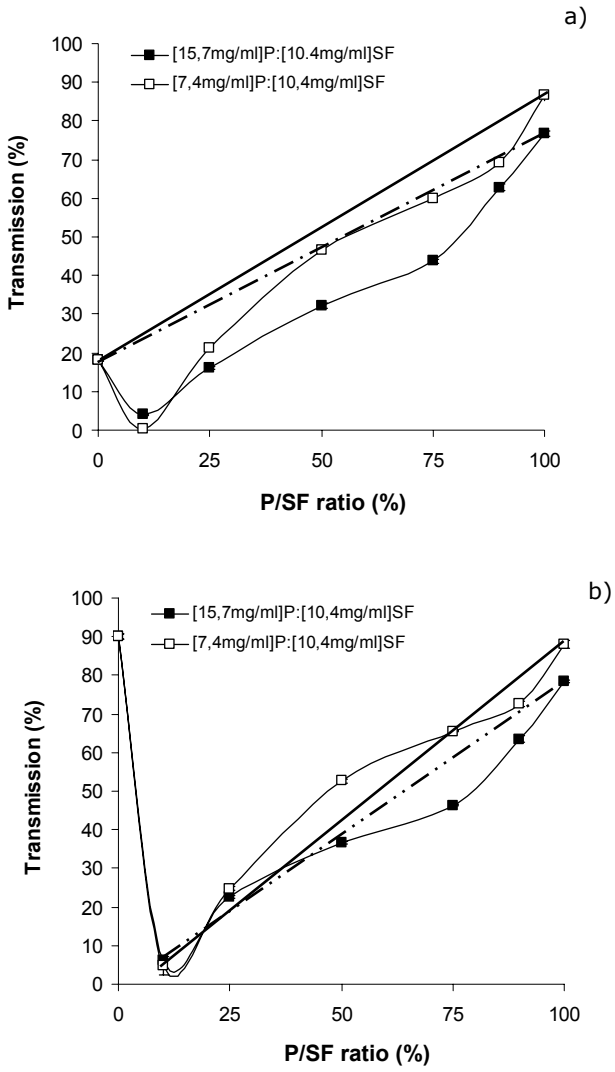
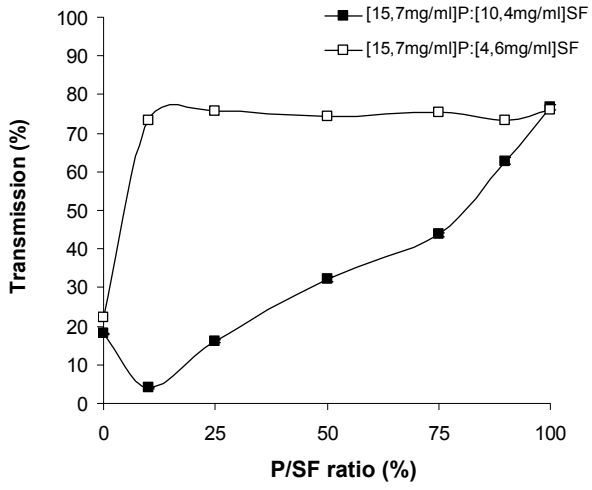


Figure 2. Transmission (%) as function of pectin/soy flour ratio for two mixtures (■)[15.7mg/ml]P:[10,4mg/ml]FS; (□) [7.4mg/ml]P:[10,4mg/ml]FS) at (a) t=0 day and (b) 10 days. T=25°C.

The Figure 3 shows the transmission percentage of water-soy flour-pectin ternary systems obtained by mixing pectin suspension at concentration to 15.7mg/ml with two soy flour suspensions at concentration 10.4mg/ml and 4.7mg/ml (A and C mixtures) just after preparation (a) and after 10 days (b). The results show that just after the mixing, the samples to 100% of soy flour

(stock suspensions of soy flour at 10.4mg/ml and 4.6mg/ml) are characterized by a transmission percentage around 20% (Figure 3a). When the pectin is added to system in the ratio of 25:75, the behaviour of the mixed system is very different depending on the soy flour concentration. For 6.6 mg/ml (75% of 10.4mg/ml initial concentration), as already comment on figure 2, the % transmission decreases down to zero, because of the formation of complexes. For the 3.5 mg/ml (75% of 4.7 mg/ml solution) the % transmission is very high and reaches a level of 80% (curve with open symbols). When the pectin/soy flour ratio increases, the mixture transparency remains constant (% transmission = 80) suggesting that as the soy flour concentration decreases and the pectin concentration increases, no complexes giving turbidity to the solution are formed. After 10 days, excepted the 100% soy flour system that becomes almost transparent (% transmission = 90) because of the precipitation of protein-polysaccharides aggregates, no change is observed in the turbidity of all the other systems is observed. There is no kinetic effect. Moreover, it seems that the formation of polysaccharide-protein aggregates can not occur. Even if entrapped in a pectin network, they should provoque an increase of the turbidity of the system (i.e. a decrease of the % transmission).

a)



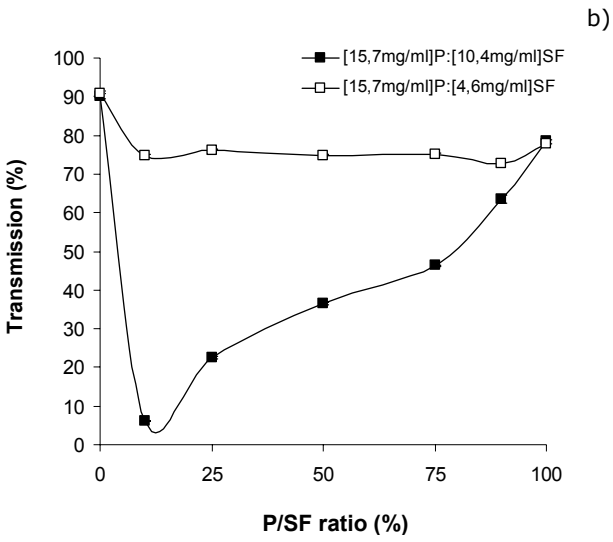


Figure 3. Transmission (%) as function of pectin/soy flour ratio for two mixtures (■) [15.7mg/ml]P:[10.4mg/ml]FS; (□) [15.7mg/ml]P:[4.6mg/ml]FS) at (a) t=0 day and (b) t=10 days. T=25°C.

Thus we can conclude that some associations between pectin and soy flour proteins and/or polysaccharide occur and leads to a stabilisation of soy flour components from precipitation. In the Figure 4 was reported the transmissions % for the [15.7mg/ml]P: [10.4mg/ml]FS mixture stored at 70°C. The stock suspension of soy flour is characterized by a higher transparency (~32% of transmission) than the same solution stored at 25°C (~20% of transmission, Fig.2a). For others systems there is no sensible difference between the % transmission measured at 25 and 70°C. A temperature effect appears after 10 days of storage. All the systems become clearer (increase of the % transmission) whereas at 25°C no difference could be seen. In the same time precipitates are found on the bottom of the tube aged at 70°C where no precipitation occur at 25°C. This, can be explained by considering that a temperature increase leads to a viscosity decrease (Andrade law : $\eta = A\, e^{-B/RT}$). At 70°C the viscosity may be not high enough to keep aggregates dispersed in the solutions.

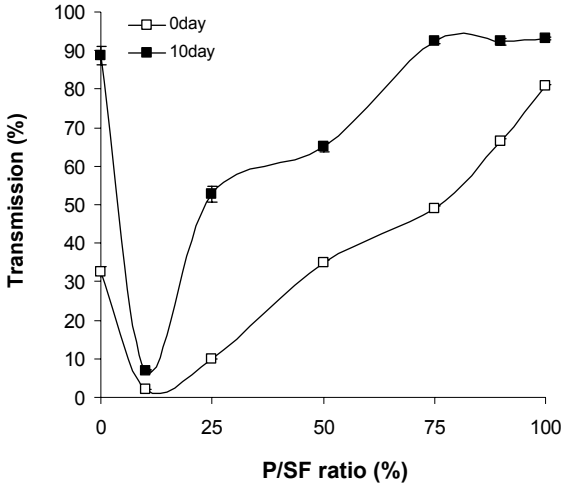


Figure 4: Transmission (%) as function of pectin/soy flour ratio for [15.7mg/ml]P:[10,4mg/ml]FS mixture at T=70°C.

1.3.3. *Viscosity results*

In figure 5 are reported the stress-rate curves obtained for 15.7 mg/ml pectin solutions mixed with 4.6 mg/ml soy flour solutions (filled symbol) or 10.4 mg/ml soy flour solutions (open symbol). All the curves follow the dotted lines with a slope of 1 for rates in the range 30-100 s^{-1}. At high shear rate, all mixed systems show Newtonian behaviour. The higher the P/SF ratio, the higher is the stress for a given shear rate, thus the higher is the Newtonian viscosity. Curves containing different level of soy flour (open and filled symbols) are superimposed in the Newtonian part (30-100s^{-1}). The flowing behaviour, at high shear rate is dominated by pectin. There is no sensible contribution of soy flour in the range of tested concentration.

All systems behaviour do not follow a Newtonian one when the shear rate decreases to values lower than 10 s^{-1}. The shear stress tends to constant value (yield stress) when the shear rate tends to zero. The yield stress value, obtained by an extrapolation of the curve at a shear rate of zero, is very small for all systems (lower than 0.01 Pa). However such a small value may be enough to suspend the aggregates and prevent their precipitation.

An effect of the soy flour concentration is observed in the small shear rate range. A decrease of the soy flour concentration leads to an increase of the yield stress. If it is hardly noticeable for the 50:50 ratio, it is very clear for the 10:90 ratio for which the yield stress is multiplied by 2 when the soy flour concentration is divided by 2.

In Figure 6 was reported the apparent viscosity measured in the shear rate range for which a Newtonian behaviour is systematically observed, versus the different pectin/soy flour ratios at 25 and 70°C.

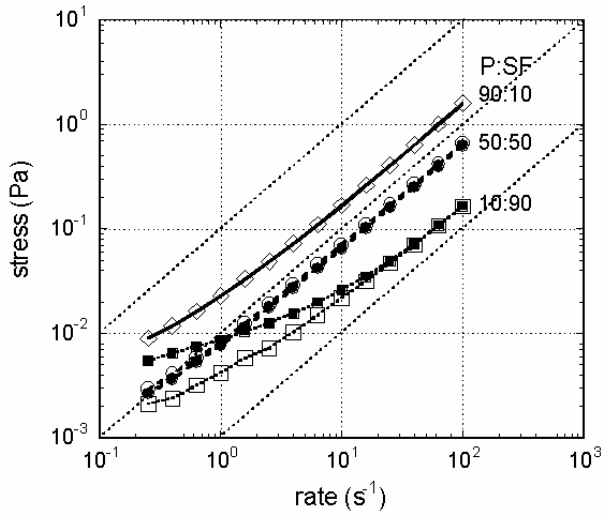


Figure 5: Flowing behavior of 15.7 mg/ml pectin solutions mixed with 4.6 mg/ml soy flour solutions (filled symbol) or 10.4 mg/ml soy flour solutions (open symbol). Ratios are written on the figure. Oblique dotted lines corresponding to the slope of a newtonian fluid were drawn to guide the eyes

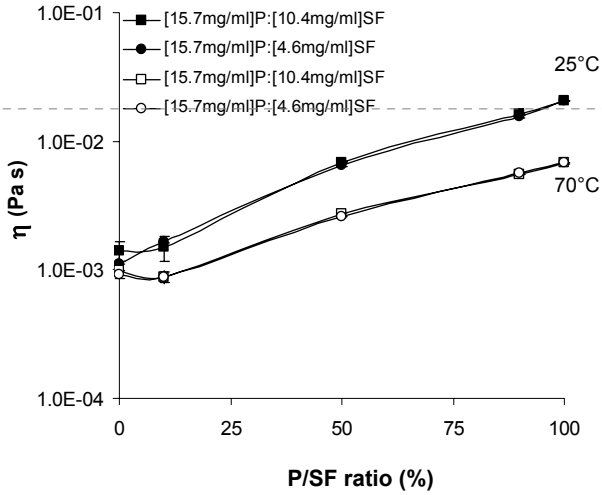


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Figure 6. Viscosity mixtures evaluated at two different temperature (25°C (filled symbols) and 70°C (open symbols)) at t=0day for two initial concentrations of soy flour 10.4 mg/ml (■□) and 4.6 mg/ml (●○).

When the pectin/soy flour ratio increase the viscosity increase for all mixtures investigated showing the dominant effect of pectin on the flowing behaviour of these systems. An increase of the temperature from 25 to 70°C decreases the viscosity which is divided by 3.

1.4. Conclusions

The results of this study prove that the turbidimetric and rheological methods are a useful tool to obtain information on pectin-soy protein interactions. The experimental data highlight that by blending charged soy protein with anionic pectin the associative interaction between biopolymers occurs. In particular, the turbidity measurements indicate the formation of soluble aggregate and that the solubility depends on the proportion of pectin and soy flours macromolecules. This results were confirmed by rheological measurements.

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2. II STUDY CASE

Effect of composition on hydrocolloid edible film network formation: use of Friedrich-Heymann model

ABSTRACT

Edible films with different compositions were submitted to dynamic mechanical analyses and microstructure analyses. The aim was to gain information on the effect of composition on network formation by means of the Friedrich-Heymann model. Results prove that the film network organization is reasonably well described by the simplified Friedrich-Heymann model and that the oscillatory tests might be a useful tools for screening among the film components for product and process design purpose.

2.1. Introduction

Functional properties of edible films can be affected by polymer structure, plasticizer concentration, solvent and other factors related to film dissolution, permeability and diffusion properties (Chen, 1994). Improved film performances are obtained with a multi-component system where hydrocolloids (proteins or polysaccharides) form a continuous and cohesive network, and the hydrophobic substances (lipids) provide the moisture barrier properties (Krochta, Baldwin and Carriero, 1994).

