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ROLE OF CONSTITUENTS ON STRUCTURE AND PROPERTIES OF POLYSACCHARIDES PROTEINS EDIBLE FILMS

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To my parents

TABLE OF CONTENTS

PART I

1. Introduction

The packaging, wrapping or coating play an important role on conservation, distribution and marketing of food stuff. One of the most important function required to a packaging when used for a food application is to protect the product from environmental factors in order to reduce mechanical damage, physical, chemical and microbiological alterations.

Petrochemical based plastics such as polyolefins, polysters, and poliamides have been increasingly used thanks to its low cost compared to classic packaging material (glass) and favourable functionality characteristics such as good tensile and tear strength, good barrier properties to oxygen and aroma compounds, a very low water vapour transmission rate and heat sealability. Nevertheless, petrochemical based plastics are totally non biodegradable, and therefore lead to environmental pollution, which pose serious ecological problem (Gennadios, 2002).

Directive EC 94/62 on packaging has been a fundamental shift in the regulation of waste. This decree, in fact, was inspired by the idea that pollution from waste should not be faced with interventions that are downstream of the processes of production and consumption and through the use of the prevailing landfill disposal, but reducing the total amount of waste management technologies and fostering self-driven recovery, reuse and recycling.

In the 94/62 EC Directive, biodegradable materials are defined as those material that under going to physical, chemical, thermal and biological degradation decompose ultimately in carbon dioxide and water and can be used as a compost in agriculture.

The Italian adaptation of European law is the Ronchi law. In the low is reported that manufacturers and users are responsible for proper environmental management of packaging and packaging waste generated by the consumption of its products.

This regulation has changed the approach to packaging and has led more and more research into alternative sources of packaging. Furthermore, our Legislative Decree of 2007 provided for the gradual replacement of plastic bags with biodegradable bags. Thus, since January 1, 2011 the marketing of non-biodegradable bags has been prohibited.

As biodegradable film, edible films and coatings can be a good answer to environmental pollution requirement. However, due to its hydrophilic nature they cannot fully replace the polymer film, but they can partially satisfy the legislation requirements.

By acting as mass transfer barriers, edible film and coating can control moisture, oxygen, carbon dioxide, lipid, flavour and aroma transfer either between food components or to/from the atmosphere surrounding the food. In fact, respect to biodegradable packaging, they can be applied on the food and consumed with it. This can reduce the requirements of the synthetic polymer. Thus, the amount of synthetic packaging is reduced, recyclables is increased, and the need of synthetic laminates is diminished (Krochta, 2002).

Edible films and coating are biopolymers based materials. Biopolymers are quite abundant in nature and have previously been regarded as surplus or waste. In order to improve film or coating properties biopolymers must be combined in new and creative ways. For this reason it is interesting to investigate different matrices, parameters and factors that may influence the properties of the films in order to optimize the performance of edible films and coatings and to understand the relationships between structure and properties.

In this work, the properties of films based on proteins and polysaccharides and their applications has been studied. In particular the work was organized in different cases studied in order to investigate different aspects of the problem.

In the first study cases to role of innovative additives on functional properties of polysaccharides based films were investigated. The objective of this first part of the work was to understand whether by adding new additives already known for their active properties as antimicrobial or antioxidant can have an effect on the structure of the edible film and thus on its functional properties.

In the second study cases, the research was focused on the role of polysaccharide-protein interactions in presence of a cross linking agent on the functional properties of the films. Understand how polysaccharide and protein can interact to form a structured film is important to optimize functional properties of edible films.

The objective of the last studied cases was to study an application on food of the studied edible film.

1.1. Definitions

In most cases, the terms edible film and coating are used interchangeably to indicate that the surface of a food is covered by relatively thin layer of material of certain composition. However, a film is occasionally differentiated from a coating for mode of application (Pavlath A.E. et al., 2009).

Films are normally regarded as stand-alone thin layers materials, being formed separate of any eventual intended use. 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; and they can be potentially formed into casings, capsules, pouches, and bags (Kroctha J.M., 2002). Generally, its thickness is less than 0.3 mm (Pavlath et al., 2009).

Coatings involve formation of films directly on the food surface of the object. They are intended to protect it or enhance it in some manner. In this sense, coatings become part of the product and remain on the product through use and consumption (Krochta J.M., 2002).

Items which are edible or are in contact with food should be generally recognized by qualified experts as being safe under conditions of its intended use, with amount applied in accordance with good manufacturing practices.

A coating must meet many demands for legality, safety, and performance. The following are areas of concern with edible coating:

- Chemical safety: as with all food ingredients and additives, safety is a fundamental requirement. However, much is still unknown about the safety of all food additives, including coating ingredients.
- Cost of ingredients and method of application
- Barrier properties: Ideal coatings form an acceptable barrier for gas exchange between food and atmosphere or between two phases of the same food item, neither too restrictive nor too permeable.
- Food quality: coating tend to change appearance, flavour, and mouth feel, and effort are need to achieve change that are good, not harmful. It must not ferment, coagulate, separate, develop off-flavors, or otherwise spoil.
- Nutritive value: same coating are so thick that they change the nutritional value.
- Environment: volatile organic compounds (usually alcohol) are sometimes released when edible coating dry (Baldwin et al., 2012).

1.2. Composition of edible films and coatings

Edible films and coatings are generally composed of protein, polysaccharides, lipid or resins, alone or, more often, in combination. Most commonly, edible films and coatings are intended to function as a barrier to moisture, oxygen, flavour, aroma, and/or oil, thus improving food quality and shelf life. An edible film or coating may also provide some mechanical protection for a food, reducing bruising and breakage and thus improving food integrity (Guilbert et al., 1996; Gennadios, 2002; Han and Gennadios, 2005, Baldwin et al., 2012).

The various naturally sources from which biopolymers can be extracted are shown in Figure 1.

Polysaccharides and protein are used for so-colled *film-forming base*, able to form a structural matrix continues with a good degree of cohesiveness. Mainly, polysaccharides and proteins provide a structural support, while lipids are used to create an effective water barrier.

Polysaccharides and protein edible 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. Lipids are not as well suited as proteins or polysaccharides because of their strong affinity for oxygen. Moreover, some lipid compounds (mainly unsaturated fatty acids) are oxygen sensitive, and can undergo rancidity, causing off-flavor development.

However, choice of which component to use, largely depends on the targeted product objectives and on technological and sensory constraints. The scientific and industrial patent literature over the last 20 years reveals a broad range of substances cited as film and coating constituents (Debeaufort et al., 1998; Avena-Bustillos and McHugh, 2009; Falguera et al., 2011; Gennadios, A., 2002; Han and Gennadios, 2005; Krochta, 1997; Zhang and Mittal, 2010). More recently, new film/coating formulations have been based on vegetable or fruit purees, including those of bananas, apples, and zucchini (Rojas Grau et al., 2007a; Sournovit et al., 2007; Debeaufort and Voilley 2009).

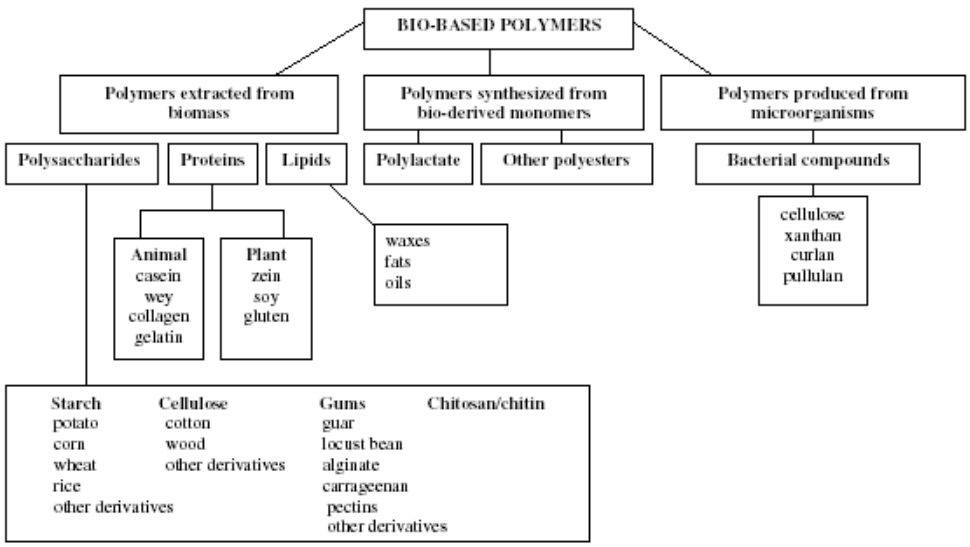

Figure 1. Different categories of bio-based material for edible and biodegradable films (From Cutter, 2006).

1.2.1 Proteins edible films

Among bio resources, protein have long been used as packaging materials. Film and coatings may be made from proteins both animal and plant origin (Gennadios, 2002; Dargaran et al., 2009; Lacroix and Cooksey, 2005; Buffo and Han, 2005).

The inherent properties of proteins make them excellent starting materials for films and coatings.

The distribution of charged and polar and non-polar amino acids along the protein chain creates a chemical potential resulting in interactive forces that can produce a cohesive protein film matrix. Most protein contain 100-500 amino acid residues. Films form and are stabilized through electrostatic interactions, hydrogen bonding, van der Waals forces, covalent bonding, and disulfide bridges. The structure of proteins can be modified by physical and chemical agent, including heat, mechanical treatment, pressure, irradiation, lipid interface, acids and alkalis, metal ions. This modification can optimize protein configuration, protein interactions, and resulting film properties. Proteins have multiple sites for chemical interaction as a function of their diverse amino acid functional groups, which can allow for property improvement and tailoring. Chemical changes can improve the stability of films and coatings (Dargaran et al., 2009; Kroch, 2002).

Proteins film-forming capabilities are best demonstrated in emulsified systems in which amphipathic proteins form films at air–water or water–oil interfaces. There are also secondary benefits for using proteins to form films and coatings.

The properties of a final film are affected by intrinsic properties of the film or coating components and extrinsic processing factor. For protein edible film, intrinsic properties includes amino acid composition, cristallinity (of protein and/or plasticizer), hydrophobic /hydrophilic, surface charge, pI, molecular size, and three dimensional shape. Extrinsic factor include processing temperature, dry conditions, pH, ionic strength, salt-type, relative humidity during process and storage, shear and pressure (Dangaran et al., 2009).

Due to hydrophilic nature, protein based film don’t have good barrier proprieties against water vapour, but they have good barrier properties against gas, such as oxygen and carbon dioxide. The majority of protein based film have good mechanical and organoleptical properties (Krochta, 2002; Dargaran et al., 2009).

The proteins studied for the development of films include whey protein (Yong Cho et al., 2002a; Muer et al., 2000; Anker et al., 2002; Shaw et al., 2002), soy protein (Pol et al., 2002; Yong Cho et al., 2002b; Yong Cho et al., 2007; Wan et al., 2005; Mariniello et al., 2003), fish protein (Bourtoom et a., 2006), proteins of lentil seeds (Bamdad et al., 2006), egg protein (Gennadios et al., 1996; Wongsaulak et al., 2006; Di Pierro et al., 2007), gelatin (Cao et al., 2007; Jo et al., 2005), zein (Pol et al., 2002; Yong Cho et al., 2002b; Oh et al., 2004; Ghanbarzadeh et al.,2008), gluten protein (Zhang and Mittal, 2010) and protein of limited availability, such as peanut protein, rice protein, pea protein, pistachio protein, lupin protein, grain sorghum protein, winged bean, cucumber pickle brine protein (Pérez- Gago, 2012).

Same properties of protein based film studied in the last years are reported in tables 1 and 2.

1.2.1.1 Casein

Casein is the major protein in milk. It is a unique protein, because it is only synthesized in the mammary gland and is found nowhere else in nature. Casein exists in the form of micelles containing all four casein species complexed with colloidal calcium phosphate. Each micelle consists of an average of 104 peptide chains with molecular weights of about 105 kDa. The casein micelles are stable to most common milk processes such as heating, compacting, and homogenization. Micellar integrity is preserved by extensive electrostatic and hydrogen bonding, and hydrophobic interactions. Four principal components of αs1-, αs2-, β-, and κ-caseins are identified. Their molecular weights range from 19 to 25 kDa. The primary structure of the four casein fractions contains many hydrophobic amino acid residues with non-polar side chains (35 to 45% total residues). The uneven distribution of these amino acids results in hydrophobic ends and patches. The caseins are amphipathic proteins having hydrophobic and hydrophilic ends and, thus, are especially used as emulsifiers.
This feature helps the formation of stable composite protein-lipid emulsions for coating wet surfaces. However, caseins are generally considered hydrophilic because their hydrophobicity values are lower than that of valine (7.05 kJ/residue). Among the casein fractions, β-casein is the most hydrophobic, and α_{s2}-casein is the most hydrophilic. Isoelectric casein is water-insoluble. Application to food field requires sodium or potassium caseinate with high water solubility. This can be also achieved by dispersing casein in water and adjusting with alkaline solution pH to between 6.5 and 7.0.
The most commonly casein product is soluble water caseinate. It is normally manufactured by dissolving fresh acid casein curd in sodium hydroxide followed by spray drying. Other soluble caseinates prepared in a similar manner include potassium, calcium, magnesium, and ammonium caseinates.

Casein can easily form films due to its open secondary structure. It is generally agreed in the literature that extensive hydrogen and electrostatic bonds, and hydrophobic associations facilitate the formation of intermolecular interactions that increase inter chain cohesion to form film (McHugh and Krochta, 1994). The chemical and physical forces that may change the balances of the intermolecular interactions can perceivably modify the film properties. Adjusting the pH, changing the drying rate, and adding functional additives such as plasticizers, hydrophobic ingredients, and cross-linking ions, are examples of approaches used by investigators. Strong covalent bonds are perceived to promote tighter intermolecular interaction, thus increasing film strength and resistance to mass transfer. Enzymatic and physical (irradiation) treatments have also been explored in forming casein films (Chen, 2002; Perez Cago, 2012).

1.2.2 Polysaccharides edible films

Polysaccharides have considerable molecular weight, and are water-soluble. They dissolve in and form intensive hydrogen bonds with water. Because of the size and configuration of their molecules, polysaccharides have the ability to thicken and/or gel aqueous solutions as a result of both hydrogen bonding between polymer chains and intermolecular friction when subjected to shear. In solution, polymer molecules may arrange themselves into an ordered structure, called a micelle that is stabilized or fortified by intermolecular hydrogen bonds. The micelle traps and immobilizes water and, depending on the extent of the intermolecular association, the water is either thickened, as measured by a parameter called viscosity, or converted into a gel that

possesses both liquid and solid-like characteristics or viscoelasticity. The formation of micelles confers upon their ability to form films because these structures are preserved during drying. Attributes of films made from various gums are influenced by the extent of intermolecular hydrogen bonding between polymer chains, arising from differences in gum molecular structures. Structural differences of gums that impact their properties include the presence or absence of branching, electrical charge, substitution (of sugar units), as well as molecular weight.

Polysaccharides can exhibit either a neutral charge (e.g., acetate esters, methyl ethers, other neutral sugars), negative charge (e.g., carboxylate, sulfate groups), or positive charge (e.g., amino groups) due to the presence various chemical groups attached to individual monosaccharide units. All of these structural features of polysaccharides contribute to their differences in solubility, synergy or incompatibility with each other or with other ingredients (e.g., proteins, minerals, acids and lipids), thickening, gelling, and emulsifying properties and, more importantly, their film-forming properties (Neito et al., 2009).

Polysaccharides edible film are generally poor moisture barriers and soluble in water, but in contrast they have moderately low oxygen permeability and, at the same time, selective permeability to O_2 and CO_2. 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.

Typically, polysaccharide based coatings have been applied very often to fruits and vegetables, either fresh or minimally processed, to reduce their respiration by creating modified atmosphere conditions inside the product, provide a partial barrier to moisture, improve mechanical handling properties, carry additives, as well as contribute to the retention and even the production of volatile compounds (Bai and Plotto, 2012; Dea et al., 2012; Gill and Gill, 2005; Oms-Oliu et al., 2008; Yingyua et al., 2006).

The polysaccharides that can be used for the production of films and coatings include starch and its derivatives (Garcia et al., 2000; Phan The et al., 2009a; Petersson et al., 2005; Bertuzzi et al., 2007; Garcia et al., 2006), cellulose and its derivate (Tong et al., 2008; Brindle et al., 2008), chitosan (Garcia et al., 2004; Rivero et al., 2009; Chillo et al., 2008), pectin (Maftoonazad et al., 2007; Liu et al., 2006; Giancone et al., 2009), agarose (Phan The et al., 2009a; Phan The et al., 2009b), arabinose (Phan The et al., 2009b) and alginate (Olivas eta l., 2008). Same properties of polysaccharides based film studied in the last years are reported in tables 1 and 2.

1.2.2.1. Cellulose

Cellulose is probably the most abundant organic substance existing in nature and is the major constituent of most land plants. It is the starting material for a wide range of modifications with uses both in the food industry, and an even greater variety of uses outside this sector.

The raw material for modified celluloses is cellulose pulp, which in turn is produced from wood pulp from specified species or from cotton linters. Cotton linters are the short fibres from the cotton ball, which are too short to be suitable for use in thread and weaving. The polymer chain length of cellulose varies with the different raw materials and hence the polymer length and the resultant viscosity required in the final product will govern the selection of the raw material.

Manufacturing process

In general terms, cellulose pulp is dispersed in alkali solution to form alkali cellulose and is then treated with appropriate reagents, under tightly controlled conditions, to substitute the anhydroglucose monomers of the cellulose chain. The substitution is at the hydroxyl groups and the substitution reagents are as follows:

- methyl cellulose – chloromethane
- hydroxypropyl cellulose – propylene oxide
- methyl hydroxypropyl cellulose – mixed substituents as above
- methylethyl cellulose – chloromethane and chloroethane mixed substituents
- carboxymethyl cellulose – monochloracetic acid.

The two stages of the reactions can be summarised as follows:

1. Cellulose + Alkali + Water→Alkali cellulose
2. Alkali cellulose + R–X → Alkyl cellulose

Alkali cellulose + $R–CH(O)CH_2$ → Hydroxyalkyl cellulose

Alkali cellulose + X–R–COOH → Carboxyalkyl cellulose

The substitution reaction is followed by purification and washing stages to remove by products
and to achieve the purity levels specified for food additives.

Structure

The structure of the cellulose molecule is shown in figure 2. It is shown as a polymer chain composed of two repeating anhydroglucose units (beta-glucopyranose residues) joined through 1,4 glycosidic linkages. In this structure, *n* is the number of anhydroglucose units or the degree of polymerisation. Each anhydrous glucose unit contains three hydroxyl groups, which in theory can be substituted. The average number of hydroxyl groups substituted per anhydroglucose unit is known as the degree of substitution (DS). Without exception, the DS required to produce desirable properties is much below the theoretical maximum.

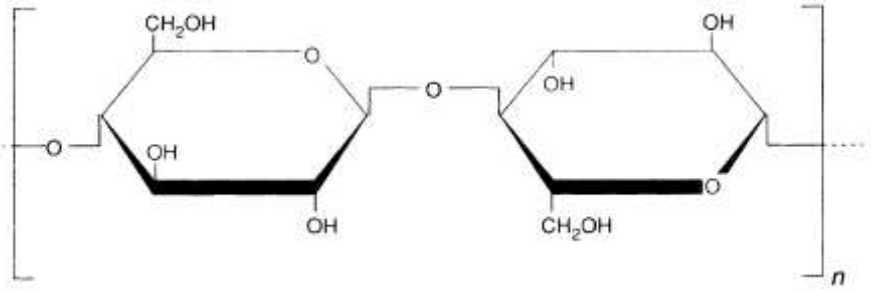

Figure 2. Structure of cellulose (From Murray, 2010)

Properties

There are three main factors which influence the properties of modified celluloses. The first and most importantly are the type of substitution of the cellulose, secondly, the average chain length or degree of polymerisation of the cellulose molecules (DP) and thirdly, the degree of substitution of the chain.

Additionally, the particle size of the hydrocolloid may be varied. Particle size and powder bulk density affect the dissolving characteristics of the product. Granular

material is less prone to clumping or balling but takes longer to dissolve. Fine powdered material can give very rapid hydration, but does not disperse so easily and good stirring or blending techniques are necessary.

Degree of polymerisation is a measure of the chain length of the polymer. Increasing DP very rapidly increases the viscosity of the modified cellulose in solution, although the viscosities of two differently substituted modified celluloses of comparable DP will not necessarily be comparable.

In general the modified celluloses give neutral-flavoured, odourless and colourless clear solutions. It should be noted that all modified celluloses, in powder or even granular form, are capable of absorbing water from the atmosphere. It is therefore desirable to store these products in airtight packs (Murray, 2010).

1.2.2.1.1. Hydroxyl propil methyl cellulose (HPMC)

HPMC is a macromolecule water-soluble and non-ionic, which is able to form gels upon heating (Yoguchi et al., 1995). The surface properties of HPMC solutions depend on the structure of the polymer (figure 3), which is a consequence of the production process that involves heterogeneous reactions.

There may be portions of the backbone of the polymer chains that are hydrophobic in nature, the rich regions of methoxyl groups, and other portions that are hydrophilic in nature, being full of hydroxy propyl groups. The hydroxy propyl methyl cellulose (HPMC) is soluble in cold water to give solutions with different viscosity characteristics, which depend on DP and DS. The solutions show a stable viscosity over a range of pH between 3 and 11.

More importantly, however, is the behavior of the solutions upon heating, because the solution becomes a gel once the solution temperature was increased to above a point known as the temperature of incipient gels or gel thermal (IGT or TGP). The IGT of hpmc varies from 63 to 80 ° C for the different types of HPMC, with increasing degree of substitution of hydroxypropyl groups increases the IGT. These gels are reversible upon cooling, although there is a pronounced hysteresis between heating and cooling (Murray, 2010)

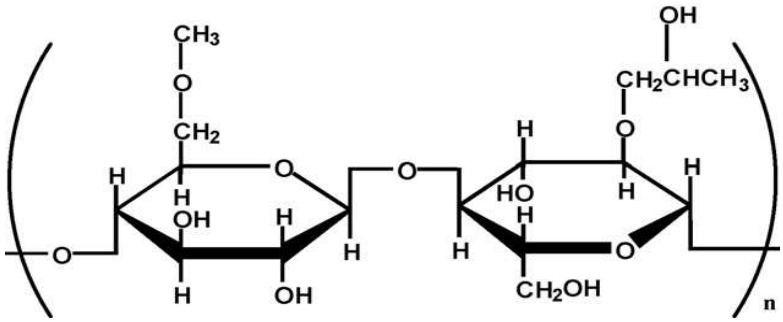

Figure 3. Structure of hydroxyl propil methyl cellulose (HPMC)

In the market there are different types of HPMC, which differ in viscosity and molecular weight (www.sigmaaldrich.it). However, not all authors report the molecular weight and viscosity of the HPMC type used for producing edible film. The first studies of this polymer back to 1995, where the effect of molecular weight and viscosity of some characteristics of the film were studied (Nokhodich et al., 1995) They report that there is a complex relationship between the size of the particles, the gel strength and

viscosity of the solution. In particular, with decreasing particle size (<45 and 45-125μm), an increase in the viscosity of the solution of HPMC resulted in a decrease of the tensile strength of the complex. In 1997, Ayrancı et al. reported that the water vapor permeability (WVP) of films based on HPMC decreases with increasing molecular weight due to the presence of extra methyl groups that make the polymer hydrophobic.

1.2.3 Lipids edible films

Lipid are used for their good water barrier properties. For their hydrophobic nature are capable of forming a structure that reduces the passage of moisture from an phase to another of food system. Because lipid and resin materials are not polymers, generally they do not form cohesive stand alone film (Morillon et al. 2002; Debeaufort and Voilley 2009), but are used in combination with polysaccharides or protein based film to improve their barrier properties.

Edible lipid include beeswax, candelilla wax, carnauba wax, triglycerides, acetylated monoglycerides, fatty acids, fatty alcohols, and sucrose fatty acid esters. Edible resins include shellac and terpene resin.

Following are the lipids most commonly used and are reported in decreasing order of efficiency as water barrier:

- Waxes;
- Lacs;
- Fatty acid and alcohols;
- Acetylated glycerides;
- Cocoa- based compounds and their derivatives.

This classification of lipids was based on chemical composition of the molecules: presence of polar components, hydrocarbon chain length, number of unsaturation or acetylation (Morillon et al., 2002, Debeaufort et al., 2000; Debeaufort and Volley 2009). For molecules having the some chemical nature, increasing chain length modifies the barrier properties because the polar part of the molecule decreases and does not favour water solubility in the film structure. This occurs because lateral packing of acyl chains is less efficient, causing a reduction in van der Waals' interaction and an increase in hydrocarbon chain mobility (Bourlier et al., 2007).

The barrier properties of lipids also depends on their physical state (solid fat content at the temperature of use, crystalline form, etc). These differences are due to several factor such as solid state morphological characteristics, including crystal size, shape, and polymorphism (Morillon et al., 2002).

1.2.4. Plasticizers

Plasticizers are small molecular weight compounds that can be added to an edible or coating solution to improve the flexibility and mechanical properties of the film matrix. Plasticizers are generally added to the protein matrix to improve process ability and to modify the properties of the final structure. As opposed to "internal" plasticizers, which are copolymerized or reacted with the polymer, "external" plasticizers consist of low molecular weight, low volatility substances that interact with the polymer chains producing swelling (Sothornvit and Krochta, 2005).

Water is the most effective plasticizer in biopolymer materials, enabling them to undergo the glass transition, facilitating deformation, and processability of the biopolymer matrix.

Cohesion and flexibility of an edible films are determined by molecular weight, branching and polarity of their constituents. The molecules with low polarity and high linearity tend to produce films with a high degree of cohesiveness and rigidity. Films and coatings formed only by the pure polymer are often very rigid and brittle, because of interactions between hydrogen bonding, electrostatic forces, hydrophobic bonds and/or cross-link. Plasticizers compete mainly for hydrogen and electrostatic bonding with the protein chain. The plasticizers reduce the brittleness, increase the flexibility of the film by interfering in the formation of hydrogen bonds between the polymer chains and increasing the molecular space. 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. Type and amount of plasticizers can decreased the ability of film to act as barrier to moisture (Gennadios et al., 1996; Yong Cho et al., 2002b; Shaw et al., 2002; Wan et al., 2005; Maftoonazad et al., 2007).

The plasticizers generally added in the edible film are polyols and they include: glycerol, propylene glycol, sorbitol, sucrose polyethylene glycol, or fatty acids and monoglycerides (Krochta 2002; Sothornvit and Krochta 2005).

How mechanical and barrier properties of an edible film can be influenced by plasticizer are reported in table 1 and 2.

1.2.5. Blends edible films

The production of edible film by combining various polysaccharides, proteins and lipid is considered beneficial because there is the advantage of the properties of each compound and the synergy between them. The attributes that each component contributes to overall film properties are different too . (Falguera et al., 2011; McHugh 2000; Pol et al., 2002; Garcia et al., 2006; Phan The et al., 2008; Ghanbarzadeh et al., 2008; Phan The et al., 2009; Rivero et al., 2009).

There are three main types of composite films:, emulsion films, bilayer films and dispersion films (Bourlier 2007, Debeaufort and Voilley 2009).

Emulsions films are colloidal system containing two partially miscible fluids and, almost invariably, a surfactant. Emulsions are comprised of fat droplets that generally is greater than 0.2 micron in diameter and less than 5 micron in diameter (Mc Hugh 2000).

Addition of non-lipid compounds or particles such as sugar crystals, fibres, and proteins as dispersed components in fat materials permits formation of fat dispersions (Debeaufort and Voilley 2009).

Bilayer edible films can be composed of protein / protein (Ghanbarzadeh et al., 2008; Pol et al., 2002; Yong Cho et al., 2002), polysaccharide / polysaccharide (Garcia et al., 2004; Garcia et al., 2006), or polysaccharide and protein / lipid (Phan The et al., 2008; Phan The et al., 2009; Rivero et al.., 2009). In the case of hydrocolloid/lipid film, films are formed by depositing a lipid layers onto the surface of the preformed hydrocolloid film.