However, polymeric films produced by physical methods often result in poor mechanical and permeability properties when compared with those obtained through chemical reactions. On the other hand, chemical cross-linking agents frequently induce toxicity or confer other undesirable effects to these materials. Therefore, the possibility to use enzymatic methods both to prepare polymeric films and to improve their features has been object of extensive studies (Lim, Mine and Tung, 1999; Jun-Hyun, Wang, Field and Aglan, 2004). In a recent paper the potential use of transglutaminase as biotechnological tool for preparing pectin-soy protein films through an enzymatic cross-linking reaction was examined (Mariniello, Di Pierro, Esposito, Sorrentino, Masi and Porta, 2003).

Modification of proteins by enzymes such as Trasglutaminase (TG, E.C. 2.3.2.13) has recently become of great interest to scientists. TG is an enzyme capable of catalyzing the formation of covalent crosslinks between peptide-

bound glutamynil residues (acyl donors) and ε-amino group of lisine residues (acyl donors) in proteins, including a variety of primary amines (Nakaoca *et al*., 1994). In the absence of amine substrate in the reaction system, water becomes the acyl acceptor and the γ-carboaximide groups of glutamine residues are deaminated, becoming glutamic acid residues (Fig.1). TG has been found in invertebrates (Singh and Mehta, 1994), vertebrates including amphibians (Zhang and Masui, 1997), fish (Yasueda, Kumazawa and Motoki, 1994) and birds (Puszkin and Raghuraman, 1985), plant tissue (Villalobos, Santos, Talavera, Rodriguez-Falcon, Tornè, 2004) and microrganisms (Ando *et al*., 1989).

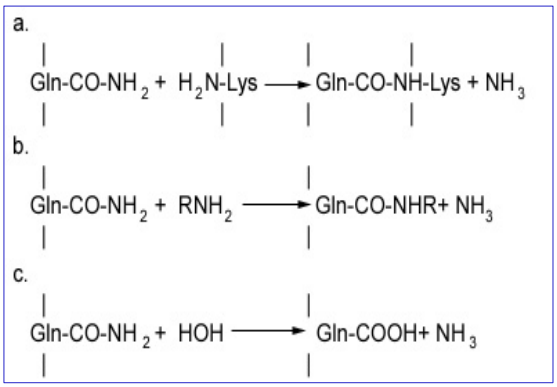

Figure 1. TG-catalysed reactions

In mammals TG are present in most tissues and body fluids and are involved in several biological processes, including blood clotting, wound healing and epidermal keratinization (Aeschilmann and Paulsson, 1994). Among these TG, the human blood coagulation Factor XIIIa, an activated form of plasma TG (Chung, Lewis, and Folk, 1974) has been most characterized. By catalyzing the cross-linking between fibrin molecules, Factor XIII forms fibrin clots for haemostasis and heals a wound (Kashiwagi *et al*., 2002). Many TG are homologous to human Factor XIII and share the common feature of Ca^{2+} dependent catalytic activity (Folk and Chung, 1973).

A microbial TG (mTG) has been isolated from the culture medium of *Streptoverticillium* sp. S-8112 (Ando *et al*., 1989), which has been identified as a variant of *Streptoverticillium mobaraense*. This enzyme is the first TG obtained from a no mammalian source. Although the physiological role of mTG is still unknown, this protein is secreted from the cytoplasm membrane as a zymogen and is activated by proteolytic processing (Pasternack, Dorsch, Otterbach, Robenek, Wolf and Fuchsbauer, 1998). A sequence analysis of mTG by Edman degradation revealed that the protein consists of 331 aminoacids with a molecular mass of 37.9 KDa (Kanaj *et al*., 1993). In contrast to other members of the TG family, the mTG exhibits Ca^{2+} independent activity. Moreover, mTG is considered to be stable over a wide pH range (4-9) and the optimum temperature for enzymatic activity is 55 °C (Yokoyama, Nio and Kikuchi, 2004). Studies have shown that mTG catalyses the cross-linking of a number of proteins, including legume globulins such as 11 S globulin (Yildirim and Hettiarachchy, 1997) and oat globulins (Siu, Ma and Mine, 2002), whey proteins (Mahmoud and Savello, 1993; Yildirim and Hettiarachchy, 1997), myosin and fibrins (Nonaka *et al*., 1989), milk proteins (Motoki, Seguro, Nio and Takinami, 1986; Cozzolino, Di Pierro, Mariniello, Sorrentino, Masi and Porta, 2003), including α-lactalbumin and β-lactoalbumin, as well as many other albumins (Nonaka, Sakamoto, Toiguchi, Kawajiri, Soeda, and Motoki, 1992; Kang, Ozaki, Takao, Kawajiri, Ide, and Motoki, 1994). For its characteristics (such as Ca^{2+} independence activity, the higher reaction rate, the broader substrate specificity for the acyl donor, the lower activity for deamidation and the small molecular size) mTG is particularly useful for a industrial and biotechnological applications (Kashiwagi *et al*., 2002). In fact it has been proved that the modification of food proteins by mTG leads to textured products, protects lysine residues in food proteins from various chemical reactions, improves elasticity, firmness and water holding capacity, modifies solubility and functional properties, and produces food proteins of higher nutrition value through cross-linking of different proteins containing essential aminoacids (Matheis and Whitaker, 1987; Kitabatake and Doi, 1993; Motoki and Seguro, 1994).

The rheological approach provides to obtain information on material structure and its organization. In particular, the small amplitude deformation oscillatory measurements are a non-destructive technique enabling measurements to be made without incurring structural damage to the sample. This allows researchers to relate dynamic rheological parameters to the sample molecular structure. Therefore, it is possible to obtain information on cross-linked network formation of edible films.

In the linear viscoelasticity regime, knowledge of the evolution of the shear relaxation modulus G(t) over the entire range of time permits calculation of all the other viscoelastic functions (Ferry, 1980):

$$G'(\omega) = G_0 + \omega\int_0^{\infty}[G(t) - G_0]\sin(\omega t)dt \qquad (21)$$

$$G''(\omega) = \omega\int_0^{\infty}[G(t) - G_0]\cos(\omega t)dt \qquad (22)$$

To solve the equations (21) and (22), G(t) was assumed to coincide with the Friedrich and Heymann model (1988):

$$G(t) = G_{0,\alpha} + \frac{S_\alpha^*}{\Gamma(1-\alpha)}t^{-\alpha}e^{\frac{-t}{\lambda_\alpha}} \qquad (23)$$

where α is the order of the relaxation function, $\Gamma(1-\alpha)$ is the Gamma function and $G_{0,\alpha}$, S_α^* and λ_α are, respectively, the equilibrium modulus, a material shear parameter and the mean relaxation time pertaining to α. Such an extended relaxation function was found to be able to reconstruct the evolution of linear viscoelasticity in oscillatory experiments during cross-linking reactions before and after the gel point.

Upon integration of equations (21) and (22), the analytical dependence of $G'(\omega)$ and $G''(\omega)$on frequency was expressed as (Friedrich and Heymann, 1988):

$$G'(\omega) = G_{\infty,\alpha} + \sqrt{\frac{2}{\pi}} S_\alpha^* \lambda_\alpha^{-\alpha}(\omega\lambda_\alpha) \frac{\sin[(1-\alpha)\arctan(\omega\lambda_\alpha)]}{\left[1+(\omega\lambda_\alpha)^2\right]^{\frac{1-\alpha}{2}}} \quad (24)$$

$$G''(\omega) = \sqrt{\frac{2}{\pi}} S_\alpha^* \lambda_\alpha^{-\alpha}(\omega\lambda_\alpha) \frac{\cos[(1-\alpha)\arctan(\omega\lambda_\alpha)]}{\left[1+(\omega\lambda_\alpha)^2\right]^{\frac{1-\alpha}{2}}} \quad (25)$$

At moderate and high frequencies, such equations exhibit the same slope for G' and G'' (i.e. $G' \propto \omega^\alpha$, $G'' \propto \omega^\alpha$), while at very low frequencies they may describe either liquid ($G' \propto \omega^2, G'' \propto \omega^1$) or solid ($G' \approx G_{0,\alpha}, G'' \propto \omega^1$) behaviour.

Moreover, Friedrich and Heymann (1988) were able to demonstrate that in the high frequency range, near the gel point or after the transition sol-gel (i.e. $\omega\lambda_\alpha \to \infty$) equations (24) and (25) can be reduced to:

$$G'(\omega) = G_{\infty,\alpha} + \sqrt{\frac{2}{\pi}} S_\alpha^* \cos\left(\frac{\pi}{2}\alpha\right)\omega^\alpha \quad (26)$$

$$G''(\omega) = \sqrt{\frac{2}{\pi}} S_\alpha^* \sin\left(\frac{\pi}{2}\alpha\right)\omega^\alpha \quad (27)$$

Both these equations are independent of the relaxation time λ_α, while the tangent of phase shift would be dependent on α only:

$$\tan\delta = \frac{G''}{G'} = \tan\left(\frac{\pi}{2}\alpha\right) \quad (28)$$

Provided that the equilibrium modulus ($G_{\infty,\alpha}$) is equal to zero (this holding for the sol state and at the gel point) or can be neglected (this holding at the gel state in a limited frequency range only).

The objective of this study was to determine the influence of composition on film structure formation. For this purpose the mechanical and micro-structural properties of pectin and pectin-soy protein films obtained in presence or in absence of TGase were detected. Information concerning the three-dimensional film structural network was derived by using the Friedrich-Heymann model.

2.2. Materials and Methods

2.2.1. Materials

Pectin from citrus fruits and soy flour was purchased from Sigma (Milano, Italy).

2.2.2. Film making procedure

Two different type of films were investigated: films made from pectin and films made by blending pectin and soy protein. Pectin were dissolved in acidified water (pH 2) at a concentration of 16mg/ml. To obtain films with the desired amount of pectin (2.5, 3.8, 5.8mg/cm^2), different volumes of solutions were pipetted into 28.26 cm^2 polystyrene Petri dishes and dried at 35°C and 50% RH overnight under air circulation. The solutions were deaerated under vacuum, prior to casting films, to prevent pinholes formation, then transferred into dishes. To obtain films from mixtures of soy proteins and pectin, soy flour and pectin were first dissolved in water obtaining two different suspensions with concentration equal to 13mg/ml and 16mg/ml respectively and then mixed in the appropriate ratio. Then, pH of mixtures was adjusted to 4.6. These films were prepared in absence or in presence of microbial TGase. The obtained films were peeled from the Petri dishes and stored at 20 °C in a desiccator (50% RH).

2.2.3. Thickness

Film thickness was measured using a micrometer model HO62 with sensitivity of ± 2 µm (Metrocontrol Srl, Casoria (Na), Italy). Film strips were placed between the jaws of the micrometer and the gap reduced until the first indication of contact. Mean thickness (µm) of films were determined from the average of measurements at 10 locations.

2.2.4. Dynamical mechanical measurements

Dynamic mechanical analyses (DMTA V, Rheometrics Inc. Piscataway, USA) were performed on rectangular film specimens (50 x 7 mm). The sample was cut whit scissors and mounted on grips so that its length was 10 mm. All measurements were conducted in dynamic mode. Before any measurements

were taken, samples were rested for 3 min, allowing the stress induced during sample loading to relax. The linear viscoelastic region was determined by performing a strain sweep test at a given frequency of 1 rad/s. Then, the frequency sweep tests were conducted by applying an oscillation amplitude of 0.01% (within the linear region) over a frequency range between 10^0 and 10^3 rad/s. The parameters used for this study were the storage modulus (E') and the loss modulus (E").

2.2.5. Microscopy

For microscopy analysis, dried strip fragments of films were mounted on specimen stubs with cross-section oriented up and coated with a thin layer of gold by DC sputtering (AGAR B7340). Digital images of film cross-section were collected by using a LEO EVO 40 scanning electron microscope (Zeiss, Germany) with a 20kV acceleration voltage.

2.2.6. Data analysis

Friedrich-Heymann model (1988) was used to describe the frequency sweep curves as described by Moresi, Mancini, Bruno and Rancini (2001). Model parameters were estimated by non linear regression method by considering the α parameter of this model, in which the relaxation function was assumed to be independent of the relaxation time and G_∞ equal to zero. The estimated parameter (α) were submitted to analysis of variance t ($p \leq 0.05$) by means of SPSS v10.1 package.

2.3. Results and Discussion

Because small-deformation dynamic measurements represent an investigation technique not destructive, the variations of E' and E" with oscillatory frequency (ω) allow a qualitative determination of material nature (Ferry, 1980). Figure 2 shows the elastic modulus (E') and E"/E' ratio (tanδ) behaviour as function of oscillatory frequency (ω) for three films at different pectin content. The results highlighted that all samples shown the same dependence to frequency. In particular, one can observe that E' changes slowly in ω region investigated and E" is considerably lower than E' (see tanδ). The elastic modulus and tanδ values were not influenced from pectin amount.

Edible films obtained by blended pectins and soy flour were shown different behaviours. As shown in Figure 3a, the elastic modulus is affected by both film composition and oscillatory frequency. The same behaviour was observed for films cross-linked with TGase, but, in this case the dependence of storage modulus on oscillatory frequency appears more much evident (Fig.3b).

Also in this case the loss modulus is lower than elastic modulus (data no shown). Therefore, all films displayed viscoelastic solid-like proprieties.