In bi layer edible film, in which a layer of film is made up of lipids, the resulting water barrier efficiency is often of the same order of magnitude as that of pure lipid. However, the hydrocolloid layer is hydrophilic, and tends to absorb water when the film is in direct contact with a moist phase. Furthermore, additional processing steps for film

making (casting and drying) make bilayer films difficult to use in high-speed commercial production (Debeaufort and Voilley 2009).

In emulsion edible films, water flow thought the portion of the film in which there is no lipid or between two lipid droplets through and water barrier efficiency is lower than bilayer films (Anker et al., 2000). In this case, barrier efficiency depends not only on the lipid type but also on the dried film emulsion structure such as the distribution and particle sizes of lipid dispersed phase (Debeaufort and Voilley, 1995; Perez-Gago, and Krochta2001; Phan The et al., 2002).

Moreover, bilayer film tend to delaminate over time, developing pinholes or crack, and exhibiting poor strength and non uniform surface and cohesion characteristics over time. In addition bi layers require multiple drying steps; whereas emulsion film require only one dehydration step (McHugh 2000; Phan The et al., 2008).

In dispersion edible film, when the continuous phase is composed of a lipid, dispersed hydrophilic particles (sucrose crystals, cocoa powder) do not significantly affect the water vapour permeability, except where there is high relative humidity, for which film structure is modified (Debeaufort and Voilley 2009).

Figure 4 is a schematic representation of the different types of composite edible films and related compounds with mechanisms of opposition to the transfer of water.

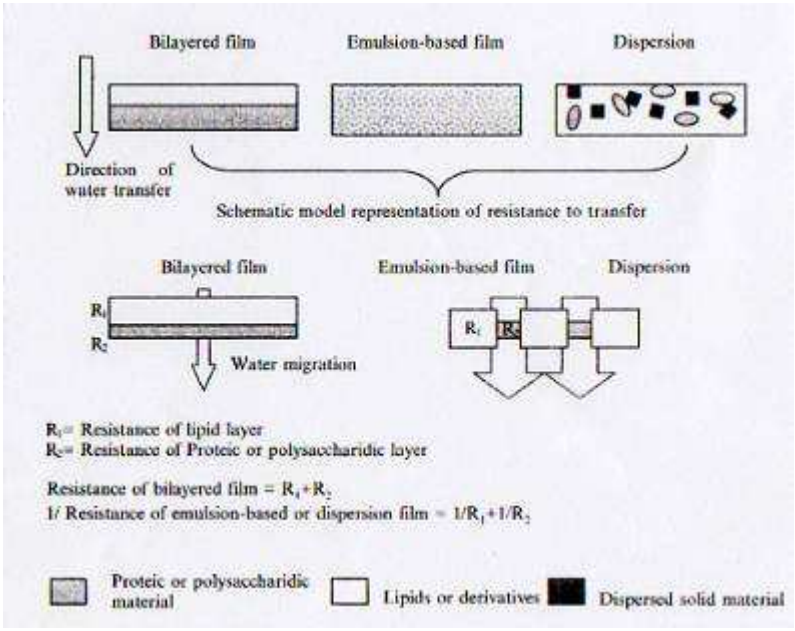

Figure 4. Schematic representation of the different types of edible films and related compounds with mechanisms of opposition to the transfer of water (From Bourlier, 2007).

The main functional properties of blend film are reported in tables 2-5.

1.2.6. Additives

1.2.6.1. Emulsifiers

To produce protein-lipid or polysaccharides –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 (Krochta, 2002).

Emulsifiers are surface-active compounds that have the ability to reduce the interfacial tension between two interfaces since they have both hydrophilic and hydrophobic ends. Emulsifiers are chosen according to hydrophilic-lipophilic balance (HLB) and phase inversion temperature (PIT).

HLB is a system of values of 1 to 40 according to the hydrophobic and hydrophilic portion of the surfactant. Surfactants with low HLB are used in water-in-oil (w/o) emulsification and surfactants with high HLB values were used in oil-in-water (o/w) emulsification.

PIT is the value where emulsions reverse from o/w to w/o depending on the temperature. It is believed that o/w interfacial tension is the smallest at PIT.

Naturally occurring emulsifiers, phospholipids, monoglycerides, soy lecithin, sodium stearoyl lactylate, sodium lauryl sulfate, propylene glycol alginate, and paraffin wax are some examples of food grade emulsifiers (Lee and Han, 2003).

Some proteins are sufficiently surface-active that no emulsifier is necessary to form well-dispersed composite films or provide good surface wetting and adhesion (Krochta, 2002).

1.2.6.2. Other additives

Edible film and coating can be carriers of additives such as anti microbial agents and antioxidants. Antimicrobials are substances that are added to edible film and coating to improve quality and shelf life of products by retarding growth of yeast, molds, and bacteria during storage and distribution (Krochta J.M., 2002; Han, 2002; Lee and Han et al., 2003; Coma et al., 2008). For edible film and coating, examples of food grade antimicrobial agents are organic acids and their salts such as benzoic acid, sodium benzoate, sorbic acid, potassium sorbate, propionic acid, lactic acid, and acetic acid (Cagri et al., 2001; Lee and Han 2003).

Another substance with antimicrobial effect is nisin. Nisin is an antibacterial peptide produced by Lactococcus lactis that effectively inhibits the growth of some bacteria (Coma eta l., 2008; Sanjurj et al., 2006).

Essential oils (EOs) (also called volatile or ethereal Oils) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). This substance have a important antioxidant and antimicrobial effects (Burt, 2004; Bakkali, 2008). An in deep description of this additives is reported in the first case studies.

Table 1. Plasticizer effect on mechanical and barrier properties of some edible film

Film*	EM (MPa)	TS (MPa)	E (%)	WVP (g mm m^{-2} h^{-1} kPa^{-1})	References
EGA+30%gly		4.2	12.4	8.77	(Gennadios et al., (1996)
EGA+40%gly		2.2	18.7	10.27	
EGA+50%gly		1.2	32.2	10.68	
EGA+50%PEG		3.8	59.7	6.22	
EGA+60%PEG		3.4	88.1	6.21	
EGA+50%Sor		3.7	15.0	4.90	
EGA+60%Sor		2.2	18.6	5.69	
EGA+60%PEG+10%YS		2.5	50.0	5.75	
EGA+60%PEG+30%YS		2.2	30.0	5.87	
EGA+60%PEG+50%YS		1.3	20.0	5.62	
EGA+60%PEG+70%YS		0.8	15.0	6.68	Wan et al., (2005)
SPI+gly:PEG=75:25		2.0	158.0	$1.15*10^{-4}$	
SPI+gly:PEG=50:50		2.4	108.0	$1.08*10^{-4}$	
SPI+gly:PEG=25:75		4.0	60.0	$1.11*10^{-4}$	
SPI+gly:PEG=0:100		5.5	0.0	$0.93*10^{-4}$	
SPI+gly:PG=75:25		2.0	125.0	$1.51*10^{-4}$	
SPI+gly:PG=50:50		2.2	130.0	$1.22*10^{-4}$	
SPI+gly:PG=25:75		6.0	70.0	$0.83*10^{-4}$	
SPI+gly:PG=0:100		5.1	0.0	$1.20*10^{-4}$	
SPI+gly:SOR=75:25		1.7	129.0	$0.97*10^{-4}$	
SPI+gly:SOR=50:50		4.2	125.0	$0.79*10^{-4}$	
SPI+gly:SOR=25:75		5.7	130.0	$0.54*10^{-4}$	
SPI+gly:SOR=0:100		7.8	0.0		
SPI+gly:SUC=75:25		2.0	110.0		
SPI+gly:SUC=50:50		3.1	80.0		
SPI+gly:SUC25:75		8.0	0.0		
SPI+gly=100		1.6	160.0		Yong et al.,(2002a)
SPI+gly:Sor=100:0 0,7g plast/gSPI		10.0	25.0		
SPI+gly:Sor=50:50		13.5	13.0		
SPI+gly:Sor=0:100		19.0	8.0		
SPI+gly:Sor=100:0 0,5g plast/gSPI		24.0	5.0		
SPI+gly:Sor=50:50		24.0	5.0		
SPI+gly:Sor=0:100		27.0	5.0		
SPI+gly:Sor=100:0 0,3g plast/gSPI		25.0	3.0		
SPI+gly:Sor=50:50		27.5	3.0		
SPI+gly:Sor=0:100		31.0	3.0		

*EGA = egg white ; YS= yolk egg ; SPI= isolate soy protein; gly = glicerol; Sor = sorbitol; PEG = polyethylene glycol; SUC= sucrose.

Table 2. Plasticizer effect on mechanical and barrier properties of some edible film

Film*	*EM* (MPa)	*TS* (MPa)	*E* (%)	*WVP* (g mm m^{-2} h^{-1} kPa^{-1})	References
NaCas:gly=0.4	280	6.5	15		Fabra et al., (2008)
NaCas:gly=0.6	50	2.5	25		
NaCas:sor=0.4	700	15	7.5		
NaCas:sor=0.6	400	9.5	15		
NaCas:gly:lip=1:0.3:0	850	14.4	4.0	4.1	
NaCas:gly:AO=1:0.3:0.25	169	5.5	32	3.7	
NaCas:gly:AO=1:0.3:0.5	84	2.9	28	3.6	
NaCas:gly:AO=1:0.3:0.75	41	1.8	24	3.0	
NaCas:gly:BW=1:0.3:0.25	193	2.8	3.5	2.6	
NaCas:gly:BW=1:0.3:0.5	222	3.2	5.2		
NaCas:gly:BW=1:0.3:0.75	ND	ND	ND	ND	
NaCas:gly:lip=1:0.3:0.5					
BW:OA=30:70	20	1.0	25	1.8	
BW:OA=50:50	100	1.8	8	1.6	
BW:OA=70:30	150	28.0	12	2.0	Shaw et al., (2002)
WPIs:gly:OS=1:0,5:0,4		4.6	44	5.3	
WPIs:gly:OS=1:0,5:0,0		4.3	33	4.8	
WPIs:gly:OS=1:0,6:0,4		3.1	71	5.4	
WPIs:gly:OS=1:0,6:0,0		3.75	39	5.7	
WPIs:gly:OS=1:0,7:0,4		23	84	56	
WPIs:gly:OS=1:0,7:0,0		3.7	51	5.8	

*WPIs = isolate whey protein; NaCas= sodium caseinate; OS= soybean oil AO= oleic acid; BW= beeswax; lip= lipid; gly= glicerol; sor: sorbitol

Table 3. Mechanical and barrier properties of some Blend edible film

Film*	*EM* (*MPa*)	TS (*MPa*)	*E* (*%*)	*WVP* (*g mm m^{-2} h^{-1} kPa^{-1}*)	References
CS		47.4	3.6	$6.37*10^{-6}$	Garcia et al., (2006)
Cs+gly		7.1	22.5	$3.13*10^{-6}$	
CS+CH		24.7	3.0	$3.17*10^{-6}$	
CS+CHgly		28.7	11.7	$1.62*10^{-6}$	
CH		60.7	3.3	$1.62*10^{-6}$	
WPI:HPMC=100:0	57	3.9	112.0	$6.60*10^{-2}$	Brindle et al., (2008)
WPI:HPMC=75:25	182	7.8	47.0	$4.40*10^{-2}$	
WPI:HPMC=50:50	258	14	27.0	$4.21*10^{-2}$	
WPI:HPMC=25:75	971	33	23.0	$3.95*10^{-2}$	
WPI:HPMC=0:100	1656	61	16.0	$4.25*10^{-2}$	
WPI:alginate (co.dried)	20.0	1.8	27.0	4.09	Coughlan et al., (2004)
WPI:alginate (D. blended)	12.5	0.9	20.5	5.21	
WPI:pectin(co.dried)	20.5	1.2	23.5	4.34	
WPI:pectin (D. blended)	11.9	0.6	15.0	5.17	
WPI:carraginine (co.dried)	20.0	1.2	19.5	4.20	
WPI:carraginine (D.blended)	14.0	0.8	18.0	5.34	
WPI:f.konjiac (co.dried)	17.5	1.0	21.0	4.21	
WPI: f.konjiac (D. blended)	11.5	0.6	15.5	5.84	
WPI (control)	12.5	0.7	19.5	4.59	Anker et al., (2002)
WPIp	96	2.2	20	13,8	
Acetem	1	0.2	13	0.2	
Laminate	36	1.0	29	0.2	
Emuls.- conc lip effect	7-52	0.3-1.2	29-50	7.7-7.4	
Emuls.- omogeniz effect	50-40	1.6-1.3	117-23	5.8-7.1	
Emuls.-effect sep.phase	8-28	0.3-0.8	19-32	8.8-8.2	Petersson et al., (2005)
NPS+AC 0% 23°C	2.5	46.2	10.3	7.8	
NPS+AC 0% 50°C	2.5	47.3	9.2	4.9	
NPS+AC 5% 23°C	2.3	39.5	3.4	6.4	
NPS+AC 5% 50°C	2.5	38.3	2.9	5.3	
NPS+AC 10% 23°C	1.9	30.0	2.3	5.7	
NPS+AC 10% 50°C	1.8	25.5	2.2	5.3	

***CS=** cornstarch; CH= chitosan; WPI= whey protein; HPMC= Hydroxyl propil methyl cellulose; co.dried= D.dried; WPIp= whey protein; NPS= native potate starch; AC= acetem

Table 4. Mechanical and barrier properties of some blend edible film

*Film	EM (MPa)	TS (MPa)	E (%)	WVP (g mm m^{-2} h^{-1} kPa^{-1})	References
AG+15%gli HB:HL=0:1		42.1	6.5	$2.6*10^{-6}$	Phan et al., (2009a)
Cas+15%gli HB:HL=0:1		35.2	2.6	$2.0*10^{-6}$	
AG+15%gli+VGB HB:HL=0.4:1		29.2	18.0	$8.6*10^{-7}$	
HB:HL=0.7:1		23.6	27.5	$8.4*10^{-7}$	
HB:HL=1:1		23.4	23.0	$6.7*10^{-7}$	
HB:HL=2:1		12.4	12.3	$5.2*10^{-7}$	
CAS+15%gly+VGB HB:HL=0.4:1		8.2	6.1	$1.0*10^{-6}$	
HB:HL=0.7:1		5.03	50.3	$9.9*10^{-7}$	Phan et al., (2009b)
AG+15%gly		42.1	6.5	$2.6*10^{-6}$	
CAS+15%gly		35.0	2.6	$2.0*10^{-6}$	
AX+15%gly		22.3	5.5	ND	
AG:CAS=8:2+15%gly		34.7	5.2	$1.2*10^{-6}$	
AG:CAS=5:5+15%gly		35.6	5.3	$1.1*10^{-6}$	
AG:CAS=2:8+15%gly		28.1	4.2	$1.4*10^{-6}$	
AG:AX=7:3+15%gly		22.2	4.5	$7.1*10^{-7}$	
AG:AX=5:5+15%gly		27.8	5.3	$7.7*10^{-7}$	
AG:AX=9:1+15%gly		29.5	5.5	$7.0*10^{-7}$	
CAS:AX=8:2+15%gly		20.8	2.8	$1.0*10^{-6}$	
CAS:AX=5:5+15%gly		17.3	3.1	$1.1*10^{-6}$	
CAS:AX=2:8+15%gly		18.1	2.9	$1.0*10^{-6}$	
B:PC=4:0+30%gly	1.8	6.0	8.4	$9.6*10^{-3}$	Sothornvit et al., (2007)
B:PC=4:1+30%gly	2.8	9.0	7.8	$1.1*10^{-3}$	
B:PC=6:0+30%gly	4.4	10.5	4.0	$1.3*10^{-3}$	
B:PC=6:1+30%gly	6.4	1.4	3.7	$1.4*10^{-3}$	
B:PC=8:0+30%gly	8.0	11.8	2.3	$1.7*10^{-3}$	
B:PC=8:1+30%gly	10.0	15.6	2.2	$1.5*10^{-3}$	

*AG= agarose; CAS= cassava starch ;AX= arabinose xylose; VGB= vegetable fat; gly= glycerol; HB= hydrophobic substance; HL= hydrophilic substance; B= banana flour; PC= pectin

Table 5. Effect of enzymatic treatments, chemical and physical performance of the films

Film*	EM (MPa)	TS (MPa)	E (%)	WWP ($g\ mm\ m^{-2}\ h^{-1}\ kPa^{-1}$)	OP ($g\ mm\ m^{-2}\ h^{-1}\ kPa^{-1}$)	References
CII		14.0	23.0			Di Pierro et al., (2006)
CH-WP(CWP)		9.5	141.0	$1.35*10^{-4}$	$8.6*10^{-4}$	
CWP+TGase		26.2	3.1	$3.67*10^{-5}$	$1.4*10^{-4}$	
Pectin-gelatin 0 kGy		106.0	20.3	0.63		Jo et al., (2005)
Pectin-gelatin 10 kGy		140.0	17.3	0.52		
Pectin-gelatin 20 kGy		116.0	17.1	0.60		
Pectin-gelatin 30 kGy		100.0	21.9	0.66		
CH	1250	15.0		$1.67*10^{-5}$		Di Pierro et al., (2007)
CH-OVA	1000	25.0		$3.83*10^{-4}$		
CH-OVA-TGase	1800	37.0		$329*10^{-4}$		
		6.8	11.6			Mariniello et al., (2003)
Pectin-soy flour		12.4	7.2			
Pectin-soy flour+TGase						
WP		4.0	20.0	$1.58*10^{-7}$		Oh et al., (2004)
WP+TGase		3.2	110.0	$1.19*10^{-7}$		
CN		3.4	215.0	$2.12*10^{-7}$		
CN+TGase		2.5	245.0	$1.76*10^{-7}$		
WP+CZ		2.3	195.0	1.33		
WP+CZ+TGase		2.1	218.0	1.48		
CN+CZ		2.2	260.0	1.55		
CN+CZ+TGase		1.9	450.0	1.51		

*****CH= chitosan; OVA= ovalbumim; WP= whey protein; CZ= zein protein; TGase= transglutaminase

1.3. Film and coating formation

Film formation generally involved inter- and intra molecular associations or cross-linking of polymer chains forming a semi-rigid three dimensional network that entraps and immobilizes the solvent. Film forming material should form a spatially rearranged gel structure with all incorporated film forming agent, such as biopolymers, plasticizer, other additives, and solvent in the case of wet casting.

There are two categories of film-production process: *dry* and *wet*.

The *Wet process* used solvent for the dispersion of film forming materials, followed by drying to evaporation of the solvent and formation of 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

The *Dry process* of edible film production does not use liquid solvent. 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 (Han and Gennadios, 2005).

For edible film and coating formation, it is essential to understand the chemical properties and structure of film forming materials, biopolymers, as well as additives, to tailor them to specific application.

Solubility in water and ethanol is very important to select a solvent for wet casting or active mixing. Thermoplasticity of biopolymers, including phase transition, glass transition, and gelatinization characteristics, should be understood for dry casting or thermoforming.

The chemical characteristics of plasticizers and any other additives should be also identified to verify their compatibility with biopolymers and to determine the changes in film structure caused by addition of plasticizers and additives. These investigations are very important to obtain critical information related to film forming mechanisms and film property modification (Han and Gennadios, 2005).

1.3.1. Film forming technology

There are many technologies that can be used to make edible films and coatings. The choice of a specific process depends on the nature of film or coating constituents and on the intended shape of the barrier layer. Polysaccharides and proteins have to be modified using processes such as polymerization, gelation, coagulation, and co-acervation. Lipids-based coatings are more often obtained from either melting and crystallization or solvent evaporation.

Edible films can be obtained by extrusion, co-extrusion, spreading, casting, roll coating, drum coating, pan coating, or laminating techniques. Edible coatings, on the other hand, are mainly applied using spraying, drum coating, spray-fluidization, pan coating, or falling film techniques.

Casting method is a technique used to produce industrial films, including non-edible film. It provides spreading of a film-forming solution followed by a roll-drying step. This technique is the most useful method for producing edible films and coatings at both laboratory and pilot scales (Debeaufort and Volley, 2009). Bio polymers power is dissolved in solvent (water or other edible). If heat or adjustments of pH are need, this

can be done after or before dissolution. 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 (Kroch, 2002). On the lab scale, spreading thicknesses and drying conditions (temperature and relative humidity) may be accurately controlled (Debeaufort and Volley, 2009).

Extrusion technologies are often used for industrial production of films, tubes, poaches, and casings. The process can involve any or all of the following operations: heating, cooling, feeding, conveying, compressing, shearing, reacting, mixing, melting, homogenizing, "amorphousizing" (converting polymer crystalline domains to amorphous domains), cooking, and shaping. For their thermoplastic properties of some protein (zein, soy protein, whey protein, wheat protein) can be used in thermoplastic process (Hernandez and Krochta, 2008).

Compression molding is one form of low-moisture processing method used to make edible film. Thermoplastic material, which softens when it is heated, is placed on one half of a mold. Heat and pressure are applied to the mold once it is closed. Film material then fills the mold cavity and polymerization occurs. The film is, then, obtained by cooling the mold. One of the differences between compression molding and extrusion is that flowability of the film-forming material for compression molding can be low, while for extrusion, the material needs to have high flowability. Because compression molding has very limited production amount, it is economical for small production (Lee and Han, 2003).

1.3.2. Coatings formation

Coating application consists of applying a liquid or a powder ingredient onto a base product. Surface properties play a key role in the success of coating application.

Application of coatings generally requires a four-step process:

1. Deposition of coating material (solution, suspension, emulsion or powder) on the surface of the product to be coated through spraying, brushing, spreading, or casting.
2. Adhesion of coating material (solution, suspension, emulsion or powder) to the food surface.
3. Coalescence (film-forming step) of the coating on the food surface.
4. Stabilization of the continuous coating layer on its support or food product through co-acervation by drying, cooling, heating, or coagulation.

Enrobing involves application of a thick coating layer by dipping the product to be coated in solution batter or in molten lipids. Coating of fresh or frozen products with a batter and/or breading can enhance palatability, add flavor to an otherwise bland product and reduce moisture loss and oil absorption during frying.

The spray-coating technique can be used alone or in combination with pan, drum, screw and fluidized-bed coaters. Spraying makes it possible to deposit either thin or thick layers of aqueous solution or suspensions and molten lipids. It is the most commonly used technique for applying food coatings. The spraying nozzle plays a critical role in the coating process. Spraying efficiency depends on the pressure, fluid viscosity, temperature and surface tension of the coating liquid, as well as nozzle shape or design. This in turn affects the flow rate, the size of the droplets, spraying distance and angle, and overlap rate (Debeaufort and Volley, 2009).

1.4. Functional properties

1.4.1. Permeability

There are many applications in the area of food packaging that use mass transfer phenomena. Example include selecting a packaging material to predict the extend product shelf-life, and to control the in-package atmosphere for protection and preservation of food products.

Permeation, absorption and diffusion are typical mass transfer phenomena occurring in food packaging systems. Permeation is the ability of permeants to penetrate and pass right through an entire material in response to a difference in partial pressure. This property of the packaging material may also be referred to as the “permeance”. To convert the permeance (which is evidently dependent on the thickness of the film) into an intensive property, it is multiplied by the film thickness to derive the permeability (P) of the film. The mass transfer of a solute from a solution thorough a (polymeric) material is a useful way to determine mass transfer coefficients experimentally, because it requires simple permeation apparatus consisting of the high and low concentration solution in chamber divided by the test film material.

Diffusion is the movement of a molecules in a medium caused by concentration differences acting as a driving force. Diffusivity (D) is a measure of how well the compound diffuses in the medium.

Absorption and its counterpart desorption measure the affinity of a given substance for two media with which it comes into contact. The affinity of a substance for a material can be expressed using the solubility (S) or partition (K) coefficient.

The permeability, solubility, and diffusivity are characteristic value for a migration component through a particular medium. These parameter are therefore essential in simulating the mass transfer profile.

The mass transfer rates of molecules through a package material or through a membrane are often described as irreversible process. A generalized thermodynamic driving force is required to induce movement of the molecules, which for the movement of gases and solute is the gradient in the chemical potential of the migration species. For most packaging and membrane applications the area through which transfer occurs is large compared to the to the thickness, so that one-dimensional flow is consider. The linear coefficient linking the flux (for unit cross-section) to the driving force can be consider as a resistance of the package or membrane material to the passage of the given species.

With the appropriate substitutions and assumptions, the gradient in chemical potential is related to the concentration gradient of the migration species. The permeation of a molecule is its movement from the region where its concentration is high (C_1) to the region where the concentration is lower (C_2).

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.

Events occurring within the material are examined first where diffusion is the dominant factor. Diffusion obeys Fick’ law, and Fick’s first law can be expressed as:

$$J_d = -D\frac{\partial C}{\partial x} \tag{1.1}$$

where J_d , D, C and x are the flux per unit cross-section, the diffusivity, the concentration of the solute, and the distance across which the molecules has to travel, respectively. Fick's second law can be used to analyze unsteady state diffusion with time t:

$$\frac{\partial C}{\partial t} = -D\frac{\partial^2 C}{\partial x^2} \tag{1.2}$$

When the steady state of diffusion has been reached, J is constant and eq. (1.1) can be integrated across the total thickness of the polymer, L, and between the two concentrations, assuming D to be constant and independent of C.

After integrating equation (1.1) for the case where C_1 and C_2 remain constant, the flux of the molecules in the steady state is given by equation:

$$J_d = \frac{Q}{A*t} = D\frac{(C_1 - C_2)}{L} \tag{1.3}$$

Where Q is the amount of diffused moving substance, A is the cross sectional diffusion area, and L is the thickness of the package or membrane. The diffusivity, D, has units of m^2s^{-1} and flux has units of mol m^{-2} s^{-1} or kg $m^{-2}s^{-1}$:

$$D = \frac{J_d * L}{\Delta C} = \frac{Q * L}{A * t * \Delta C} \tag{1.4}$$

Before gas diffuse through the packaging material from C_1 to C_2 it must first dissolve into material. The sorption of a gas component into a packaging material generally has a linear relationship to the partial pressure of the gas as show in Henry' law under conditions where the gas concentration is lower than its saturation concentration or maximum solubility:

$$p = \sigma X_S \tag{1.5}$$

Where p and X_s are the partial pressure of the gas in the atmosphere and molar fraction of gas in the packaging material respectively, and σ is the Henry's law constant in P*a*. If the permeable gas molecule has an affinity to the packaging material matrix, or is immobilized in the micro voids of the matrix polymer at a relatively low pressure, the sorption behaviour follow a logarithmic non linear relationship, which is expressed as a Langmuir type sorption. Following equation show the linear relationship between the concentration at the surface of the packaging material and the partial pressure of the gas:

$$C_S = H^{-1} p_1 \tag{1.6}$$

Where p_1 is the partial pressure of the gas on the high concentration (C_1) side.

Since the driving force for gas penetration through a packaging material is the difference in gas concentrations or partial pressure between the two sides of the packaging material, the gas flux J of both permeation and diffusion can use partial pressure term instead of the concentration gradient.

In the mass transfer situation, the concentration can be substituted for the partial pressure p and the solubility S in:

$$Q = \frac{D * S(C_1 - C_2)At}{L} \tag{1.7}$$

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

$$P = \frac{Q * L}{A * t * (p_1 - p_2)} \tag{1.8}$$

Or

$$\frac{Q}{t} = \frac{P}{L} A * (\Delta p) \tag{1.9}$$

The term P/L is called the *permeability* or *permeance* (Han and Scanlon et al., 2005).

1.4.2. Viscoelasticity and mechanical properties

1.4.2.1. Viscoelasticity

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. It deals with the predictions of mechanical behaviour based on the micro- or nanostructure of the material, e.g., the molecular size and architecture of food polymers in solution or particle size distribution in a solid suspension.

The ideally *elastic material* exhibits no time effects and negligible inertial effects. The material responds instantaneously to applied stress. When this stress is removed, the sample recovers its original dimensions completely and instantaneously. In addition, the induced strain, ε, is always proportional to the applied stress and is independent of the rate at which the body is deformed. For the ideal elastic material, the mechanical response is described by Hooke's law:

$$\sigma = \frac{F}{A} = E\varepsilon \tag{1.10}$$

Where σ the force divided by the cross-sectional area of the specimen, ε, is the strain and E is Young's 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. Thus in contrast to the ideal elastic response, for ideal *viscosity material* strain is a linear function of time at an applied external stress. The material will not recover from

its deformation when the load is removed. On the release of the applied stress, a permanent set results. According to Newton's law, the response of a fluid to a shearing stress τ is viscous flow, given by:

$$\tau = \eta \frac{d\gamma}{dt} \tag{1.11}$$

Where η is viscosity and dγ/dt is strain rate (ε).