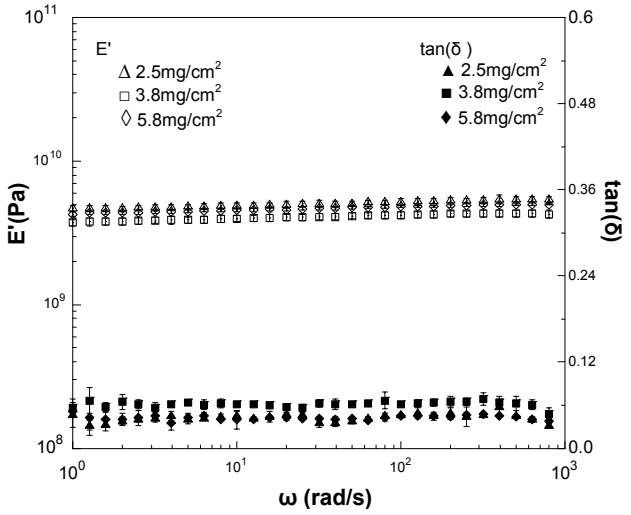

Figure2. E' (Pa) and tanδ as function of ω (rad/s)for different pectin content

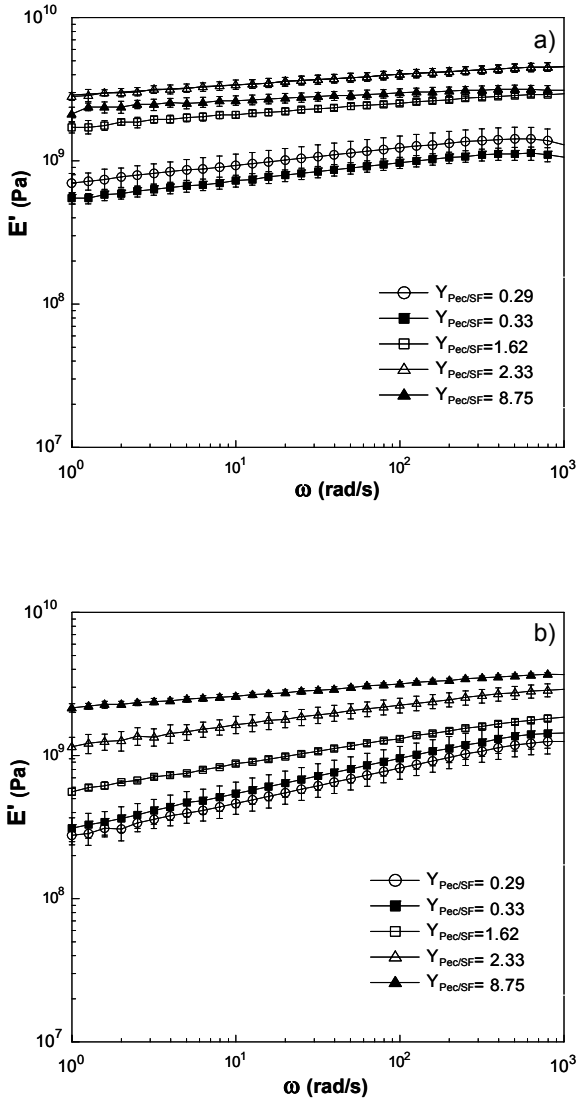

Figure 3. E' (Pa) as function of ω (rad/s) for different pectin-soy flour films: a) without Tgase; b) with TGase

In order to obtain information on structural organization of film network the frequency sweep curves were described by the Friedrich-Heymann model (1988).

Figure 4 shows, as sake of example, E' curves relative to pectin film at different concentration (a) and pectin soy and proteins film with and without

TGase (b), in the range of frequency 1-1000 rad/s. The dots represent the experimental data and the solid line the storage and loss modulus curves as predicted by the Friedrich and Heymann model. The agreement between the model and the experimental data is very satisfactory (R^2>0.99). Figure 5a shows the α parameter of the model as a function of pectin concentration. ANOVA proves that the α parameter is independent from the pectin concentration (P>0.05), on the other hand, the pectin/soy protein ratio and the TGase have a significant effect on the α parameter (p<0.01). α decreases as the PEC/SP ratio increases and increases in presence of the enzyme TGase (Figure 5b).

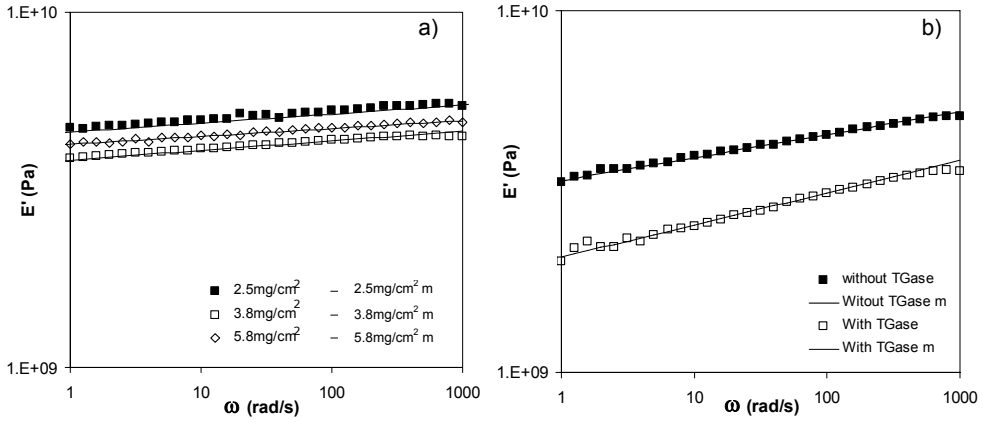

Figure 4. E' (Pa) as function of ω (rad/s) for pectin film at different concentration (a) and pectin and soy proteins film with and without TGase (b).

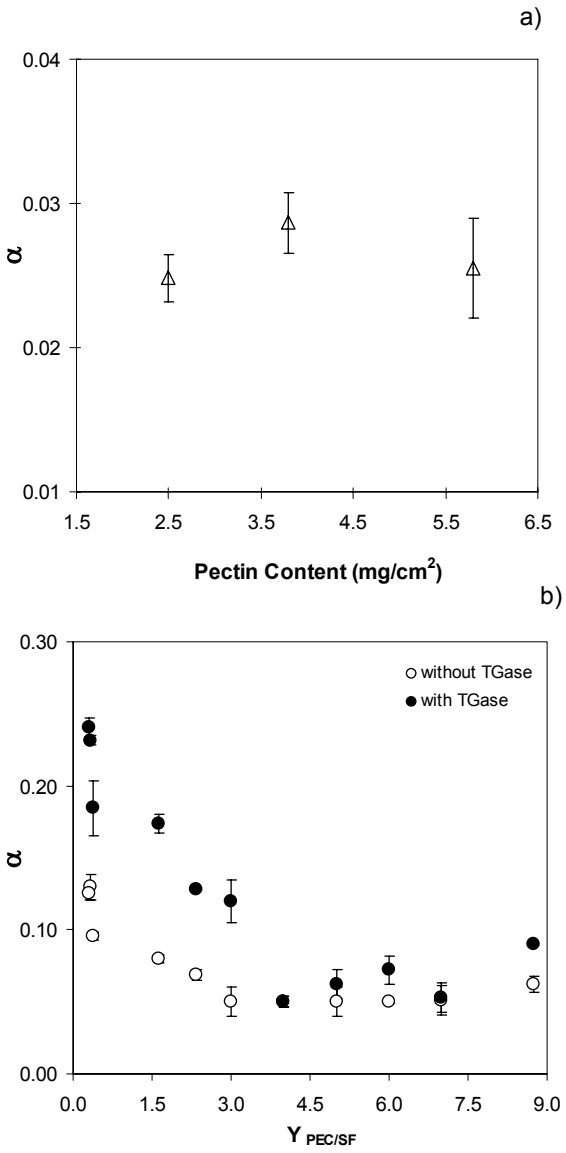

Figure 5. The α parameter as function of pectin content for the pectin films (a) and pectin/soy protein ratio ($Y_{P/SP}$) for the pectin-soy flour films (b).

In order to evaluate the effect of composition on the film microstructure, the films were analyzed by SEM.

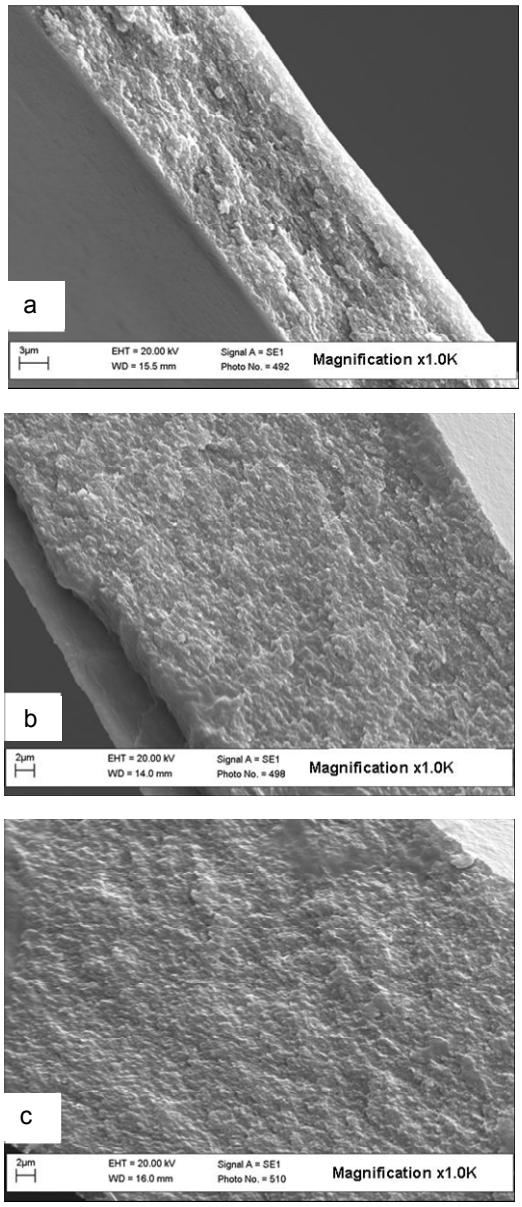

Figure 6. SEM micrographs of pectin films with different pectin content: a) 2.5 mg/cm^2, b) 3.8 mg/cm^2, c) 5.8 mg/cm^2.

One can observe, from the micrographs shown in Figure 6, that by varying the pectin content in the film-forming suspension the microstructure of the film does not changes. This is in agreement with the fact that the α parameter was

independent from pectin concentration. The pectin films were characterized by the absence of a homogeneous structure and the film structure appears to be the result of pectin clusters packed in a more or less tied way. In particular, the films with pectin content of 5.8 mg/cm^2 presents a more compact network than the one with the lowest pectin content (2.5 mg/cm^2). When pectin and soy flour proteins are blended together an improvement in the network formation of the film is observed (Figure 7a).

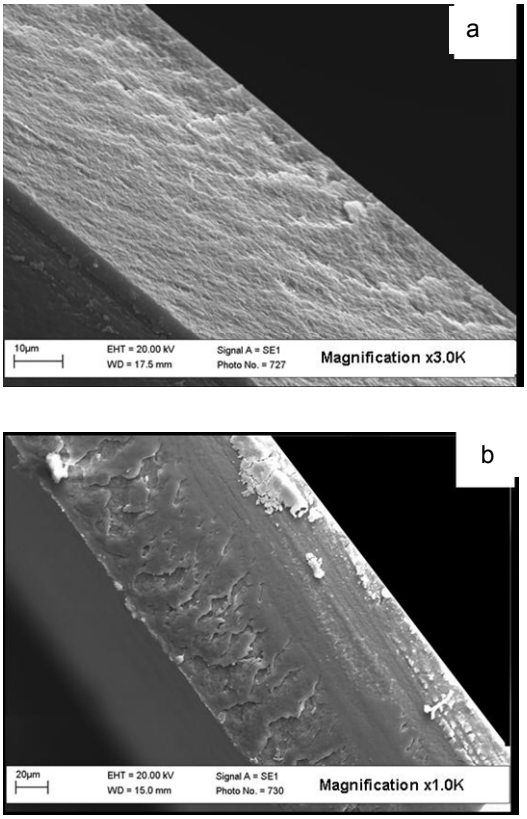

Figure 7. SEM micrographs of pectin-soy flour film: a) without TGase, b) with TGase

This result suggest that the ionic interactions between charged soy protein and pectic polysaccharides have the potential to contribute to the structural

assembly of pectin network according to MacDougall, Brett, Morris, Rigby, Ridout and Ring (2001b).

Besides, the films cross-linked by TGase show a more compact and homogeneous structure then the corresponding film without TGase addition (Figure 7b). These results are in according with α parameter values.

2.4. Conclusions

The results obtained in this study suggest that in film made by a pectin suspension the pectin held together as a result of physical entanglements that establishes when the solvent in which pectin are dissolved is removed. Therefore, by increasing the pectin content in the film-forming suspension the network link density does not changes. By adding soy proteins a more dense structure forms with link density that depend on both pectin and soy protein content. Moreover, the film obtained in presence of TGase exhibits a even more compact and homogeneous structure.

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3. III STUDY CASE

Performance of pectin-based edible films.

ABSTRACT

In this study mechanical, thermal, and some diffusion properties of pectin-based edible film were detected. Mechanical measurements were performed by means of an Instron Universal Testing Machine. Water vapour permeability was evaluated by a gravimetric test according to ASTM E96 (1993) and film permeability to oxygen and carbon dioxide was examined by using a manometric method according to Di Pierro et al. (2005). Thermal analysis was performed by means of thermo-gravimetric analysis (TGA). The experimental results indicated that the functional properties of pectin-based films strongly depend on film composition.

3.1. Introduction

Edible films are receiving considerable interest since they can act as selective barriers of gases, vapours and solutes as well as for mechanical protection in several food systems. As a consequence, research interest in the study of thermo-mechanical behaviour and diffusion properties of glassy biopolymers and biopolymers blends, used in foods as edible films and coatings or as bioactive ingredients, has recently increased. The diffusion and the thermo-mechanical properties are of paramount importance in producing a new material for food packaging. In fact, an adequate mechanical strength of an edible film is necessary to protect the integrity of packaging throughout distribution. Moreover, the mechanical properties of such materials are equally important to provide physical protection and controlled rates of release of additives in the food. The control of mass transfers involves preventing foods from desiccation, regulating microenvironments of gases around foods and controlling migration of ingredients and additives in the food systems (Chen, 1994).

The use of polysaccharides as coating materials for food protection has grown extensively in recent years. These natural polymers can prevent the product deterioration extending the shelf life and maintaining the sensory quality and safety of several food products (Robertson, 1993). Generally, these systems

are designed by taking advantage of their barrier properties against physical/mechanical impacts, chemical reactions, and microbiological invasion. In addition, the use of polysaccharides presents advantages due to their availability, low cost, and biodegradability. Furthermore, polysaccharides can be easily modified in order to improve their physiochemical properties (Lacroix and Le Tien, 2005).

The development of coatings from water-soluble polysaccharides has brought a surge of new types of coatings for extending the shelf-life of fruits and vegetables because of the selective permeabilities of these polymers to O_2 and CO_2. In fact, polysaccharide coatings or films applied to respiring products should allow for the right modification of the gaseous environment inside the package, i.e. allowing O_2 to penetrate into the package and excessive CO_2 to escape from it. Polysaccharide-based coatings can be utilized to modify the atmosphere, thereby reducing fruit and vegetable respiration. (Guilbert, Contard and Gorris, 1996).

Water-soluble polysaccharides are long-chain polymers that dissolve or disperse in water to give a thickening or viscosity-building effect. These compounds serve numerous diverse roles such as providing hardness, crispness, compactness, thickening quality, viscosity, adhesiveness, gel forming ability, and mouthfeel (Nisperos-Carriedo, 1994).

A wide variety of polysaccharides have drawn attention for their film-forming ability (cellulose and derivates, starch, chitosan, pectins, etc.).

In particular, the pectins have been widely used as gelling agents, stabilizers, thickeners, and emulsifiers in many food products. They exhibits excellent film–forming properties.

Blends of pectin and starch can be used to make strong , self-supporting films (Fishmann, Coffin, Konstance and Onwulata, 2000). Pectin has been used in making biodegradable drinking straws in which coloring and flavouring substances incorporated in a pectin layer are released when liquids pass through the straw. Plasticized blends of citrus pectin give strong, flexible films, which are thermally stable up to 180°C. Pectin are also miscible with poly(vinylalcohol) in all proportions. These films are solution-produced by air-drying after casting at ambient temperatures. Pectin coatings have been

investigated for their ability to retard moisture loss and lipid migration, and improve handling and appearance of foods (Lacroix and Le Tien, 2005).
In this work edible films made whit different pectin content were examined. Techniques such as calorimetry and mecchanical measurements together with a polymer science approach provide information on composition/function relationships in order to study the structural properties of edible materials. Manometric and gravimetric method were used to study chemical-physics properties of films such as permeability to gases O_2 and CO_2, water vapour permeability and water solubility. The material properties of such networks should show a strong concentration dependence. Therefore, the aim is to evaluate the composition effect on functional properties of pectin edible films.

3.2. Materials and Methods

3.2.1. Materials

Pectin from citrus fruits and soy flour were purchased from Sigma (Milano, Italy). Pectin characteristics: Galacturonic acid content 93.5% (as is); methoxy content 9.4%; loss on drying 7.3%.Film-making procedure.

3.2.2. Film-making procedure

Pectin were dissolved in acidified deionised water (pH 2) at a concentration of 16mg/ml. To obtain films with the desired amount of pectin (2.5, 3.2, 3.8, 4.5, 5.1, 5.8mg/cm^2), different volumes of solutions were pipetted into 28.26 cm^2 polystyrene Petri dishes and dried at 35°C and 50% RH overnight under air circulation. The solution was de-aerated under vacuum, prior to casting films, to prevent pinholes formation, then transferred into dishes.

3.2.3. Thickness

Film thickness was measured using a micrometer model HO62 with sensitivity of ± 2 µm (Metrocontrol Srl, Casoria (Na), Italy). Film strips were placed between the jaws of the micrometer and the gap reduced until the first indication of contact. Mean thickness (µm) of films were determined from the average of measurements at 10 locations.

3.2.4. Film solubility

Film solubility was tested with a procedure similar to that described by Stuchell and Krochta (1994). Small pieces of films (20-25 mg) were dried at 70°C and 50 Torr in a vacuum oven for 24 h and then weighed in the nearest 0.0001g to determine the initial dry weight of the film. Each film piece was incubated at 25°C for 24 h into a screw-top tube (150 x 15 mm) with 10 mL of 0.1 M acetate (pH 4.0), phosphate (pH 6.0) or Tris-HCl (pH 8.0) buffer solution. At the end of the incubation the samples were poured onto Whatman #1 qualitative filter paper. The non-dissolved material, taken off by the filter with 10 mL of distilled water, was dried at 70 °C and 50 Torr in a vacuum oven for 24 h and then weighed. The percentage of soluble matter was calculated as follows:

Soluble matter (%)= (initial dry weight - final dry weight) x 100 / initial dry weight.

3.2.5. Barrier Properties

Water vapor permeability (WVP) of films was evaluated by a gravimetric test according to ASTM E96 (1993) by means of a Fisher/Payne permeability cup (Carlo Erba, Italy). Three grams of silica gel were introduced in each cup. The film samples having diameter of about 6 cm were put on top of the cup and sealed by means of a top ring kept in place by three tight clamps. The film area exposed to vapour transmission was 10 cm^2. The cups containing silica gel were weighed and then placed in a desiccator containing a saturated KCl solution which provided a constant water activity at 25 °C equal to 0.8434. The desiccator was stored in a Heareus thermostated incubator at 25.0 (±0.1 °C). Cups were weighed at scheduled times, and the amount of water vapour transmission rate through the film was estimated by the linear portion of the diagram obtained by plotting the weight increment of the cup as a function of time. It was assumed that the steady state was reached once the regression analysis made by using the last four data points resulted in $r^2 \geq 0.998$. The WVP was calculated from the equation

$$WVP = \frac{X}{A\Delta p}\frac{dm}{dt} \qquad (29)$$

where dm/dt is the slope of the cup weight versus time curve once steady state was reached, X is the film thickness, A is the film exposed area, and Δp is the water vapour pressure across the film. By assuming that the vapour pressure inside the cup, due to the presence of silica gel, can be taken equal to zero, Δp becomes equal to the vapours pressure inside the desiccator and was calculated by multiplying water activity and the water tension (P_0) at 25 °C (P_0=3.167 kPa).

Permeability of films to oxygen (PO_2) and carbon dioxide (PCO_2) were examined at 30°C by using a modified manometric standard method according to Di Pierro, Mariniello, Giosafatto, Masi and Porta (2005). The tests were performed at 51.4%HR and a ΔP of 100kPa for every gas. Ten independent tests for each film were performed.

3.2.6. Mechanical measurements

Tensile strength (TS, MPa) and percentage of elongation (ε, %) at breakpoint of the films were measured uniaxially by stretching the specimen (10mm x 80mm) in one direction at 30mm/min by using an Instron Universal Testing Machine (Instron Ltd., mod. 4467 High Wycombe, GB), equipped with a 1KN load cell.

3.2.7. Calorimetric analysis

The analysis of all film samples was performed by differential scanning calorimetry, using a DSC 7 (Perkin-Elmer Corp. Norwalk, CT).
Edible Films was cut and weighed (20-25 mg) into aluminium punctured pans by means of analytical balance (Sartorius, BP61). Samples were heated at 30°C/min, from -80 to 200°C, in inert atmosphere (20 ml/min of N_2) and cooled at -80°C whit same rate. After cooling a third scan was started. A indium sample (T_m=156,4°C, ΔH_m=28,54 J/g) was used as reference standard to calibrate the instrument.

3.2.8. Thermo-gravimetric analysis

Thermo-gravimetric analysis was performed whit a thermo-balance TGA 7 (Perkin-Elmer Corp. Norwalk, CT). Edible films were cut and placed into platinum sample pans, then, the weight loss, as function of temperature, was monitored at 10°C/min from room temperature to 500°C, in inert atmosphere (20ml/min N2).

3.2.9. Data analysis

Data were submitted to analysis of variance and Duncan's test ($p \leq 0,05$) by means of SPSS v10.1 package.

3.3. Results and Discussion

3.3.1. Total soluble matter

The water resistance of edible films is an important characteristic in applications such as food protection in the case of high water activity or when the film is in contact with water during food processing (e.g. edible coatings for osmotic dehydration).

The Total soluble matter (TSM) is a measure of the water resistance and the integrity of a film. In order to value the film solubility in real condition (food contact) the TSM was valuated at three different pH condition. In Table 1, the TSM data obtained at different pH from films made by different amount of pectin are reported. In particular, film solubility was negatively related to pectin content for all pH detected. This behaviour is according to previous results about the structure formation more packed duo to an increase of pectin content. Moreover, the solubility was greater at pH 4.0 and 6.0 than pH 8.0. The lower solubility observed at pH 8.0 can be explained by the stabilization of network due to the formation of hydrogen bonds between the hydrogen of methoxyl groups and the dissociated carboxyl groups according to model proposed by Okefnfull (1991).

Table 1: Total soluble matter of films obtained by casting of different amount of pectin

Pectin (mg/cm^2)	Total soluble matter (%)		
	pH 4.0	pH 6.0	pH 8.0
2.5	99.0 ± 0.6	97.3 ± 1.3	78.0 ± 3.3
3.2	97.3 ± 2.2	92.8 ± 6.3	61.3 ± 4.2
3.8	95.1 ± 3.2	89.6 ± 3.4	60.1 ± 5.2
4.5	90.3 ± 4.2	86.4 ± 1.9	59.5 ± 4.6
5.1	87.5 ± 2.1	84.7 ± 4.2	58.5 ± 2.1
5.8	83.3 ± 4.6	81.8 ± 3.6	53.3 ± 4.6

3.3.2. Mechanical properties

Figure 1a shows film thickness as a function of pectin content. The results highlighted that when the pectin content increases the thickness increases, but, as one can observe the film thickness does not vary proportionally to the pectin amount used (the experimental data are lower than the solid line). This result confirm that by increasing the pectin amount in film forming solution a structure more dense one can obtain. Mechanical investigation prove that the tensile strength of the films increases with increasing the pectin content whereas no significant ($P>0.05$) variation in elongation at break is observed (Fig. 1b). Moreover, pectin films are quite brittle as the elongation at break is near 1%. This result suggest that an increases of packing degree duo to an increase of pectin content per area (mg/cm^2) involve an increase of cohesion forces and, as consequence a decrease of flexibility of film.

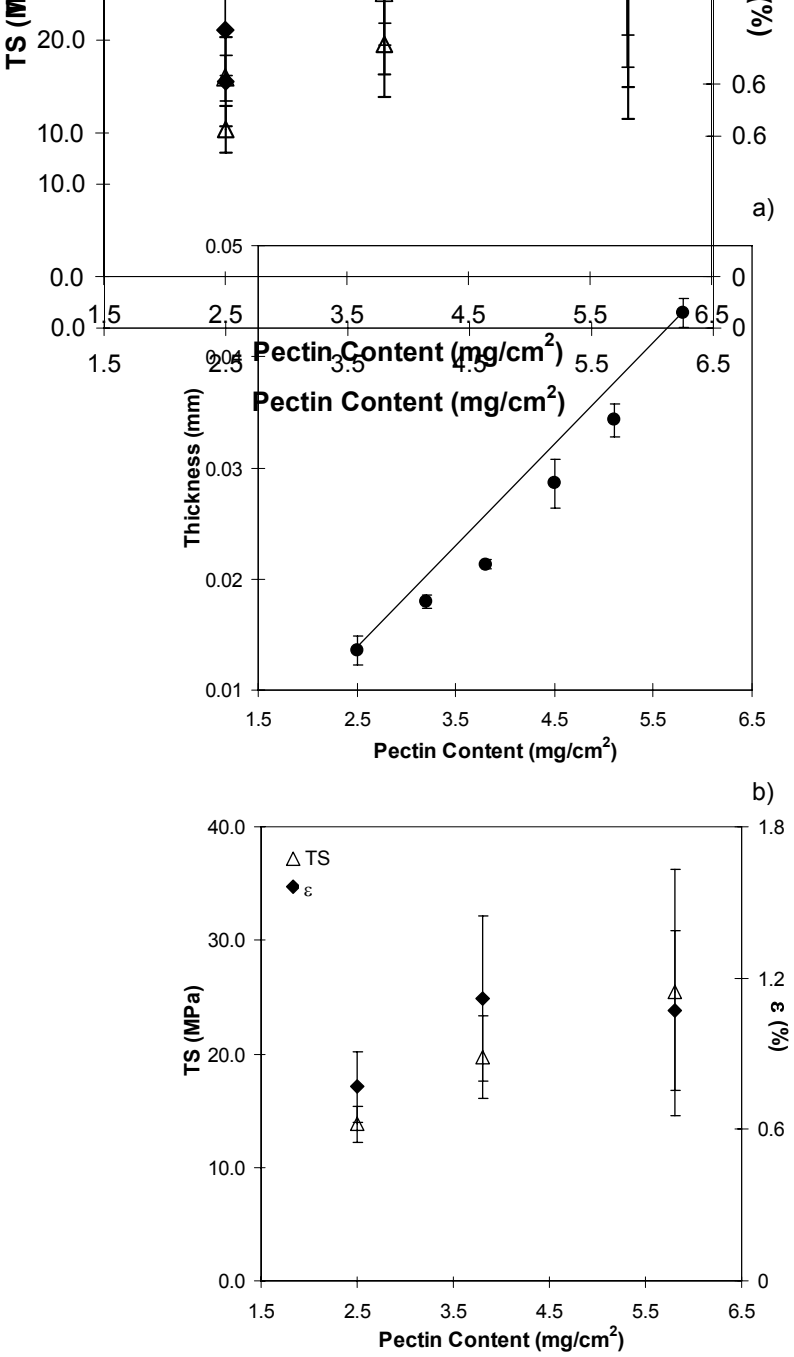


Figure 1. Thickness (a), Tensile Strength and Elongation at break (b) of films obtained from different amount of pectin.