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 (an instantaneous strain is applied to the sample. The stress required to maintain this strain is measured as a function of time) and creep test (the sample is subjected to an instantaneous constant stress and the strain is monitored as a function of time).

The viscoelasticity studied by transient methods can be represented by two mechanical model: Hookean 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 mechanical tests provide useful information about the viscoelastic nature of a polymer. It is a versatile tool for studying the effects of molecular structure on polymer properties. In dynamic mechanical tests, the response of a material to periodic stress is measured. Dynamic mechanical properties of viscoelastic polymers are measured when the applied stress or strain is oscillatory in nature with a specific frequency.

Data from dynamic mechanical measurements can provide direct information about the elastic modulus and the viscous response of a polymer. This can be illustrated by considering the response of elastic and viscous materials to imposed sinusoidal small strain (or stress), ε , and measuring the resulting stress (or strain):

$$\varepsilon = \varepsilon_0 \sin(\omega t) \tag{1.12}$$

Where ε_0 is the max amplitude and ω is the frequency (rad/s).

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.

For a purely elastic body, Hooke's law is obeyed (phase angle, δ , is equal to 0). Consequently,

$$\tau = G^{'}\varepsilon_0 \sin(\omega t) \tag{1.13}$$

where *G'* is the *elasticity modulus* and represent the material resistance to deformation. It is evident from two last equations that for elastic bodies, stress and strain are in phase.

Now consider a purely viscous fluid. Newton's law dictates that the shear stress is given by $\tau = \eta\dot{\varepsilon}$, that is,

$$\tau = \eta\varepsilon_0\omega\cos(\omega t) \tag{1.14}$$

In this case, the shear stress and the strain are 90° out of phase. The response of viscoelastic materials falls between these two extremes with $0° < \delta < 90°$.

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

$$\tau(t) = \varepsilon_0 G^{'}(\omega)\sin(\omega t) + \varepsilon_0 G^{''}(\omega)\cos(\omega t) \tag{1.15}$$

The frequency dependent functions *G'* (ω) and *G*"(ω) are shear elastic (storage) modulus and shear viscous (loss) modulus respectively. *G'* (ω) 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*"(ω) is a measure of the energy dissipated as heat for cycle of deformation per unit volume. *G*"(ω) is the property that relates to molecular events of viscous nature.

The linear viscoelastic behaviour of a fluid, or polymer, is completely characterized when we know the frequency dependence of two functions, such as $|G^*|$ and the loss tangent, $\tan\delta = G^{''}/G^{'}$, or the dynamic moduli *G* ' and *G*", or any other combination of two quantities.

$|G^*|$ corresponds to the ratio between the maximum stress τ and the maximum strain applied ε^0. At constant frequency, it does not depend on ε_0 and then the stress response is linearly proportional to the applied strain only if the oscillation amplitude is enough small, within the linear viscoelastic regime (Ebewele, 2000; Grassi et al., 2007).

1.4.2.2. Mechanical properties

Mechanical behaviour involves the deformation of a material under the influence of applied forces. The mechanical properties of polymers are affected by their chemical composition, surrounding conditions and test conditions. The various factors that affect the mechanical properties are:

1. Molecular weight and molecular weight distribution
2. Cross-linking and branching
3. Crystallinity and crystalline morphology
4. Copolymerization (random, block or graft)
5. Plasticization
6. Fillers, type and amount
7. Blending and related morphology of the blend

8. Molecular orientation

In any given polymer system one or more of the above factors would be operative. The effect of these factors can be correlated with the mechanical behaviour, which help in tailoring properties.

In addition several environmental and external variables that affect the mechanical behaviour of polymers are:

1. Temperature
2. Time, frequency, rate of stressing /straining
3. Pressure
4. Stress and strain amplitude
5. Type of deformation
6. Thermal history
7. Nature of surrounding atmosphere, such as moisture level, ozone level, etc.

Processing methods and the conditions of processing play an important role in governing the polymer morphology and hence the resulting mechanical properties. The micro-structure produced during processing affects the viscoelastic nature and thus the response to applied stress during testing. It is, therefore, important to also correlate processing with structure and properties.

The tensile properties of polymers are normally determined by studying the stress-strain behaviour at relatively high strains. A typical plot of stress strain curve is shown in Figure 5. The initial part of the curve has a linear stress-strain relationship exhibiting elastic deformation of the polymer. The slope in this linear region gives the tensile modulus of the material. The point at which it begins to deviate from linearity is called the proportionality limit. At slightly higher strains, the yield point is reached and after this the polymer deforms in a plastic manner and all the strain is not recoverable. The stress at this point is called the yield stress with a corresponding elongation at yield. Beyond the yield point, the material is permanently deformed. If the stress is removed after the yield point the polymer exhibits some recovery and some permanent deformation. At a higher strain level the polymer breaks giving the ultimate tensile strength and the corresponding strain at break. Another feature of importance is that during the plastic deformation there is an increase in stress which is called strain hardening.

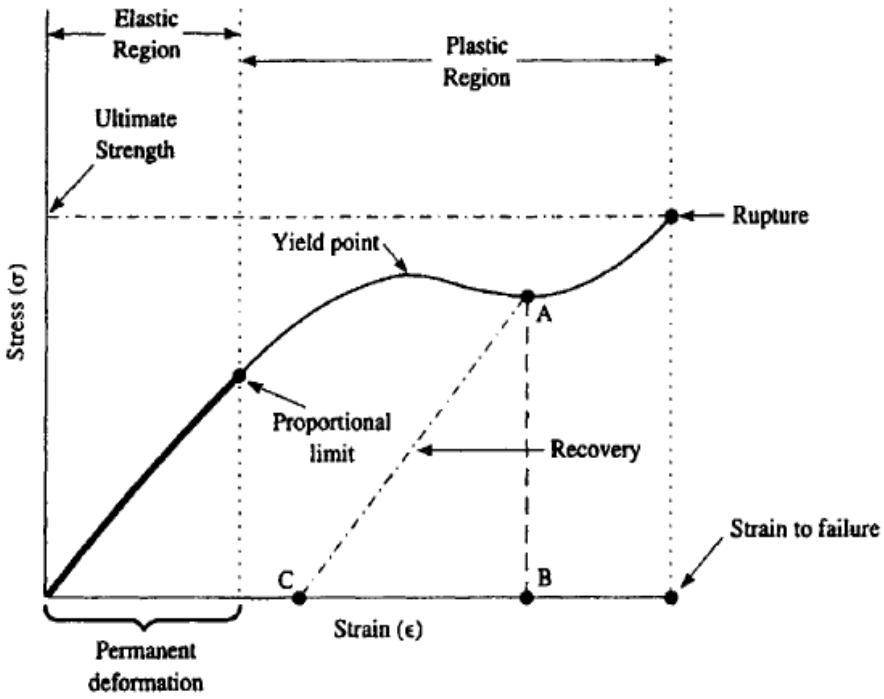

Figure 5 Stress-Strain behaviour of Polymeric Materials (From David and Mirsa, 2001)

The common tensile test involves elongation of a dumbbell shaped sample held in jaws that pull the sample at a constant rate and the load required for this is measured as a function of time. The load-elongation curve obtained on a uniaxial tensile testing machine is plotted and then converted to stress-strain curve. Ductile materials have a relatively higher elongation at break while brittle materials have a lower elongation at break. Similarly stronger materials have higher yield stress and ultimate strength values. Stiffer materials have a higher modulus as compared to softer materials. Amorphous polymers in their glassy state are generally brittle and in the rubbery state they are ductile. Another feature of importance is that during the plastic deformation there is an increase in stress, which is called work hardening. The stress-strain relationship at large values of deformation gives an idea about the type of behaviour a polymer has (David and Misra, 2001) .

1.5. Factor affecting films properties

The main properties of interest for films and coatings are tensile properties (tensile strength, elongation at break and elastic modulus), gas permeability, water vapour permeability and appearance. All of these properties can be affected by the extrinsic conditions used to process and produce the films (Dangaran et al., 2009).

1.5.1. Drying condition

Drying conditions may influence the final properties of the material. For example, proteins can change their structure as a function of processing parameters (Tapia-Blacido et al., 2005). In this sense, temperature is a strong denaturing factor for proteins, although the thermal stability and conformation of each protein depend on the amino acid composition. During the drying period, when water is progressively

eliminated, proteins conformation changes, and the degree of protein unfolding determines the type and proportion of covalent (S–S bonds) or non-covalent (hydrophobic interactions, ionic and hydrogen bonds) interactions that can be established between protein chains. It is known that chains can interact more strongly and easily, especially by disulfide bonds, when proteins are denatured (Mauri and Anon, 2006). So the cohesion of the final network would be a function of these bonds and determines the properties of the films obtained (Denavi et al., 2009).

The effect of dry conditions (e.g., temperature, relative humidity, type of energy source, etc.) on the properties of edible based films have been investigated for different proteins. Just as an examples the results of the work of Denavi et al. (2009) on the influence of drying condition (air temperature and relative humidity) on properties of commercial soy protein isolate (CSPI) films and other obtain in laboratory (LSPI) is reported. Authors report that different drying condition could promote different interaction. CSPI films were dried at high temperature (70°C) and low relative humidity (30%), which could have promoted the formation of a higher number of hydrophobic interactions within the film structure, while LSPI films were dried at 60 °C and high relative humidity (60%), which would promote a higher number of hydrophilic interactions. Thus, the higher number of hydrophobic interactions in the CSPI film would hamper water diffusion through the film and would confer it better barrier properties to water vapour and lower solubility than LSPI films. On the other hand, the presence of a higher number of pores in the structure of LSPI films would explain the higher oxygen permeability of such films.

1.5.2. pH

If protein is one of the components of the film forming solution, the pH should be adjusted not to be extremely acidic or extremely alkaline, since intra molecular protein repulsive force develops under extremely acidic and alkaline conditions. Therefore, films formed in these conditions will be less dense and more permeable. Film opacity, solubility, WVP, and mechanical properties of wheat gluten films were affected by pH. It is reported that the films made at pH 5 were the strongest, while films made at pH 6 had the lowest WVP (Lee and Wan, 2003). pH affects the mechanical properties of soy protein/gelatine film (Cao et al., 2007). The authors reported that tensile strength was most strong at pH 8-9. And the films were most opaque when pH increased from 6 to 9.

1.5.3. Heat denaturation

The protein film network may be improved through heat-denaturation, which improves the tensile and barrier properties of solvent casted films by induction of cross-linking between the protein chains (Dangaran et al., 2009).

Heat denaturation effect was studied for whey protein and soy protein. Temperature denatured unfolded whey protein structure and increased exposure of free thiol group (Dangaran et al., 2009). Perez-Gago and Krochta (2002) reported that S–S bonds, whether intra- or intermolecular, play a very small role in determining the moisture barrier properties of WPI-based films, even if these films possess different oxygen permeability (OP), solubility, and mechanical properties. OP of native WPI films is significantly higher than OP of heat-denatured films, but of the same order of magnitude. The lower OP values for heat-denatured films may be related to their more linear (unfolded) structure, leading to higher cohesive energy density and lower free volume among polymer chains. The unfolded structure of heat-denatured whey proteins

and the covalent S–S bonding during drying leads to film insolubility in water and produces films that are stronger and can withstand higher deformations. The degree of protein denaturation and unfolding as heating time and temperature increase affects the degree and nature of protein–protein cross-linking and, as a consequence, the solubility and mechanical properties of the films.

For soy protein films formation is believed to involve development of hydrophobic, disulfide, and hydrogen bonds between protein polymer chains. Heating of the film-forming solution is very important to disrupt the protein structure, cleave native disulfide bonds, and expose sulfhydryl groups and hydrophobic groups, and then to form new bonds between protein chains during film drying.

1.5.4. Enzymatic treatment

Cross-linking of proteins has been induced by both chemical and enzymatic means. Formaldehyde, glutaraldehyde, and lactic acid have been used to cross-link whey proteins through lysine residues.

Transglutaminase (TGase) is a food grade enzyme that uses the acyl-transferase mechanism to link the gamma-carboxyamide (acyl donor) of a glutamine residue to the gammaamine (acyl acceptor) of lysine residues along protein chains. This enzyme is known to improve elasticity in foods. Originally, transglutaminase was extracted from Guinea pig liver, making it expensive and cost-prohibitive for large-scale production. It is now possible to be obtained it at a lower cost from microbial sources, and it has been used in both homogenous protein systems and mixtures of proteins to affect tensile and permeability properties. Whey protein, casein, soy, egg albumin and wheat gluten have all been investigated for treatment with this enzyme. The molecular weight of alpha-lactalbumin, beta-lactoglobulin and alpha-lactalbumin/betalactoglobulin mixtures was shown to increase after TGase treatment, indicative of cross-linking. Moreover, TGase-treated proteins were also more heat stable relative to untreated ones (Truong et al. 2004). The cross-linked protein networks were less soluble, which may improve the water vapour permeability properties of films formed from the cross-linked proteins (Dangaran et al., 2009; Mariniello et al., 2003; Di Pierro et al., 2007).

1.6. Proteins polysaccharides interaction

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 equilibrium, strength and fracture of materials, and surface and transport properties (Aguilera et al., 1999).

1.6.1. Polymer solution

Knowledge of the role of protein-polysaccharide interactions, in relation to their functionality in complex multiphase systems, such as food mixed solutions, bio polymeric 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 bio polymeric 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 (Aguilera and Stanley, 1999). A necessary (but insufficient) condition for a homogeneous solution to be formed after mixing is given by this expression:

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix} < 0 \qquad (1.16)$$

where ΔG_{mix} (or $G_{\text{mixture}} - G_{\text{pure components}}$) 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:

$$\frac{\Delta G_{mix}}{N} = \Delta H_{mix} + RT\left(x_1 \ln x_1 + x_2 \ln x_2\right) \qquad (1.17)$$

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, if they behave as an ideal solution ($\Delta H_{mix} = 0$).