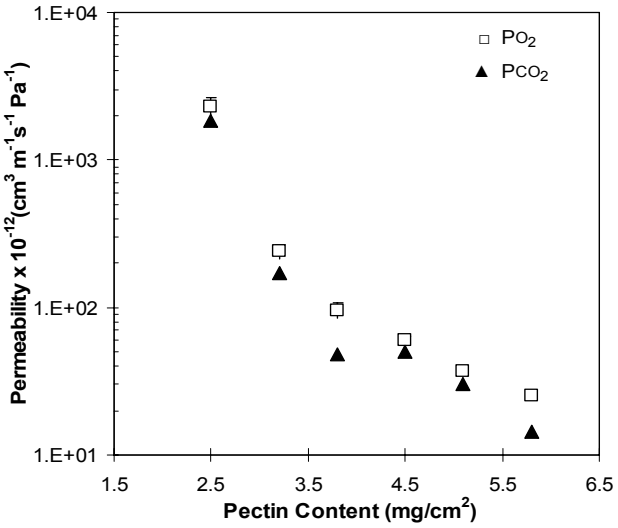
3.3.3. Barrier Properties

Figure 2 show O_2, CO_2 and water vapour permeability of all investigated films. One can observe that the pectin amount has a marked influence on barrier

proprieties, in particular, the film permeability to O_2 and CO_2 decreases as pectin content increases (Fig.2a), and the film permeability to water vapour increases as pectin amount increases (Fig.2b). The enhancement of film permeability to O_2 and CO_2 can be explained by considering that a free volume reduction occurs due to a microstructure more compact to effect of higher content of pectin per area (as previously observed).

The increased WVP showed by the pectin-based materials after addition of increasing amounts of pectins could be directly related to the availability of more polar groups due to the higher content of pectin structures. Barrer (1951), and Banker, Gore and Swarbrick (1966) attributed this effect to swelling film for effect of attractive forces between film and water.

a)



b)

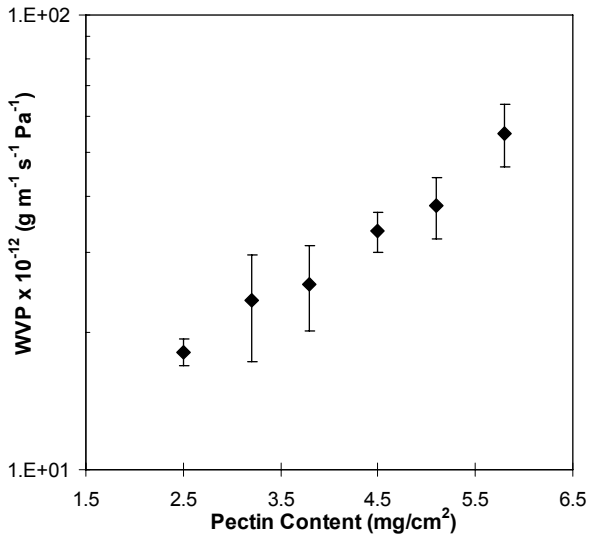


Figure 2. Barrier properties (a: PO_2, PCO_2; b: WVP) of films obtained from different amount of pectin.

3.3.4. Thermal properties

The films formulated with different pectin content (from 2.5 to 5.8mg/cm^2) were submitted to thermogravimetric analysis and differential scanning calorimetry. In particular, the thermogravimetry was used to determine the sample moisture content and the decomposition temperature, whereas the calorimetry was employed to detect the pectin thermal transition. As the films were submitted to temperature running in thermobalance, weight loss as function of temperature was observed. For all samples the weight loss became to temperature of 30°C and carried off to ~150°C. Then, the weight kept on constant until ~250°C and successively the samples degraded (Fig.3). The highest rate of water loss (~2%/min) was observed to temperature of 62-65°C whereas, the pectin degradation occur to temperature of around 260°C (Td). Both thermal parameters were not influenced by pectin content in film forming-solution as shown by the variance analysis results ($p>0.05$) (table 2). The moisture content, calculated at 200°C, for all samples was around 12-13%.

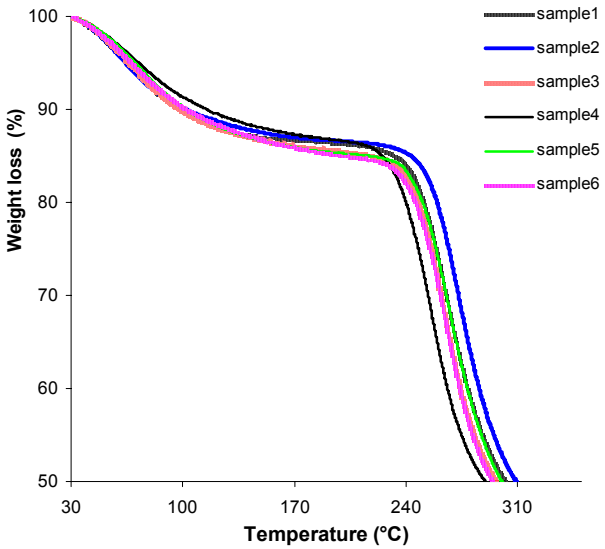


Figure 3. Weight loss as function of temperature for pectin films with different pectin content (from 2.5mg/cm^2 (sample 1) to 5.8mg/cm^2 (sample 6)).

Table 2: Thermal parameters and ANOVA results

Films	dW/dt (%/min)	T (°C)	dW/dt d (%/min)	Td (°C)
2.5 mg/cm^2	1.84	64.33	8.30	262.87
3.2 mg/cm^2	1.65	65.07	8.85	256.20
3.8 mg/cm^2	1.89	65.45	8.25	259.95
4.5 mg/cm^2	1.60	70.45	8.25	256.43
5.1 mg/cm^2	1.78	72.90	7.95	263.10
5.8 mg/cm^2	1.78	67.85	8.45	260.73
F	1.34	1.84	1.36	0.77
p	0.31	0.18	0.31	0.59

Those results suggested that an increase of pectin amount did not involve an increase of pectin-water interaction. The calorimetric analysis did not allow to obtain information on pectins transitions.

3.4. Conclusions

The results of this study reveal that the permeation and mechanical properties depend on pectin content in film formulation. The films with 5.8mg/cm^2 of pectins show best water resistance and good barrier properties to O_2 and CO_2. The mechanical measurements demonstrate that an increase of pectin content leads to stiff and little flexible films. Then, the use of a plasticizer can be useful to overcome the brittleness of these films. Besides, it was possible to prove that the pectin increase did not determine a rise of interaction pectin-water and then, an increase of link density in film network according to previous results.

3.5. References

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4. IV CASE STUDY

Performances of soy protein-pectin films cross-linked by TGase enzyme.

ABSTRACT

The aim of this study was to investigate the influence of composition on the engineering properties of hydrocolloids films. Films obtained from pectin-soy flour mixtures prepared in absence or in presence of microbial TGase were detect. The experimental results indicated that the mechanical properties were mainly affected by pectin content. The water vapour permeability of the films was not signifinantly different in presence and in absence of TGase. Finally, the TGase treatment improves the flexibility of films.

4.1. Introduction

Mass transfer of various molecules (water, gases, lipids, flour compounds, or solutes) can occur between a food and its surrounding medium, leading to physico-chemical changes and food deterioration. In most cases, synthetic food packaging is used to prevent these migrations and to maintain and improve food quality and to increase food product shelf-life. However, in some cases, such as for composite foods (i.e. association of different phases that create concentration gradients favouring molecular migration), edible films and coatings could be used to prevent and to control mass transfer (Karbowiak, Debeaufort and Volley, 2006).

Edible and biodegradable films must meet a number of specific functional requirements (moisture barrier, solute and/or gas barrier, water or lipid solubility, colour and appearance, mechanical and rheological characteristics, etc.). These properties are dependent on the type of material used, its formation and application. For example, polysaccharides and proteins are good film-forming materials giving rise to excellent mechanical and structural properties, but poor moisture barrier efficiency. Plasticizer, cross-linking agents, antimicrobials, antioxidants, texture agents, etc. can be added to enhance the functional properties of the film. The barrier properties of edible films have been studied by many research groups. Studies have focused on

films coating lipids, which are less permeable, or proteins which are more permeable.

Biopolymer films have application as packaging materials, and as pharmaceutical coatings. There is a continuing need to modify properties of such materials for improved and new uses. In the synthetic polymer area modifications of material characteristics usually involve a use of various polymer blends. Mostly, these are phase-separated systems with domains of one polymer dispersed in a matrix of the other. Less frequently there is an attractive interaction between chemically dissimilar polymers with single-phase materials (Tolstoguzov, 1986). There is relatively less information available on biopolymer blends. Polysaccharides in their blends are usually not miscible. Between proteins and anionic polysaccharides there is an attractive interaction if these components are electrically compatible.

The suitable use of edible packaging strongly depends on their favourable mechanical and barrier properties. Little information exists in the literature on the film-forming ability of such combinations of protein and polysaccharide components. Parris, Coffin, Joubron and Pessen (1995) formed films from whey proteins and alginate or pectin and reported that the films formed from protein-polysaccharide blends had lower water-vapour permeability (WVP) than those formed from protein alone. Di Pierro, Chico, Villalonga, Mariniello, Masi and Porta (2006) have reported that edible films made by chitosan and ovalbumin showed higher tensile strength and lower elastic modulus when compared with those prepared with the polysaccharide alone. Moreover, the addition of protein to the chitosan matrix noticeably increased the water vapour permeability of these materials.

Transglutaminase can be used to modify the functional properties of edible films (Yildirim and Hettiarachchy, 1998;Lim, Mine and Tung, 1999; Oh, Wang, Field and Aglan, 2004; Di Pierro *et al.*, 2006). Recently, Mariniello, Di Pierro, Esposito, Sorrentino, Masi, and Porta (2003) reported that the treatment with mTG improved the mechanical properties of a film constituted by apple pectin and soy flour proteins.

The objectives of this study were to investigate the effect of both film composition and TGase treatment on the physical properties of protein-polysaccharide–based edible films.

4.2. Materials and Methods

4.2.1. Materials

Pectin from citrus fruits and soy flour were purchased from Sigma (Milano, Italy). *Pectin characteristics*: Galacturonic acid content 93.5% (as is); methoxy content 9.4%; loss on drying 7.3%. *Soybean flour characteristics*: 52% protein (85+% dispersible) and 1% fat.

4.2.2. Film-making procedure

Defatted soy flour, containing all proteins occurring into soy seeds, was dissolved into distilled water at concentration of 13 mg/ml. Pectin were dissolved in water at a concentration of 16 mg/ml. To obtain films with the desired ratio of each component, different volumes of the two solutions were gently mixed (Tab.1). Then, pH of mixtures was adjusted to 4.6. The solutions were de-aerated under vacuum prior to casting films, in order to remove small bubbles which could form pinholes in the finished film. Films were cast by pipetting the solution into 28.26 cm^2 polystyrene Petri dishes. Solutions were allowed to dry at 35°C and 50% RH overnight under dry air circulation. The obtained films were peeled from the Petri dishes and stored at 20°C in a desiccator (50% RH). For films obtained with TGase-crosslinked soy proteins, the enzyme was added after mixing pectin and soy flour solutions into which the enzyme was easily dispersed.

Table 1 Formulation of pectin-soy protein films

Film	$Y_{PEC/SP}$	Glycerol (mg/cm^2)	Thickness (mm)
A	0.33	0.020	0,053±0,007
B	1.61	0.040	0,120±0,013
C	2.33	0.022	0,092±0,008
D	8.75	0.034	0,097±0,001

4.2.3. Thickness

Film thickness was measured using a micrometer model HO62 with sensitivity of ± 2 μm (Metrocontrol Srl, Casoria (Na), Italy). Film strips were placed between the jaws of the micrometer and the gap reduced until the first indication of contact. Mean thickness (μm) of films were determined from the average of measurements at 10 locations.

4.2.4. Barrier properties

Water vapor permeability (WVP) of films was evaluated by a gravimetric test according to ASTM E96 (1993) by means of a Fisher/Payne permeability cup (Carlo Erba, Italy). Three grams of silica gel were introduced in each cup. The film samples having diameter of about 6 cm were put on top of the cup and sealed by means of a top ring kept in place by three tight clamps. The film area exposed to vapor transmission was 10 cm^2. The cups containing silica gel were weighed and then placed in a desiccator containing a saturated KCl solution which provided a constant water activity at 25 °C equal to 0.8434. The desiccator was stored in a Heareus thermostated incubator at 25.0 (±0.1 °C). Cups were weighed at scheduled times, and the amount of water vapour transmission rate through the film was estimated by the linear portion of the diagram obtained by plotting the weight increment of the cup as a function of time. It was assumed that the steady state was reached once the regression analysis made by using the last four data points resulted in $r^2 \geq 0.998$. The WVP was calculated from the equation

$$WVP = \frac{X}{A\Delta p}\frac{dm}{dt} \qquad (29)$$

where dm/dt is the slope of the cup weight versus time curve once steady state was reached, X is the film thickness, A is the film exposed area, and Δp is the water vapour pressure across the film. By assuming that the vapour pressure inside the cup, due to the presence of silica gel, can be taken equal to zero, Δp becomes equal to the vapours pressure inside the desiccator and was calculated by multiplying water activity and the water tension (P_0) at 25 °C (P_0=3.167 kPa).

4.2.5. Mechanical measurements

Tensile strength (TS, MPa) and percentage of elongation (ε, %) at breakpoint of the films were measured uniaxially by stretching the specimen (10mm x 80mm) in one direction at 30mm/min by using an Instron Universal Testing Machine (Instron Ltd., mod. 4467 High Wycombe, GB), equipped with a 1KN load cell.

4.2.6. Dynamical mechanical measurements

Dynamic mechanical analyses (DMTA V, Rheometrics Inc. Piscataway, USA) were performed on rectangular film specimens (50 x 7 mm). The sample was cut whit scissors and mounted on grips so that its length was 10 mm. All measurements were conducted in dynamic mode. Before any measurements were taken, samples were rested for 3 min, allowing the stress induced during sample loading to relax. The films were submitted to strain sweep tests performed at a given frequency of 1 rad/s, over a deformation amplitude range between 0.001-1%.

4.2.7. Data analysis

Data were submitted to analysis of variance and Duncan's test ($p \leq 0,05$) by means of SPSS v10.1 package.

4.3. Results and Discussion

4.3.1. Mechanical properties

As reported in Figure 1, in absence of TGase enzymes, the thickness of films increased with the increase of the pectin added until a given pectin/soy flour ratio. Then, the thickness decreases and successively its keep on constant until to reach a value of 0.09-0.10 mm. This behaviours can be explained by means of previous results about the effect of pectin on film network formation. In particular, the thickness decrease, as a consequence of a pectin content increase, appears to be the result of a density reduction. When the cross-linking of proteic fraction were promoted by transglutaminase reaction, the film thickness was shown similar behaviour. Moreover, as one can observe, the TGase treatment determine an increase of film thickness.

Two different mechanical properties, i.e. tensile strength and elongation to break, were investigated for pectin-soy flour films cross-linked or not by TGase. The tensile strength provides a measure of film strength, whereas the elongation to break is an indicator of the flexibility of the materials.

Figure 2 show the mechanical measurement results of pectin-soy protein films. One can observe that when pectin and soy flour proteins are blended together a certain effect of composition on Tensile Strength and Elongation at break of film is observed. In according to literature works (Krochta *et al*., 1994; Zsivanovits et al., 2004), when the pectin/soy protein ratio increases the TS arises (Fig. 2a) and the resistance at break decreases (Fig. 2b).

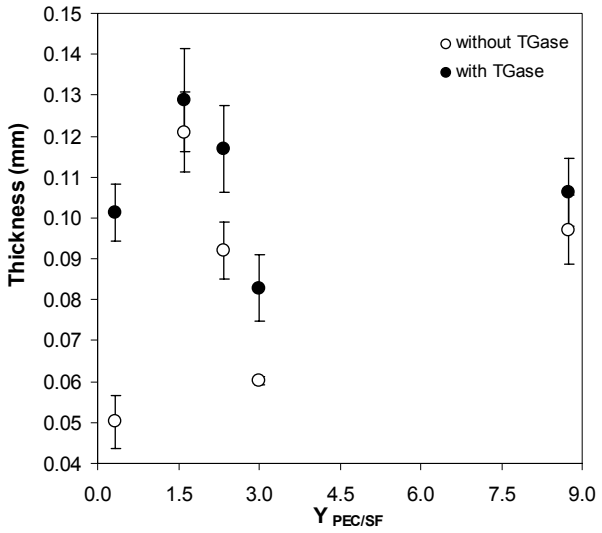


Figure 1. Thickness of pectin-soy proteins films ($Y_{PEC/SP}$= pectin/soy protein ratio).

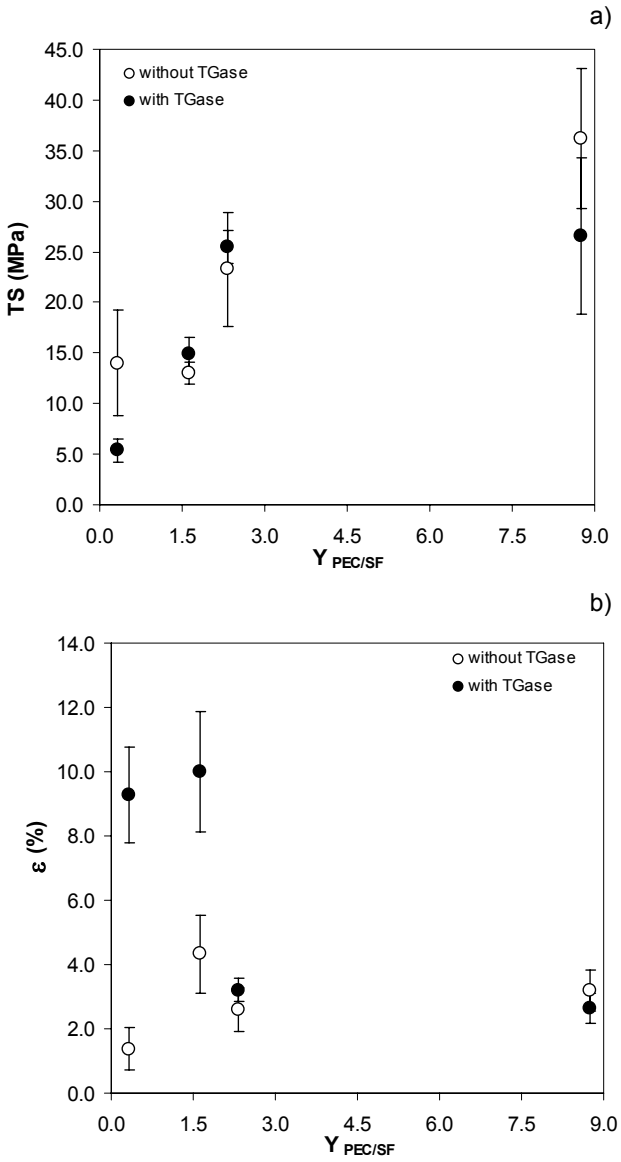


Figure 2. Tensile Strength (a) and Elongation at break (b) of pectin-soy protein films ($Y_{PEC/SP}$= pectin/soy protein ratio).

The elongation at break depend on plasticizer content, in fact, the film B ($Y_{PEC/SP}$=1.61) with glycerol content of 0.040 mg/cm^2 show the highest ε value (Gennadios et al., 2002; Krochta, 1997). Transglutaminase does not cause significant changes in tensile strength whereas improves the elongation at break that increase when the protein content arises. A study reported by Oh,

Wang, Field and Aglan (2004) showed that the transglutaminase increased the elongation of the film fabricated with casein and zein hydrolysate, but the TS did not affect by TGase treatment. The same results were reported by Lim and Tung (1999) for gelatin-based films.

The mechanical results are in according with the first investigation concerning the study of interactions in pectin-soy protein-water mixtures. When pectin and soy protein were blending together in electrically compatibility condition (pK_a of pectin carboxyl group<pH<soy protein pI) the soluble complex formation occurs. So, when these solutions are submitted to casting the presence of these domains could reduce protein-protein interactions duo to hiding pectin effect. This, could avoid the interconnect network formation between domains. These results can explain why the protein addition and then, the TGase treatment, dos not involve an enhancement of tensile strength as observed by comparing the Figure 2a with the mechanical results obtained for pectin films in previous study.

In Figure 3 was showed the elastic modulus (E') behaviour as function of oscillatory amplitude (ε) for all samples investigated. The elastic modulus gives an indication about the elastic nature of the sample under test. As we can note, the E' values are constant in deformation spectrum observed (Fig.3). All edible films have showed a linear viscoelasticity region rather wide suggesting a solid-like behaviour. According to mechanical results, obtained in large deformation, film composition significantly affect the E' values ($p<0.001$). In particular, films with highest pectin content show highest E', on the contrary, except for C film characterized by the highest plasticizer content, when the pectin content decreases the E' values decrease. Figure 4 compare elastic modulus of films cross-linked in absence and in presence of TGase at deformation of 0.01%. Introduction of covalent iso-peptide bonds into the protein structure involve a loss of E' for all films investigated as reported by mechanical results in large deformation.

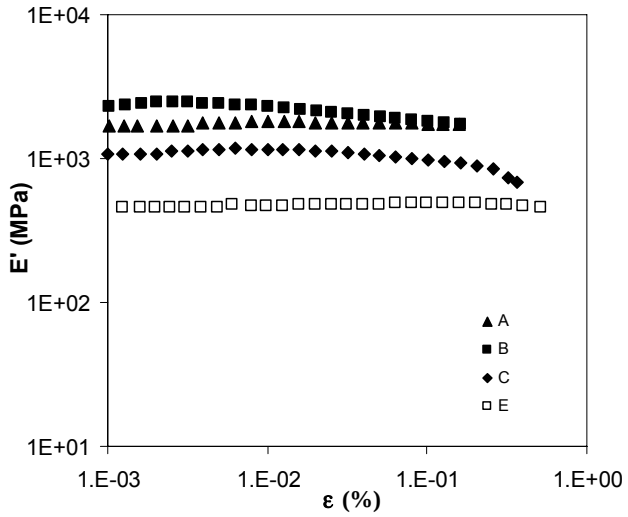


Figure 3. Elastic modulus (E') as function of oscillatory amplitude (ε) of pectin-soy proteins films.

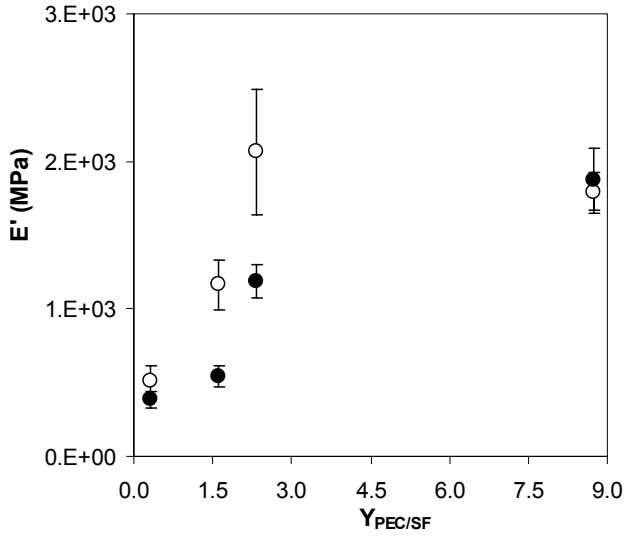


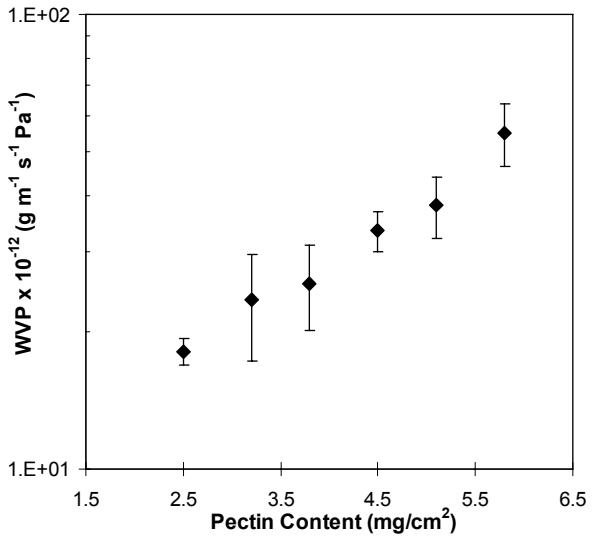
Figure 3. Elastic modulus (E') of pectin-soy proteins films ($Y_{PEC/SP}$= pectin/soy protein ratio). (E' values were compared at strain of 0.01%).

4.3.2. Water vapour permeability results

It is well known that many factors affect the film barrier properties besides the intermolecular cross-linking. Among these the polarity and the density of the molecules constituting the film, as well as the high level of chain-to chain packing, are the most important. In fact, these factors determine the film free volume that is a measure of the interstitial space among the different molecules (Miller and Krochta, 1997).

Concerning the WVP characteristics of films prepared in the presence or absence of TGase, it should be considered that this property is supposed to be dependent on the number of ''available'' polar (-OH, -COOH, $-NH_2$) groups that the polymeric components possess (Miller and Krochta, 1997). Thus, the increased WVP showed by the pectin-soy protein based materials than pectin films (Fig.4) could be directly related to the availability of new polar groups due to the higher content of hydrophilic structures. In fact, generally, protein films are ineffective water vapour barriers due to the inherent high hydrophilicity of proteins (Yildirim and Hettiarachchy, 1998). Also in this case the TGase treatment does involve an enhancement of functional propertie investigated (Fig.4b) according to previous results.

a)



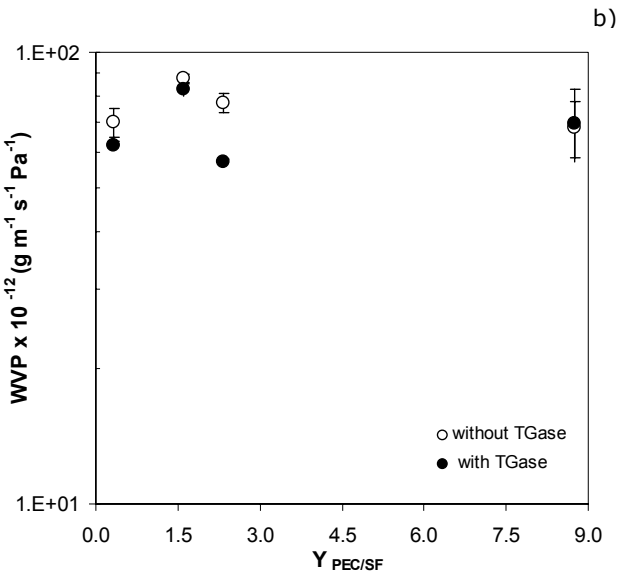


Figure 4. WVP of pectin (a) and pectin-soy proteins (b) films. ($Y_{PEC/SP}$= pectin/soy protein ratio).

4.4. Conclusions

The results of this study reveal that the mechanical properties depend on pectin content. In fact, the tensile strength was not affected by TGase and the soy protein addition in film-forming suspension. The elongation to break depend on both glycerol content and TGase treatment. In particular, the performed mechanical studies have shown that the introduction of covalent iso-peptide bonds into the protein framework considerably increased the elongation to break of films. Therefore, it could be used to improve the flexibility of the films with reduced plasticizer usage without sacrificing their water vapour permeability. Finally, the WVP of pectin-soy protein films was negatively affected to soy protein addition. These results were according to complex formation between pectin and soy protein in film forming suspension that could hinder the continues network formation.

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5. V STUDY CASE

Food application of pectin-based edible film: oil absorption reduction on French fried potatoes

ABSTRACT

The objective of the present work was to assess the oil barrier properties of pectin film coated on potato surface. Pectin coating at three concentration were prepared and applied on the potato surface by immersion for 5 minute. The product was dried to allow the film formation on the food surface. Coated and uncoated potato were fried for 3 minute in oil at 190°C and then the absorbed oil was determined by extraction with n-hexane. Micrographics were also carried out on samples before and after the frying process.

Results showed that the pectin film is a good barrier again oil absorption during frying process. A oil absorption reduction from 14 to 41% was obtained in function of potato moisture content and pectin composition. Also, it was showed that oil absorption depends by the homogeneity of the coated surface.