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:

$$\frac{\Delta G_{mix}}{N} = RT\left(x_{12}\phi_1\phi_2 + \phi_1 \ln\phi_1 + \frac{\phi_2}{x}\ln\phi_2 \right) \qquad (1.18)$$

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 $\chi 12$ is called the Flory-Huggins interaction parameter and is equal to:

$$x_{12} = \frac{\Delta H_{mix}}{RTN_1\phi_2} \qquad (1.19)$$

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:

$$x_{12c} = \frac{1}{2} + \frac{1}{2x} + \frac{1}{\sqrt{x}} \qquad (1.20)$$

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), χ_{12c} =2 , whereas for large polymer ($x \rightarrow \infty$) 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; Aguilera, 1999).

1.6.2. Consequences of mixing solution

On mixing two biopolymers in solution, for instance a polysaccharides and a protein, one may observe either one of the following possibility as depicted in figure 6.

The interaction of the of the two biopolymers may be:

- Associative (the biopolymers attract one another)
- Or segregative (the biopolymers repel each other and are denote as incompatible)

(de Kruif et al., 2001)

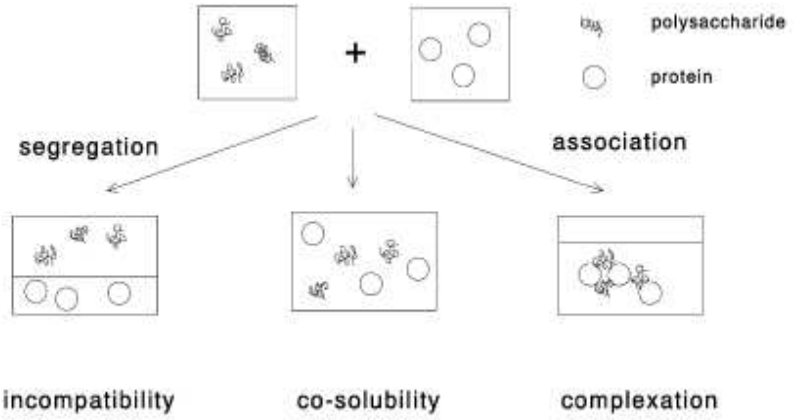

Figure 6. Main trends in the behaviour of protein/ polysaccharides mixture (From de Kruif et al., 2001)

For very dilute solutions the system is stable since entropy dominates and proteins and polysaccharides are co-soluble. Upon increasing the concentration of the biopolymers the system may become unstable, depending on the type of interaction. As a rule biopolymer mixtures tend to segregate. For polymers of a similar and expanded structure this is classically ascribed to the difference in interaction energy between polymer segments and is at the core of Flory theory.

For polymers dissimilar in shape and structure segregation lead to a reduction of the polymer concentration near the other (protein) particle. Exceeding a certain polymer concentration lead to a phase separation into a protein-enriched and a polysaccharide enriched phase. A special case is a segregative interaction of very large polymers and relatively small colloidal sphere.

A mixture of polysaccharides and proteins can also unstable when associative interactions are operational. In that case the polysaccharides adsorb onto the protein surface. If the amount of polymer is not large enough to completely cover protein, a polysaccharides may adsorb onto more than one protein surface (de Kruif et al., 2001)

Phase separation in mixed polymer solution is quite common 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.

The mixing process is spontaneous when changes in Gibbs free energy ($\Delta G_{mix} = \Delta H_{mix} - T\Delta S$) 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.

1.6.3. Associative phase separation

Associative phase separation between proteins and polysaccharides refers to a demixing phenomena induced either by direct interactions between bio-polymers, e.g. electrostatic interactions (the famous complex coacervation phenomenon) or hydrogen bonding, or by bad solvent conditions without requiring the involvement of interactions between molecules.

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 (Doublier et al., 2000).

Electrostatic complex between proteins and anionic polysaccharides generally occurs in the range between the pk value of the anionic groups (carboxyl groups) on the polysaccharide and the protein isoelectric point (IEP). Generally, electrostatic complexes dissociate when the ionic strength exceeds 0.2-0.3, or when the pH value is above the protein IEP. At pH value above the IEP, e.g., at neutral pH, electrostatic interaction may still occur between anionic carboxyl containing polysaccharides and positively charged subunits of oligomeric proteins and between proteins and sulphated polysaccharides.

The process of mutual neutralization of macro- reactants leads to an electrostatically neutral insoluble complex. Electrostatic biopolymer interactions are enhanced with an increase in the net opposite charges of biopolymers and when the ratio of net charges of charges of the polymers reactants approaches unity. In other words the composition of insoluble interbiopolymer complexes tend to satisfy the condition of electrical neutrality. The net charge of anionic polysaccharides decreases

with gradual attachment of each successive protein macro ion during formation of electrostatic complexes. This diminishing net opposite charges, reduces both the hydrophilicity and the solubility of the resultant complex and decreased its IEP compared to that of the initial protein. The higher the relative content of polysaccharide, the lower the pH at which the complex precipitates. The composition of an insoluble inter biopolymer complex usually depends on the pH of the system, but not on the ratio of the biopolymers.

Since the net charges of proteins and anionic polysaccharides are differently changed with pH, the stoichiometry of the complex is greatly changed with pH. When the pH is decreased below the protein's IEP, the net charge of the protein increases, while that of anionic polysaccharide macro-ion decreases. As a result the insoluble complex is enriched with polysaccharide.

Aggregation of neutral insoluble complex particles is mainly due to intermolecular hydrogen bonds and hydrophobic, dipole-to dipole and charge - to dipole interactions. Formation of the concentrated phase of a complex leads to a decrease in electrostatic free energy of the system. The loss of entropy on complexing of rigid polymer molecules may be counter –balanced by the enthalpy contribution from interactions between macro-ions by liberation of counter –ions and water molecules.

Non electrostatic interactions play an important role in composition property relationships of complex coarcevates. In particular, non electrostatic interaction can cause non equilibrium effect in the complexing and can lead to an irreversible complexing. After the precipitation of one of the polymer components remains in solution.

The composition of soluble complexes depend on the initial ration of protein and polysaccharide concentration. Normally, soluble complexes form at low bulk concentrations when the ratio of biopolymer reactant is far from equivalent. Normally, soluble complex are metastable and are aggregated when the concentrations increased. The aggregation of soluble complexes when the concentration is increased. The aggregation of soluble complex can be induced by the addition of a small amount of salt. At high salt concentration these complexes are dissociated.

Since the composition and properties of a complex depend on its formation condition, e.g., on the way of their preparation and the time of aging, inter-biopolymer complexes can be not at equilibrium. Not equilibrium complexes are especially typical of polyelectrolytes with a high charge density (Tolstoguzov et al., 2007).

1.6.3.1. Functional properties of electrostatic complex

Interaction of protein with polysaccharides and of various proteins between each other govern the stability, solubility and co-solubility of biopolymers, their to form viscous solutions and gels and their behaviour at interfaces. Even a small alternation of the between macro-molecules may result in a change in food texture. Functional properties of inter-biopolymer complex differ strongly from those of the macromolecular reactants.

The nature and number of interacting side-groups and size and stability of junction zones determine the stability and functional properties of the interbiopolymer complex, the non equilibrium nature of the complex and dispersed systems stabilizes by the complex.

Since biopolymers macromolecules difference in shape, size, conformation, flexibility and net charge at a given pH and ionic strength, the formation of structurally

regular junction zones in interbioplymer complexes is very unlikely. Formation of interbiopolimers junction zones decreases hydrofilicity and provides a more compact conformation to the complex, increased the critical concentration for gelation and decreases the viscosity of soluble complexs.

In other words, an increase in junction zone size (especially multichain interbiopolymer zone) decreases the solubility of the complex. Macromolecular segments that are not incorporated into the junction zone play a key role in dictating the hydration, solubility, surface activity, and gelation and other functional properties of the complex. Neutral insoluble interbiopolymer complexes may be dissociated in either a salt solution or at alkaline pH to recover and re use the polysaccharide.

Thus, the hydrophilic/hydrophobic character of a complex particle is controlled by the junction zone /polisaccharides chain ratio. An increase in junction zone content can decrease the solubility (dispersibility) and improve the surface actability of functional protein complexes.

The two methods, namely the cross-linking of proteins and anionic polysaccharides by the binding of multivalent cation (such as Ca, Fe, Cu, etc) and thermal denaturation of the bound globular protein can increase the stability of protein-anionic polysaccharide complex. These two treatments can increase the stability of protein –polysaccharide complexes against high ionic strength and pH values above the IEP. This is mainly due to coordinate, hydrogen bonds and hydrophobic interaction. The composition and properties of protein multivalent cation anionic polysaccharide complexes depend on their preparation condition (Tolstoguzov et al., 2007) .

1.6.4. Aggregative phase separation: Thermodynamic incompatibility of proteins and polysaccharides

Grinberg and Tolstoguzov (1997) reported about 100 protein –polisaccharide-water systems, which show that the thermodynamic incompatibility of protein and polysaccharides is a very general phenomenon.

This means that under certain conditions, any protein-polysaccharide-water system is spontaneously demixed into two liquid phases with separation of the protein and the polysaccharide. The conditions necessary for phase separation vary according to the biopolymers. They are dependent on specific structural and compositional features, as well as on the molecular weight and conformation of the biopolymers.

Incompatibility of mixed polymers in solution depends on interactions between the two polymers, and it was measured by the Flory-Huggins interaction parameter $x_{P_1-P_2}$ and interactions of each polymer with the solvent, measured by x_{P_1-S} and by x_{P_2-S}. A positive value for $x_{P_1-P_2}$ is indicative of the exclusion of one polymer from the neighbourhood of the other (net repulsion between the polymers). Clearly, solvent-biopolymer1 (or 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 ($x_{P_1-P_2} < 0$). 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 et al. 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.

Sufficiently concentrated solutions of biopolymers slightly differing in chemical composition and conformation are usually immiscible.

The mixing of proteins and polysaccharides under repulsive conditions often results in phase separation phenomenon. Thermodynamic incompatibility was identified as the driving force of this phenomenon. Resulting systems present two phases enriched in one of the polymer with a partition of solvent between these two phases.

The thermodynamic incompatibility of biopolymers take place when the Gibbs free energy of mixing is positive. Since the mixing entropy 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 then that corresponding to monomers.

The maximal co-solubility of polymers that are required to phase separation. Phase separation threshold for mixture of polysaccharide and for mixture of synthetic are usually below2%. The phase separation threshold exceeds 4% for polysaccharide-globular protein mixture and 12% for mixture of globular proteins.

Incompatibility usually increases with molecular weight and salt concentration. Phase separation threshold in mixed solutions of a large number of biopolymers studied in very sensitive to entropy factors given by the excluded volume of the macromolecules. The phenomenon of incompatibility relates to the occupation of the solution volume by macromolecules and repulsion between unlike macromolecules. The differences in excluded volume effects between incompatible biopolymers and their competition for space determines the critical condition of a system phase separation and contributes to asymmetry of phase diagrams of protein- polysaccharide systems. The water is always is higher in the phase of a more hydrophilic biopolymer with a higher excluded volume effect. The degree of asymmetry of phase diagrams reflects a higher hydrophilicity and larger effective volume of polysaccharide macromolecules compared to compact molecules of globular proteins. Accordingly, a less concentrated phase rich in polysaccharide is usually in equilibrium with a concentrated phase rich in protein.

The symmetry of phase diagrams may be characterized by (i) the ration of critical point coordinates, (ii) the angle made by tie lines with the concentration axis of one of the system components (iii) the length of the bimodal segment between the critical point and phase separation threshold.

Normally, phase separation of a mixed solution is accomplished by a non-equal partition of water between the co-existing phase formed. Usually, a higher concentrated protein-rich phase is in equilibrium with a diluted polysaccharide phase. This phenomenon of water transfer between immiscible water solutions of biopolymers ca be used for the concentration of protein solution. This method is called “membraneless osmosis”

Incompatibility of protein with polysaccharides may result in depletion flocculation of protein particles and protein stabilized dispersions. The phenomenon of depletion flocculation is similar to the phenomenon of limited thermodynamic incompatibility of biopolymer of limited thermodynamic incompatibility of

biopolymers in solutions, the basic difference between the two phenomenona is that depletion flocculation is of a non equilibrium nature.

1.7. Edible film application

Edible film and coating can be used in same process food, minimally processed food, meat food, dried food, nuts and fried foods. These coating can improve the quality and prolong the product shelf life (Mc Hugh et al., 2012).

The use of edible film and coating in food applications and especially highly perishable products, such as horticultural ones, is conditioned by the achievement of diverse characteristic such as cost, availability, functional attributes, mechanical properties (flexibility, tension), optical properties (brightness and opacity), the barrier against gases flow, structural resistance to water and microorganisms and sensory acceptability.

1.7.1. Fresh fruits and vegetables

Since fruits and vegetables consist of living tissue, subsequent physiological and biochemical changes which cause detrimental changes in quality and shelf life of produce are common after harvesting. Respiration, transpiration and ethylene production are main factors contributing to deterioration of fruits and vegetables. Problems mentioned above increase with partial or total loss of skin in minimally processed fruits and vegetables. Skin protects produce against water loss and pathogen invasion and provides partial barrier to gases. Slicing, chopping, and peeling, etc., of fruits and vegetables can cause injury not only to cells immediately exposed by the action, but also to unexposed cells deep within the tissue, increasing extent of damage (Olivas et al., 2009).

Edible coatings improve the quality and extend the shelf life of lightly processed fruit and vegetables by acting as a barrier to water loss and gas exchange, creating a micromodified atmosphere around the product.

In addition, edible coatings can serve as carriers for other generally recognized as safe (GRAS) compounds, such as preservatives and other functional ingredients from natural sources. For example, the addition of a texture enhancer, such as calcium chloride, in an edible coating formulation may enhance fruit quality during storage by maintaining firmness. Furthermore, calcium in the form of calcium ascorbate provides a dual function of cross-linking (from Ca^{++}) and preventing the cut surface from browning (from ascorbate). The incorporation of natural antioxidants, such as ascorbic acid (AA), citric acid, cysteine, and antimicrobials such as lactic acid, acetic acid, can help in reducing enzymatic browning and controlling microbial growth of fresh-cut products.