5.1. Introduction

Since consumer interest in low fat products continues to increase, significant pressure is mounting to reduce or replace fat in cooked products. Fried foods still remain popular although excess fat consumption is considered as the key dietary contributor to high blood cholesterol, high blood pressure and coronary heart disease. Deep fat frying is a dry cooking process where the fat serves as the heat transfer medium and also migrates into the food providing nutrients and flavour.

The deep-fat frying process is well described according to Figure 1. Upon addition of the food to the hot oil, the surface temperature of the food rises rapidly. The water at the surface immediately starts boiling. Surrounding oil is cooled down but this is quickly compensated for by convection. Only if the amount of added food exceeds a critical value, will the temperature of the oil be significantly affected. As the boiling commences, the convection will be further intensified by the turbulent water vapour. Due to the evaporation, surface drying will occur. The evaporation will also lead to shrinkage and development of surface porosity and roughness. Especially explosive evaporation can lead to the formation of large pores (see Fig. 1, right). Water

deep inside the food will become heated and will be cooked. As the food is fried for a longer period of time the moisture content in the crust slowly diminishes, thereby reducing the amount of steam bubbles leaving the surface.

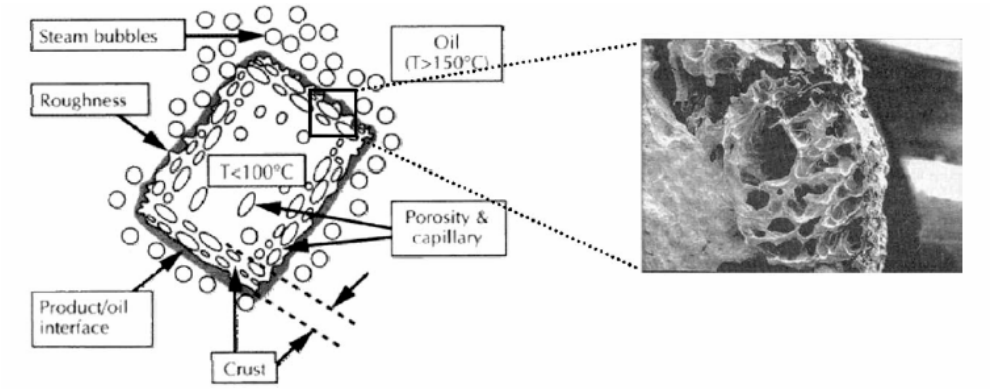

Figure 1. (left) Schematic cross-section of a piece of food during deep fat frying, taken from Saguy, Ufheil & Livings, 1998. (right) Scanning electron microscope image of a cross-section of the crust of a fried potato, taken from Singh, 1995.

The surface temperature can rise above the boiling temperature of water. Several physicochemical changes take place (starch retrogradation, Maillard reactions, glass transitions). This will lead to beneficial organoleptic properties and colour of the crust. Note that for large pieces of food like French fries or meatballs the temperature of the food core will not rise above 100°C. For thin potato crisps the core temperatures will be higher. During the frying not only water vapour but also other compounds will go from the food to the fat. This, combined with long-lasting high temperatures, will lead to degradation of the frying fat (Mellema, 2003).

During deep-fat frying water in the crust will evaporate and move out of the food. In order for the flow of vapour to continue, sufficient water has to be able to migrate from the core of the food to the crust and the crust has to remain permeable. The fact that the vapour leaves voids for the fat to enter later, is the reason why fat uptake is largely determined by the moisture content of the food (Saguy and Pinthus, 1995).

Research into reducing the fat/oil absorption during deep-fat frying has been intense in recent years. Edible coatings might offer a potential solution to form a barrier to fat absorption during the frying process. Hydrophilic cellulose derivatives, certain proteins and gums form films that are able to lower the migration of lipids (Albert and Mittal, 1997). Components of edible films can be hydrocolloids, lipids or composites of both. Hydrocolloids are of special interest because they possess good oil barrier properties (Mallikarjunan, Chinnan, Balasubramaniam and Phillips, 1997; Williams and Mittal, 1999).

Applicable hydrocolloids include proteins, cellulose derivatives, alginates, pectins, starches, and other polysaccharides.

Mallikarjunan, Chinnan, Balasubramaniam and Phillips (1997) working with mashed potato balls reported a reduction, compared to uncoated balls, of 14.9%, 21.9% and 31.1% in moisture loss and of 59.0, 61.4 and 83.6% in fat uptake for samples coated with corn zein, hydroxypropylmethyl-cellulose (HPMC) and methylcellulose (MC) films, respectively. Williams and Mittal (1999) also found that MC films showed the best barrier properties, because it reduced fat uptake more than hydroxypropylcellulose (HPC) and gellan gum films applied to a pastry mix. They also reported difficulties to evaluate potato products. Albert and Mittal (2002) have used different film materials as coatings on deep-fat fried foods, and have compared their oil/fat and water barrier properties. The results of their research proved that from eleven edible films tested only nine coatings were found to be suitable for deep-fat frying. Soy protein isolate, whey protein isolate and methyl cellulose provided the best results.

Less intensive studied materials are: proteins such as serumalbumin and ovoalbumin; polysaccharides such as pectin, acacia gum, gelatinized amylase, cellulose gum and xanthan gum.

The focus of this study was to obtain a reduction in oil retention of potato french fries by forming a coating with a pectin suspension prior to frying. Three different concentration of pectin were detected.

5.2. Materials and Methods

5.2.1. Materials

Pectin from citrus fruits and soy flour were purchased from Sigma (Milano, Italy). Pectin characteristics: Galacturonic acid content 93.5% (as is); methoxy content 9.4%; loss on drying 7.3%.Film-making procedure.

Potatoes (variety Bintje) were purchased from Del Gaudio Luigi Import-Export (Napoli, Italy). Potato tubers were stored at 0°C ±1°C and 95% relative humidity prior to be processed.

5.2.2. Film-making procedure

Pectin were dissolved in acidified deionized water (T=80°C) by stirring slowly for 5h at three different concentrations (16mg/ml, 24mg/ml and 51mg/ml). The solutions were de-aerated under vacuum in order to remove small bubbles.

5.2.3. Pre-treatments

Potato strips (10x10x40mm) were rinsed immediately after cutting for 1min in distilled water (1L) to eliminate some loose starch adhering to the surface prior to frying. Blanched samples were prepared by heating raw strips in hot water at 80°C for 4min (potato-to-water ratio 1:6 w/w) (Pedreschi and Moyano, 2005). Then, potatoes were blotted with paper towel to eliminate loose material adhering to the surface prior to frying.

5.2.4. Sample preparation and frying conditions

Samples of potato strips were dipped in the coating suspensions for 5 min and then, sprayed with a $CaCl_2$ solution (5% w/w). In order to allow the film formation on potato surface, the potato strips were dried under air circulation. During a first experiment, the drying process was carried out by using a domestic phone. Then, to optimize the process, a desiccator tunnel at controlled air velocity was used (UOP 8 tray dried, Armfield, UK).

Because of the oil absorption during frying depend on moisture content of potato (Saguy and Pinthus, 1995), the uncoated sample, used as reference, was submitted to the same treatment.

Coated and uncoated (control) samples were fried in a controlled temperature deep-fat fryer (Delonghi, Italy) filled with commercial sunflower oil. Potato-to-oil weight ratio was maintained as low as possible (~0.06) in order to keep constant the frying temperature (190±1°C). The frying time was 3min. The oil was preheated for 1h prior to frying, and was replaced by fresh oil after four frying batches. Experiments for oil uptake were run in triplicate.

5.2.5. Microscopy observations

For microscopy analysis, the samples were fixed in a solution formed by: a ethanol solution (50°) (90ml), formaldehyde (5ml) and acetic glacial acid (99.9%) (5ml). Samples were fixed cold (4°C) in the same buffer for 16h, then rinsed three times for 30min and dehydrated in ethanol. Potato samples were dried with CO_2 under vacuum. Dried sample fragments were mounted on specimen and coated with a thin layer of gold by DC sputtering (AGAR B7340). Digital images of film cross-section were collected by using a LEO EVO 40 scanning electron microscope (Zeiss, Germany) with a 20kV acceleration voltage.

5.2.6. Water content

Water content (WC) for all samples (Coated and uncoated) was determined measuring weight loss of fried products, upon drying in an oven at 105 °C until constant weight and expressed as water content /total weight %.

5.2.7. Lipid content

Lipid content (LC) of fried products was determined by Soxhlet extraction (Universal extraction system B-811, BÜCHI) with n-hexane. Two grams of homogenized samples were placed in each cellulose thimbles (30x100mm, Delchimica, Italy). After extraction for 2h, the solvent was released to a rotary-evaporator and the extract was dried under a nitrogen stream until the difference between two consecutive weightings was smaller than 1mg.

5.2.8. *Data analysis*

Data were submitted to analysis of variance and Duncan's test ($p \leq 0,05$) by means of SPSS v10.1 package.

5.3. Results and Discussion

5.3.1. Microscopy

Figures 1 and 2 show the micrographs of surface and cross section of a potato sample prior to be fried coated with a pectin suspension at concentration of 16mg/ml. As one can observe, the surface of samples is not completely wrapped from the coating (Fig.1). In fact, next to smooth and homogenous zone (on the left of figure) characterized by presence of film, one can note a potato portion where appears quite evident the typical structure of raw material (on the right of figure), that is a closed cell structure as reported by Lisińska (2005).

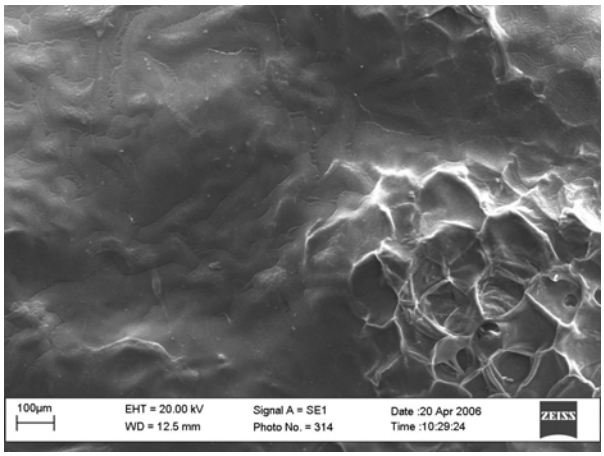

Figure 1. Micrograph of potato strip surface coated by pectin suspension at concentration of 16mg/ml prior to frying (Magnification 200x).

In Figure 2 was reported the surface of a uncoated (a) and coated (b) French frayed potato. As one can note, during the frying process, when the potatoes were wrapping by a pectin coating at concentration of 16mg/ml, a thin protective layer of film was formed on surface of samples (Fig. 2b). By comparing the two micrographs (a and b) one can note that through the thin layer formed on samples it is possible to observe the typical potato structure. Moreover, the micrograph 2b shows cracks in the film. This protective layer can inhibit the transfer of moisture and fat between the sample and the frying medium.

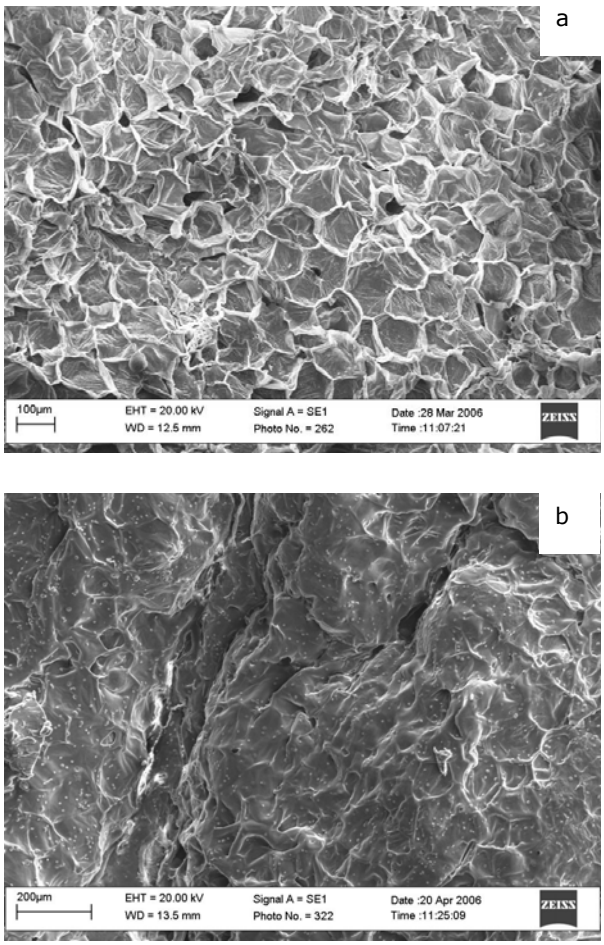

Figure 2. Surface micrographs of a uncoated (a) and coated (b) potato french fries (Pectin coating concentration= 16mg/ml) (Magnification 200x).

Coating integrity is an important factor because the presence of cracks may reduce barrier properties of coatings (García, Ferrero, Bértola, Martino and Zaritzky, 2002).

On the other hand, the cross-section micrographs, reported in Figure 3, show that there are not significantly differences between the uncoated and coated sample. Two samples show similar structure.