On a general basis, edible coatings used with fresh-cut products must be transparent, tasteless, and odourless, in addition to containing safe and food-grade substances. They must have an appropriate water vapour permeability (WVP), solute permeability, and selective permeability to gases and volatile compounds. Further, the cost of technology and raw materials from which coatings are made has to be relatively low (Rojas-Grau et al., 2007b; Oms-Oliu et al., 2008, Emmambux et al., 2003; Yaman et al., 2002; Simoes et al., 2009; Dea et al., 2012) .

For minimally processed food, coatings have been formulated from several major chemical classes including lipids, resins, carbohydrates, and proteins. Composite film

can be advantageous because have been also combined the good water barrier properties of lipid coatings and the good gas permeability properties and non greasy texture of polysaccharide or protein coatings (Bai et al., 2012).

Interestingly, fruit and vegetable coatings are generically referred to as “waxes” even though some do not contain any wax components. Most or many commercial coatings are wax or lipid based (Bai et al., 2012).

Generally, lipid-based coatings offer resistance to water vapour and, therefore, reduce water loss from coated fresh produce. This, in turn, reduces weight loss, shriveling, and shrinkage of fruit and vegetable products, which, if left unchecked, render these commodities unmarketable.

These coatings, however, are relatively permeable to gases and, therefore, result in less modification of the fruit internal atmosphere. As a result, they have less of an effect on ripening and senescence. On the other hand, lipid coatings are less likely to result in anaerobic conditions and the accompanying off-flavors (Baldwin et al., 2002).

Polysaccharides show effective gas barrier properties, although they are highly hydrophilic, and they also show high water vapour permeability in comparison with lipid and resin coatings. The polysaccharides used in edible coatings are starch and derivatives, cellulose derivatives, chitosan, gums, pectin, and other compounds (Bai et al., 2012).

Protein coatings, similar to carbohydrates due to their hydrophilic nature, are not effective in reducing water loss. But, certain protein materials such as soy protein and casein, which contain higher levels of hydrophobic amino acids, present more effective moisture barriers, especially in combination with lipids. Protein coatings are more effective for ripening control via creation of a gentle MA. Only the corn protein and zein, can result in a high-gloss appearance that equals that of resin-based coatings (Baldwin et al., 2002; Bai et al., 2012).

1.7.2. Meat food

The aim of any packaging system for fresh muscle foods is to prevent or delay undesiderable change to appearance, flavour, odour, and texture (Gill and Gill, 2005).

Cutter (2006) has analyzed the opportunities of edible films to improve food quality and safety of meat. Films and coatings based on polysaccharides can be used to prolong the shelf life of foods based on meat to prevent dehydration, oxidative rancidity and superficial browning. When applied to products such as meat packaged and subjected to the smoke and steam, the polysaccharide-based film melts and becomes an integral part of the surface of the meat. Meat treated with the film in this way showed a better structure and texture, and have low moisture loss (Yingyuand et al., 2006; Wu et al., 2000).

Fats have been used for coating chicken, shrimp, meat and sausages. Waxes and other fat-based oils have also been added to films based on proteins or polysaccharides to impart the flexibility to improve the characteristics of the coating.

Several studies have demonstrated the benefits of lipid-based coating to preserve the quality of meat-based foods (fish or meat), fresh, frozen or processed. Meat treated with wax exhibits a longer shelf life in refrigerated, reduced drying surface, and retains colour. Despite the advantages of using protein-based films, research has shown that the enzymes associated with muscle (meat) can degrade proteins of the film. In addition, proteins of the film may have health problems, especially for people with food allergies associated with milk proteins, eggs, peanuts, soy, rice or protein.

1.7.3. Fried food

Deep-fat frying is a widely used method for preparing foods with an attractive and tasty surface. The soft, moist interior and the porous, crispy crust increase food palatability. In most industrialized countries, deep-fat frying is one of the most rapidly growing culinary techniques.

One of the main problems associated with fried food is its high oil content; as a result, owing to its association with the high incidence of diseases such as obesity, high cholesterol levels or high blood

pressure, fried food consumption is a cause for concern. Different ingredients have been proved to be effective in reducing the amount of oil absorbed by fried food. Among these, edible coatings based on proteins and other hydrocolloids can play a role in reducing oil absorption during frying (Antonova et al., 2002).

Many factors have been reported as affecting oil uptake, including oil quality, frying temperature and process duration, the product's shape, its moisture, solids, fat or protein contents and porosity, pre-frying treatments (drying, blanching) and coating, among others. Since covering the surface of the food removes the differences in surface properties and also protects it, this is an interesting field of study.

Figure 7 is a schematic diagram representing the moisture and lipid transfer during frying of a product coated with an edible film.

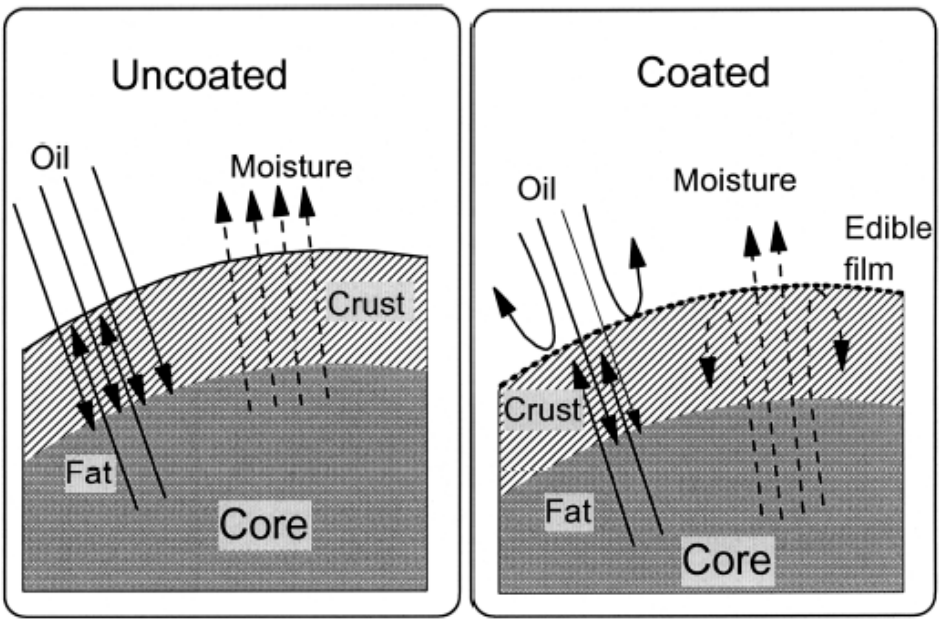

Figure 7. Schematic representation of reduction in oil uptake and of moisture retention during deep-fat frying of a food coated with an edible film (from Antonova et al., 2002).

The coating has to be designed to minimize water loss, thus preventing oil from entering. The crust may act as a diffusion barrier that limits mass transfer, but inner moisture converted to steam may find selective channels in the structure and escape through open capillaries, pores, and crevasses, and oil may enter the voids left by the water (Valera et al., 2011 Albert et al., 2002; Williams et al., 1999;).

1.8. Film and coating as carrier of active substances

Edible films and coatings have received increasing interest because films and coatings can carry a diversity of functional ingredients. It is possible to incorporate antioxidants, antimicrobials, nutraceuticals, flavors, and color agents to enhancing food quality, stability, and safety (Avena-Bustillos and McHugh, 2012).

The term “active packaging” may be defined as packaging which performs some desiderate function other than merely providing a barrier to the external environment. The active packaging solutions interact consistently and actively with the atmosphere inside a package, changing the qualitative and quantitative, or directly with the product it contains, through the release of useful substances to improve their quality or by the seizure of unwanted substances. The functions that can have an active packaging are antimicrobial functions, O_2 scavenger, absorbing moisture, or ethanol.

Generally, antimicrobial agents control the deterioration of foods and the growth of pathogenic microorganisms.

The non-edible packaging can contain any kind of preservatives in their food packaging materials to create an antimicrobial activity. They may contain organic acids and their salts, fungicides, bacteriocins, antibiotics, enzymes, alcohols, thiols, antioxidants, or gas.

In the case of edible films and coatings, the choice of antimicrobial agents is limited to edible compounds because they must be consumed together with the film or coating, and food, so their edibility and safety are essential.

Antimicrobial agents that can be incorporated into edible films and coatings are organic acids and their salts, such as benzoic acid, sodium benzoate, sorbic acid, potassium sorbate, propionic acid, lactic acid and acetic acid (Han, 2002). Recent studies had demonstrated that also essential oil have antimicrobial activities against alterative and some pathogens microorganism (Burt, 2004; Bakkali, 2008).

Antioxidants can be added into the coating matrix to protect against oxidative rancidity, degradation, and discoloration of certain foods. For examples, nuts were coated with pectinate, pectate, and zein coatings containing BHA, BHT, and citric acid to prevent rancidity and maintain their texture.

Edible films and coatings are excellent vehicles to enhance the nutritional value of fruits and vegetables by delivering basic nutrients and nutraceuticals that are lacking or are present in only low quantity in fruits and vegetables. These substances are calcium, zinc, vitamin E, and beta carotene (Avena-Bustillos and McHugh, 2012).

Among the factors to consider in designing antimicrobial edible film and coating, was need to controller release of these substances. Figure 8, shows the structure of edible film and coating systems.

Packaged food may be contaminated by microorganisms before packaging or by post-process contamination after the sealed package is opened. Therefore, surface contamination is the most probable and needs to be prevented. Contaminating microorganisms will locate themselves on the food surface, in the area between the package and the food. In the case of edible coating systems, they can completely cover the food. However, the food surface may potentially get contaminated before the coating process. Microorganisms positioned between the coating layer and the food product may not become active and grow due to lack of oxygen and to direct contact with the antimicrobial agents. Therefore, coated foods are most likely to become contaminated on the external coated surface where microorganisms will position themselves and start to grow. In both film and coating systems, the food layers that do not contain antimicrobial agents initially have very large volume compared to the

volume of the thin films or coatings. Because of the almost infinite volume of the food layer compared to the film/coating layer and the migration of antimicrobial agents from the films/coatings into the food, the concentration of the antimicrobials in the edible film and coating layers will be reduced. Eventually the antimicrobials will be depleted from the edible films/coatings. Therefore, the release rate must be controlled to prevent the early depletion of antimicrobial agents due to fast migration.

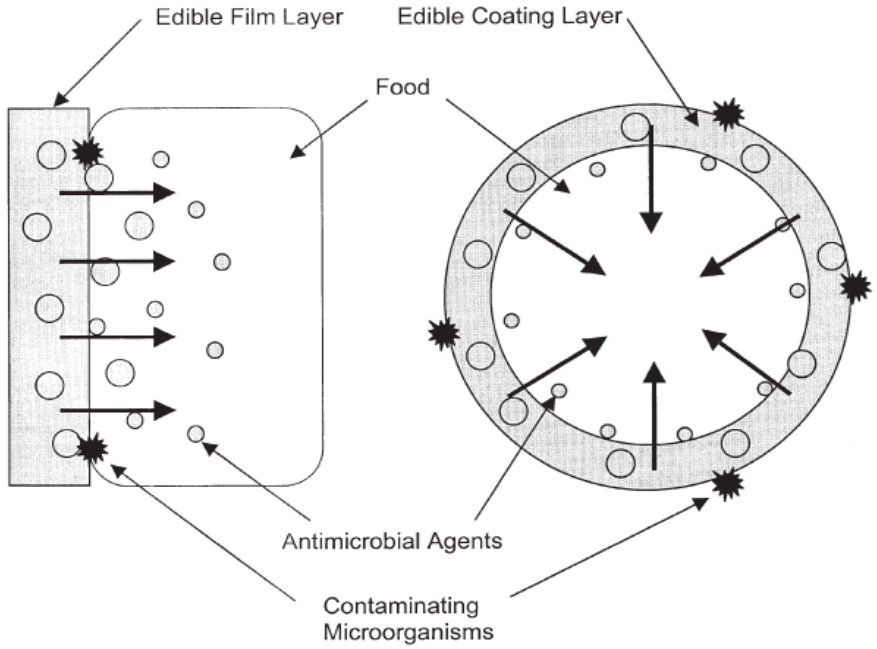

Figure 8. Structure of edible film and coating systems and migration of antimicrobial agents in food (From Han, 2002)

Therefore, figure 8 suggests that the two systems (the film system and the coating system) should have different protective functional designs. In the film system, the incorporated antimicrobials should migrate slowly from the film layer into the food, thereby acting against contaminating microorganisms. In contrast, in the coating system, the antimicrobial agents must remain in the coating layer to protect the food product from invasion of contaminating microorganisms. Controlled release of the antimicrobials with an intermediate diffusion rate is expected to achieve effective antimicrobial activity at the food surface in a film system. In a coating system, preserved high concentration of the antimicrobials is required with a very slow diffusion rate to maintain the efficiency of the antimicrobial functions against spoilage and pathogenic microorganisms.

A mass transfer model of the migration phenomena can be used to describe the concentration profile in the film/coating layer and food over time. Figure 8 shows that a two-layer diffusion model can represent both the film and coating systems. When volatile antimicrobial agents are incorporated, they can evaporate out from the system to the surrounding environment. Most non-volatile antimicrobial agents would penetrate into the food layer during storage and distribution (Han 2002).

Controlled release and mass transfer models have been proposed. That may be used to describe the migration of antimicrobial agents through food packaging systems

consisting of single, double, or triple layers (Han 2000; Mastromatteo et al., 2009; Flores et al., 2007; Guillard et al., 2009).

Because the mass transfer model describes the relationship between concentration and time, it allows for calculating the storage period that maintains the antimicrobial concentration above the critical inhibitory concentration and permits the estimation of the microbiologically safe shelf life.

1.9. References

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2. Aim of thesis

In recent years the concept of using edible film and coating to extend the shelf-life of food has been increased. The latest regulations concerning the production and disposal of packaging (94/62 EC, 1935/2004 EC, 87/2007 EC) have directed research towards the study of renewable raw materials. Furthermore, much interest has been placed on the use of natural ingredients that can replace some chemical traditional components.

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.

For these reasons it is interesting to investigate different matrices, parameters and factors that may influence the properties of the films in order to optimize the performance of edible films and coatings and to understand the relationships between structure and properties.

The aim of this work was to investigate the effect of compounds on structure and functional properties of hydrocolloids based films. The results will help to develop a film or coating system 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.