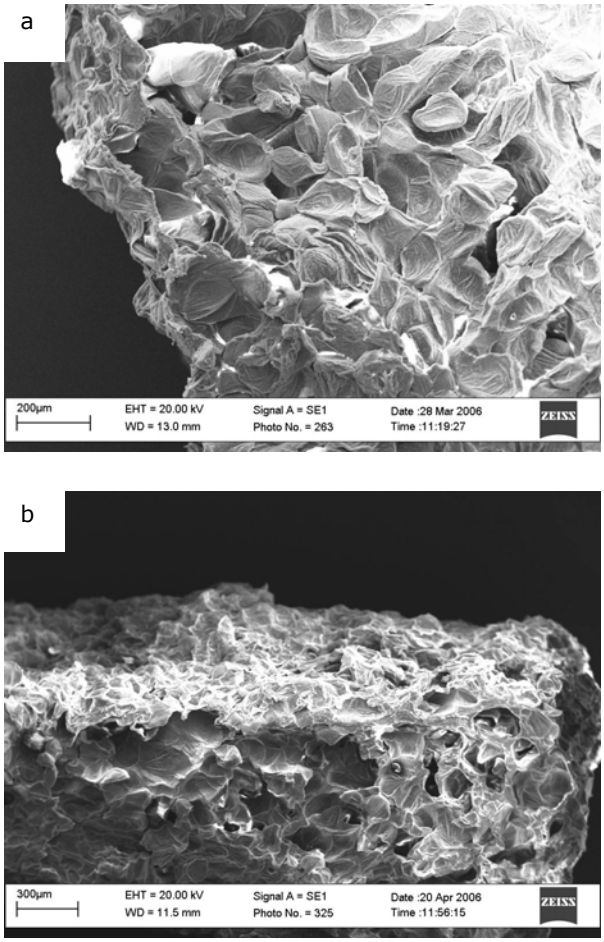

Figure 3. Cross-section micrographs of uncoated (a) and coated (b) potato french fries. (Pectin coating concentration= 16mg/ml) (Magnification:200x (a); 100x (b)).

When the potato strips were dipping in pectin suspension at concentration of 26mg/ml a layer of coating much more thick were formed during frying (Fig. 4a and 4b). As a consequence, the typical potato cell structure disappears. Moreover, the Figure 4 shows that an increase of pectin content in film forming suspension involve the hole formation duo to water migration from potato to frying medium.

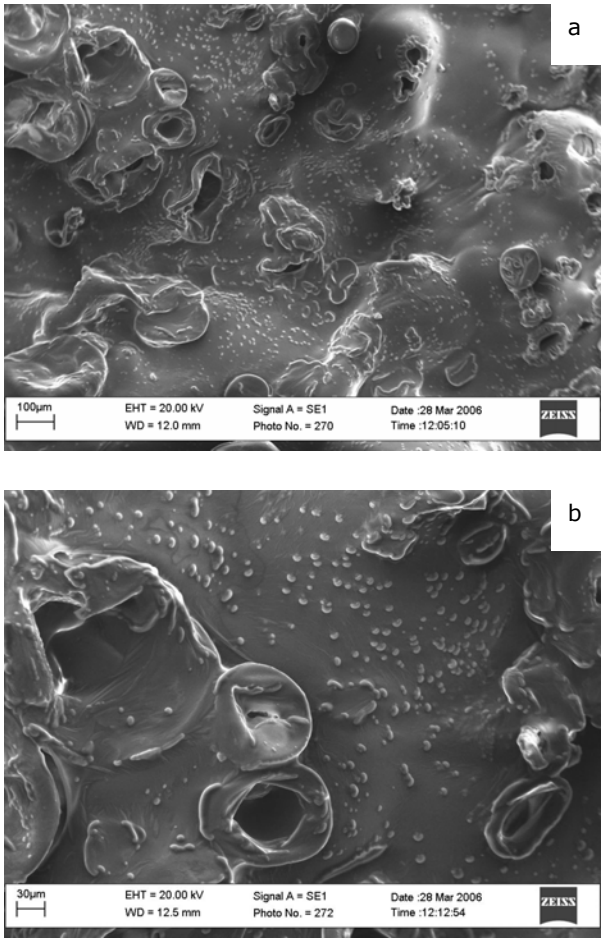

Figure 4. Micrographs of potato french fries surface, coated by pectin suspension at concentration of 26mg/ml: a) Magnification 100x and b) 500x.

In Figure 5 the cross-section at two magnifications of previous sample was reported. Also in these pictures, the formation of a thick and homogeneous film layer on potato french fries was clearly observed.

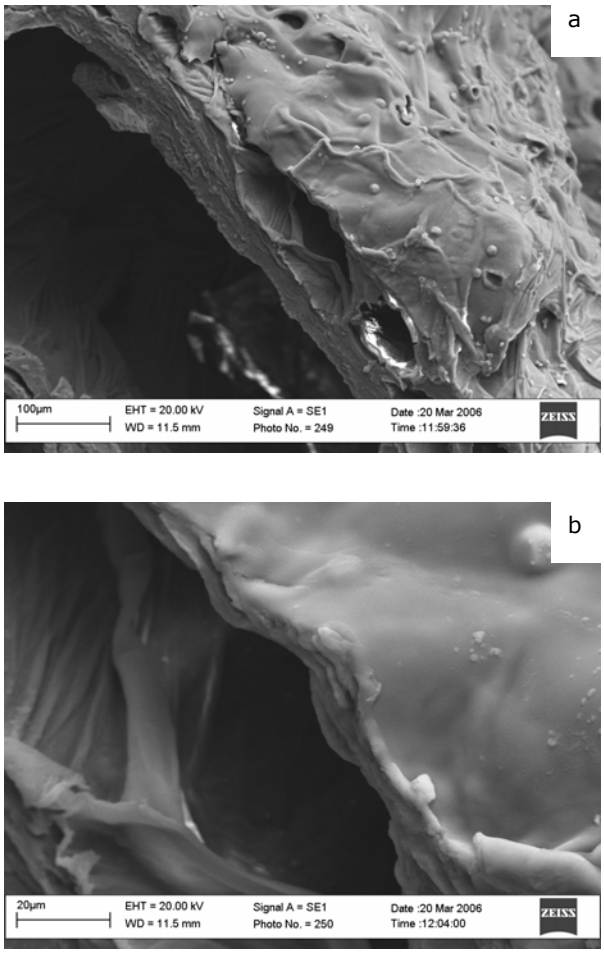

Figure 5. Micrographs of potato french fries cross-section, coated by pectin suspension at concentration of 26mg/ml: a) Magnification 500x and b) 2.5kx.

Finally, in Figure 5 is reported the surface of a fried sample coated with pectin suspension at concentration of 51mg/ml. It is interesting to note that film integrity and homogeneity does not depend linearly with suspension concentration. In fact, appears evident that the crack and hole formation increases with pectin concentration. This, can have a considerable impact on barrier properties during thermal process. This results can be explained by considering that an increase of pectin concentration can involve an increase of cohesion forces (forces between the film-forming polymer molecules). In fact,

a strong cohesion reduces flexibility, gas and solute barrier properties and increases porosity (Guilbert, Gontard, and Gorris, 1996).

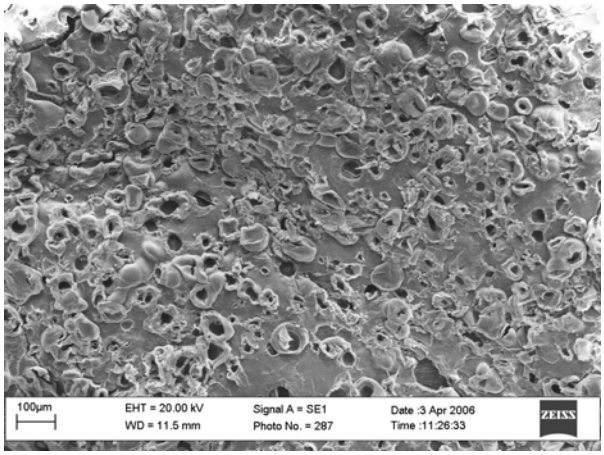

Figure 6. Surface micrograph of potato french fries coated by pectin suspension at concentration of 51mg/ml (Magnification 200x).

5.3.2. Lipid content determination

The figure 7 show the oil absorption, during frying process, in presence and absence of coating for two different pectin formulation (16 and 24mg/ml). In this case the film formation on potatoes was obtained by using a commercial phone. As one can note, during thermal process the potato oil absorption was of 11%, whereas, in presence of coating oil absorption decreased of 17%. The pectin concentration does not influences the oil uptake.

In order to enhance the oil absorption reduction the coating was dried by using a tunnel drying system. The drying times was set up as function of film formulation. In particular, we have found that, the drying times were: 1.5h for samples coated with pectin suspension at concentration of 16mg/ml; 2h for samples coated with pectin suspension at concentration of 24mg/ml; 2.5h for samples coated with pectin suspension at concentration of 16mg/ml. This treatment involves changes in moisture content of samples prior to frying. In fact, the humidity of potatoes changes from 80% (after the blanching) for uncoated sample, to 69% (after drying in tunnel) for sample coated with film solution at pectin concentration of 51mg/ml.

The Figure 8 report the effect of coating formulation on oil uptake of french fried potato in absence and in presence of coating.

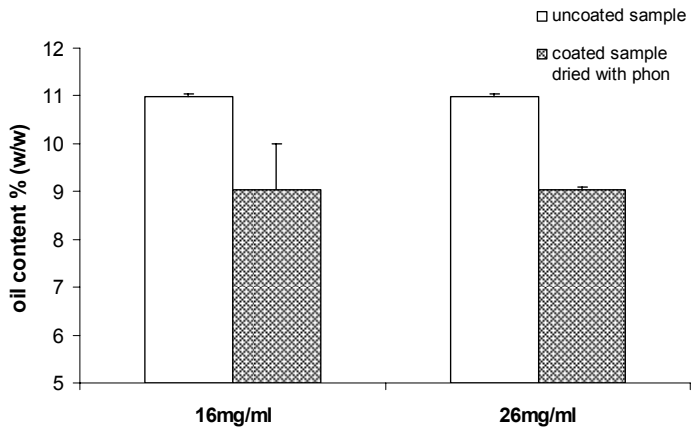

Figure 7. Effect of coating formulation on oil uptake of uncoated and coated fried potatoes.

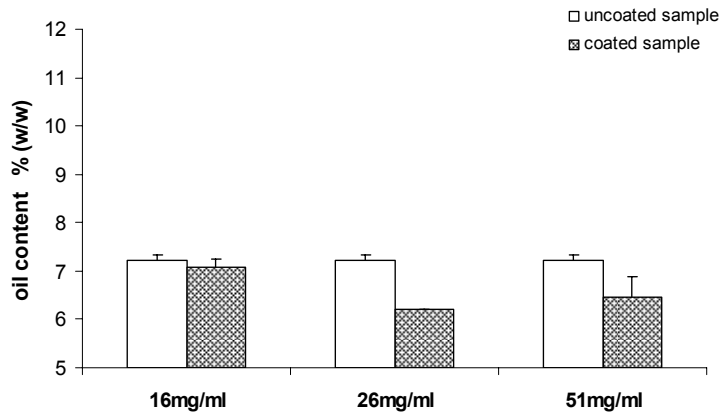

Figure 8. Effect of coating formulation on oil uptake of uncoated and coated fried potatoes. (drying film =tunnel)

These results highlight that the samples coated with pectin suspension at concentration of 26mg/ml shows the highest oil uptake reduction (14.2%). This result may be explain by scanning electron microscope photographs seen

previously. In fact, the micrographs of these samples show, on potato surface, the formation of a coating more compact and homogeneous than samples coated with pectin coatings at concentration of 16 and 51mg/ml, which show an oil absorption reduction of 1.8 and 10.4% respectively.

The Figure 9 compare the oil uptake of uncoated sample submitted to drying tunnel for 1h, prior to frying with the same sample not submitted to drying. The results show that the potatoes submitted to drying are characterized by a lower oil absorption (7%), by confirming that the moisture content in raw material, affect notably the oil uptake during the deep-fat frying.
Finally, by comparing the oil content results of coated samples (dried in tunnel) with uncoated sample (without drying) we have an oil absorption reduction of 35.5%, 43.6% and 41.2% for coatings at pectin concentration of 16, 26 e 51 mg/ml respectively (Fig.10).

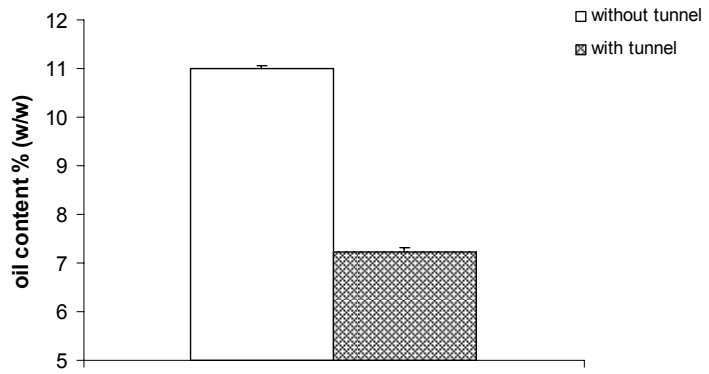

Figure 9. Oil uptake of uncoated fried potatoes with and without tunnel treatment.

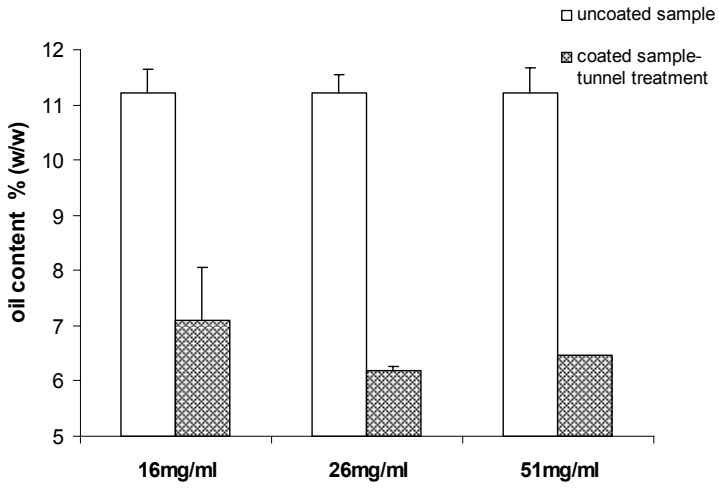

Figure 10. Effect of coating formulation on oil uptake of uncoated and coated fried potatoes. (drying film =tunnel)

5.4. Conclusions

This work proves that the pectin-based edible films are very promising as coating materials for oil uptake reduction during deep-fat frying of foods.

A oil absorption reduction from 14 to 41% was obtained in function of potato water content and film composition. Considering that the film was not homogeneous on the food surface, the results let suppose that the pectin film is barrier to the oil transport and that the oil absorption move towards the food surface not covered by the film.

5.5. References

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