The objective of the first part of the work was to review the results on the main polymer used to produce edible film and coating, with the objective of studying the knowledge's on the edible film properties and structure-functional properties relation.

Then, the objective of the work was to study the effect of constituents on the structure and properties of films based on proteins and polysaccharides. In particular the work was organized in different cases studied in order to investigate different aspects of the problem:

I Study case: Effect of rosemary oil on functional properties of hydroxyl propyl methyl cellulose films.

II Study Case: Structure and properties of Hydroxy propyl methyl cellulose-sodium caseinate film cross-linked by Transglutaminase.

III Study Case: Food application of sodium caseinate cross linked with trasglutaminase edible film: oil absorption reduction on French fried potatoes.

PART II

1. I STUDY CASE

Effect of rosemary oil on functional properties of hydroxyl propyl methyl cellulose films

ABSTRACT

Edible films based on hydroxyl propyl methylcellulose (HPMC) obtained with different concentrations of rosemary oil were prepared. In order to study the impact of the incorporation of rosemary oil into the HPMC matrix, mechanical properties, water vapour permeability (WVP) and microscopy analysis were evaluated. Results showed that the different amount of rosemary oil did not have a significant effect on mechanical properties at small and large deformation. For film at 6% of HPMC, at concentration of oil equivalent to 0.4% a reduction of WVP was achieved.

1.1. Introduction

Active packaging technologies involve interactions between the food and the packaging material to extend the shelf life of foods while maintaining their quality and safety. These materials are designed to deliberately incorporate 'active' components intended to be released into the food or to absorb substances from the food (Regulation (EC) No 1935/2004).

A variety of antimicrobial agents have traditionally been used for food preservation and may be added to the film materials as "active" components to provide antimicrobial functions. In the last years, the demand for replacing synthetic chemicals with natural compounds it is increased. In this context, the essential oils (EOs) are interesting for their potential use as natural preservatives.

EOs are natural complex compounds characterized by a strong odour and they are formed by aromatic plants as secondary metabolites. They can be synthesized by all plant organs, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretors cells, cavities, canals, epidemics cells or glandular trichomes. They are liquid, volatile, limpid and rarely coloured, soluble in organic solvents with a generally lower density than that of water. Since ancient time known for their antiseptic, i.e. bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they has been used in preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anaesthetic remedies. Nowadays, more is now known about some of their mechanisms of action, particularly at the antimicrobial level. They can contain about 20–60 components at quite different concentrations, but are characterized by two or three major components at fairly high concentrations (20-70%) compared to others components present in trace amounts. The main group is composed of terpenes and terpenoids and the others of aromatic and aliphatic constituents, all characterized by low molecular weight.

To measure the antimicrobial activity of essential oils, in the literature usually defined the MIC and MBC. MIC is defined as the minimum inhibitory concentration of EOs, which allows a reduction of microbial population growth; MBC is defined as minimum bactericidal concentration, which allows a reduction in the number of microbial cells at least 99%. The in vitro efficacy of EOs against food borne pathogens and spoilage bacteria and their action mechanism has been extensively reviewed. Among the most common oils that have been proven antimicrobial properties against

spoilage microorganisms the main are Cilantro oil, Coriander oil, Oregano oil, Rosemary oil, Sage oil, Clove (bud) oil and Thyme oil (Burt, 2004; Bakkali, 2008).

Among essential oils, rosemary oil shows a strong antimicrobial action against spoilage and pathogenic microorganisms and in addition it shows also antioxidant properties (Fernandez-Lopez et al. 2005; Estevez et al., 2007; Fu et al., 2007).

Edible films and coatings may carry antioxidants, antimicrobial agents, colorants, flavours, fortified nutrients, and/or spices. Recently, it is increasing the interest in using these molecules as active compound in edible film or coating to preserve and prolong the shelf life of food as meat and fish and minimally processed fruit (Lanciotti et al., 2004; Gutierrez et al., 2008; Raybaudi-Massilia et al., 2008; Rajas-Grau et al., 2006; Valencia-Chamorro et al., 2009; Villalobos-Carvajala et al., 2009; Oussalah et al., 2004). The only limitation in using edible film or coating as carrier material is that incorporated antimicrobial agents must be selected among edible compounds (Han et al., 2002; Sanchez-Gonzales et al., 2011)

Polysaccharides have been extensively used as film forming solution for edible films or coating. Cellulose is probably the most abundant organic substance existing in nature and is the major constituent of most land plants. HPMC is a macromolecule water-soluble, non-ionic, which is able to form gels upon heating (Yoguchi et al., 1995). Residual group along the backbone of the polymer chains can be hydrophobic in nature, like methoxyl groups, and other portions ban be hydrophilic in nature, being full of hydroxypropyl groups. Thus, the properties of this polysaccharide depend of the nature of the substitution groups, the degree of substitution (DS) and the distribution of the substitution groups. Derivatives with DS below 0.1 are generally insoluble, but if the DS is increased up to 0.2–0.5 (depending on the type of substituting group) the product becomes soluble in aqueous alkali (Richardson & Gorton, 2003).

Hydroxypropyl methylcellulose (HPMC) yields films that are flexible, odourless, tasteless, water soluble, and resistant to oils and fats, and present good oxygen and aroma barrier properties (Miller & Krochta, 1997). However, their hydrophilic nature makes them rather ineffective moisture barriers.

Several lipids have been tested as depressors of water vapour permeability in composite films based on polysaccharides. In this respect, the incorporation of plant extracts to composite films represents an interesting alternative. Like other lipids, essential oils (EO) may improve the water barrier properties of the films, because of their hydrophobic nature, (Sanchez_Gonzales et al., 2009; Atares et al., 2010a; Atares et al., 2010b; Lim et al., 2010; Mahdi Ojagh et al., 2010). However, functional properties of edible film depends on the type of constituents and also on their interaction (Phan The et al., 2002; Phan The et al., 2009; Giancone et al., 2011). Regarding the role of EOs on film functional properties the results reported on literature are still contradictory (Sanchez Gonzales et al., 2011; Du et al., 2009; Du et al., 2010; Hosseini et al., 2009). Few works have been focused on the effect of rosemary oil on functional properties of polysaccharides based edible films. Thus, the objective of this work was to study the effect of rosemary oil on the functional properties of HPMC edible films.

1.2. Materials and Methods

1.2.1. Materials

Hydroxypropyl methyl cellulose (HPMC) (1.8-2.0 methyl substitution (DS); 0.20-0.3 hydroxypropyl substitution (MS)), rosemary essential oil (*Rosemary officinalis*) and Tween 80 were purchased by Sigma Aldrich (Milan, Italy).

1.2.2. Film making procedure

HPMC film were prepared by dissolving 2%, 4% and 6% of HMPC powder in deionised water at room temperature for all night. Rosemary oil- and Tween 80 were emulsified, at 6:1 ratio rosemary oil/tween 80, by using a vortex (IKA MS 3 Digital) for 5 min at 2000rpm. Different concentration of rosemary oil (0.0, 0.4; 0.7; 1, 2%) were added to the HPMC solutions at 2% and 6%. This solutions were emulsified by using a Blender (Osterizer) to maximum power for 15 minutes.

Prior to film casting, solutions were de-aerated under vacuum to prevent pinhole formation. 20 ml of film forming solutions were poured onto levelled 56.7 cm^2 polystyrene Petri dishes and allowed to dry at 20 °C and 50% relative humidity (RH) for 48h under air circulation. The dried films were peeled from the Petri dishes and stored at 20°C and 50% relative humidity prior to testing.

1.2.3. Film thickness measurement

Film thickness was measured using a micrometer model HO62 with a 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 instrument feel in contact with the film. Mean thickness (µm) of films was determined by averaging 10 measurements at different locations.

1.2.4. Scanning electron microscopy analysis

Microstructural characteristics of film samples were examined using an LEO EVO 40 scanning electron microscope (Zeiss, Oberkochen, Germany). All film samples were dried in a desiccator containing lithium chloride (a_w=0.113±0.003) and then manually fragmented. Dried strip fragments of films were mounted on specimen stubs with the cross-section oriented up and coated with a thin layer of gold by a DC sputter coater (AGAR B7340, Agar Scientific Ltd, Stansted, UK). Digital images of film cross-section were collected at a tilt angle of 0° to the electron beam using an acceleration voltage of 20 kV.

1.2.5 Dynamical mechanical measurement

Dynamical 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 strain sweep tests at an angular frequency (ω) of 1 rad s^{-1}. Then, all the films were submitted to frequency sweep tests by increasing ω from 10^{-2} to 10^{3} rad s^{-1} under a constant strain amplitude (ε) of 0.01% (within the linear viscoelastic region) to monitor the storage (E') and the loss (E'') tensile moduli.

1.2.6. Film mechanical properties

The tensile strength of the films was measured by using an Instron Universal Testing Machine (Instron Ltd., Model 4467, High Wycombe, GB) equipped with a 1,000-N load cell. Film samples were cut into 25 wide and 100 mm length strips using a sharp razor blade. The strips were equilibrated overnight at 50±5% RH and 23±2 °C in an environmental chamber. Ten samples of each film type were tested. Tensile properties of the films were measured according to the ASTM (1991) Standard Method D882 using Test Method A, the StaticWeighing, Constant Rate-of-Grip separation test. The initial grip separation was 50 mm and crosshead speed was 15 mm/min in a Tension Mode. Tensile strength (TS) and percent elongation at break (ε%), Young modulus (EM) were calculated. Results are reported as average of ten replications of each sample.

1.2.7. Water vapour permeability

Water vapour permeability (WVP) of films was evaluated by gravimetric test according to ASTM E96 (1993) by means of a Fisher or Payne permeability Cup (Carlo Erba, Milan, Italy). Eight grams of silica gel were introduced in each cup. Film sample having diameter of about 6 cm was placed 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 desiccators containing a saturated KCl solution which provided a constant water activity of 0.8434 at 25 °C. The desiccators was stored in a Heareus thermostated incubator (Binder KBF240, Turin, Italy) at 25.0 (±0.1 °C). Cups were weighed at scheduled times, and the amount of water vapour transmission rate (WVTR) 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 $R^2 \geq 0.998$.

From WVTR data, the value of vapour pressure on film's inner surface (p_2) was obtained taking into account the method proposed by Mc Hugh et al. (1993) to correct the effect of concentration gradients established in the stagnant air gap inside the cup.

$$WVTR = \frac{P \cdot D \cdot Ln\left[\frac{P - P_2}{P - P_1}\right]}{R \cdot T \cdot \Delta z} \qquad (1.1)$$

Where, P is the total pressure; D is the diffusivity of water through air at 25°C; R is the gas law constant; T is the absolute temperature; Δz is the mean stagnant air gap height, considering the initial and final z value; P_1 is the water vapour pressure on the

solution surface; P_2 is the corrected water vapour pressure on the film's inner surface in the cup. Then permeance was calculates as followed:

$$Permeance = \frac{WVTR}{(P_2 - P_3)} \quad (1.2)$$

Where P_3 is the water vapour partial pressure at the film outer surface. Permeability was obtained by multiplying the permeance by the average film thickness. Results are reported as average of three replications of each sample.

1.2.8. Statistical analysis

To assess the effect of HPMC concentration and essential oil concentration on the functional properties of HPMC-based films, three HPMC concentration levels (2%, 4%, 6%) and 5 oil concentration level (0%, 0.4%, 0.7%, 1%, 2%) were tested, each level being replicated three times. The reliance of HPMC and oil concentration on film functional properties was assessed by ANOVA analysis by using SPSS 13.0 for window (SPSS, Milan, Italy). Duncan's test was carried out to find the source of the significant differences within the samples examined. Significance of differences was defined at $p \leq 0.05$.

1.3. Results and Discussions

1.3.1. Thickness

Thickness of HPMC film is reported in Figure 1. By increasing the HPMC concentration from 2% to 6%, the film thickness increased from 0.062 ±0.005 mm to 0.23 ± 0.01 mm. ANOVA highlighted a significant effect of HPMC concentration on film thickness ($p<0.01$), and results showed that the thickness increased linearly with HPMC concentration ($R^2>0.999$). As reported for pectin film (Giancone et al., 2010), an increase in HPMC concentration lead to a denser structure which can justify the increment of thickness.

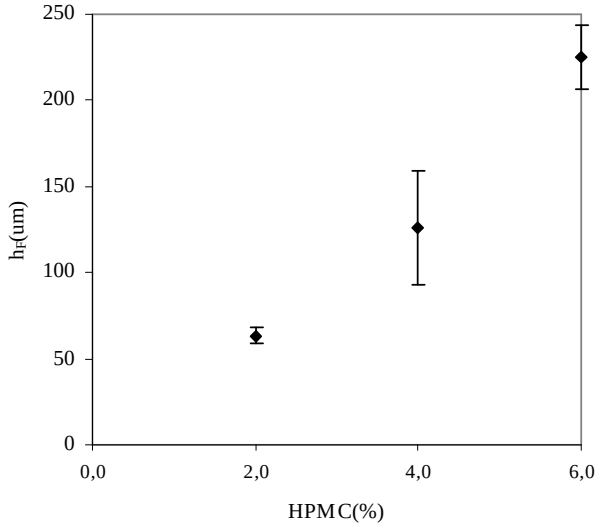


Figure *1*. thickness (h_F) of HPMC film at different concentration at 20°C.

The addition of rosemary oil to HPMC films did not have a significant effect on the thickness of the film ($p>0.05$). This result were in accordance with Du et al., (2010) who reported that the thickness of tomato pectin films does not increase in presence of oregano oil, garlic and allspiece up to 3%. As reported by Sanchez-Gonzales (2011a) a possible losses of oil could occur during film drying which can reduce the total amount of solids contributing to the film thickness. However, the effect of EOs on thickness is reported to be a function of the kind of oil and its concentration. In fact, in the case of apple and pectin edible film it was showed that the thickness increased in presence of clove bud oil at a minimum concentration of 3%, whereas in the case of cinnamono and allspice oil the thickness increased for 1% of oil (Du et al., 2009).

1.3.2. Scanning electron microscopy analysis

Characteristic SEM images of cross sections of the HPMC films at different concentration (2%-4%-6%) was shown in Fig. 2. A continue structure was observed for HPMC film at different concentration HPMC.

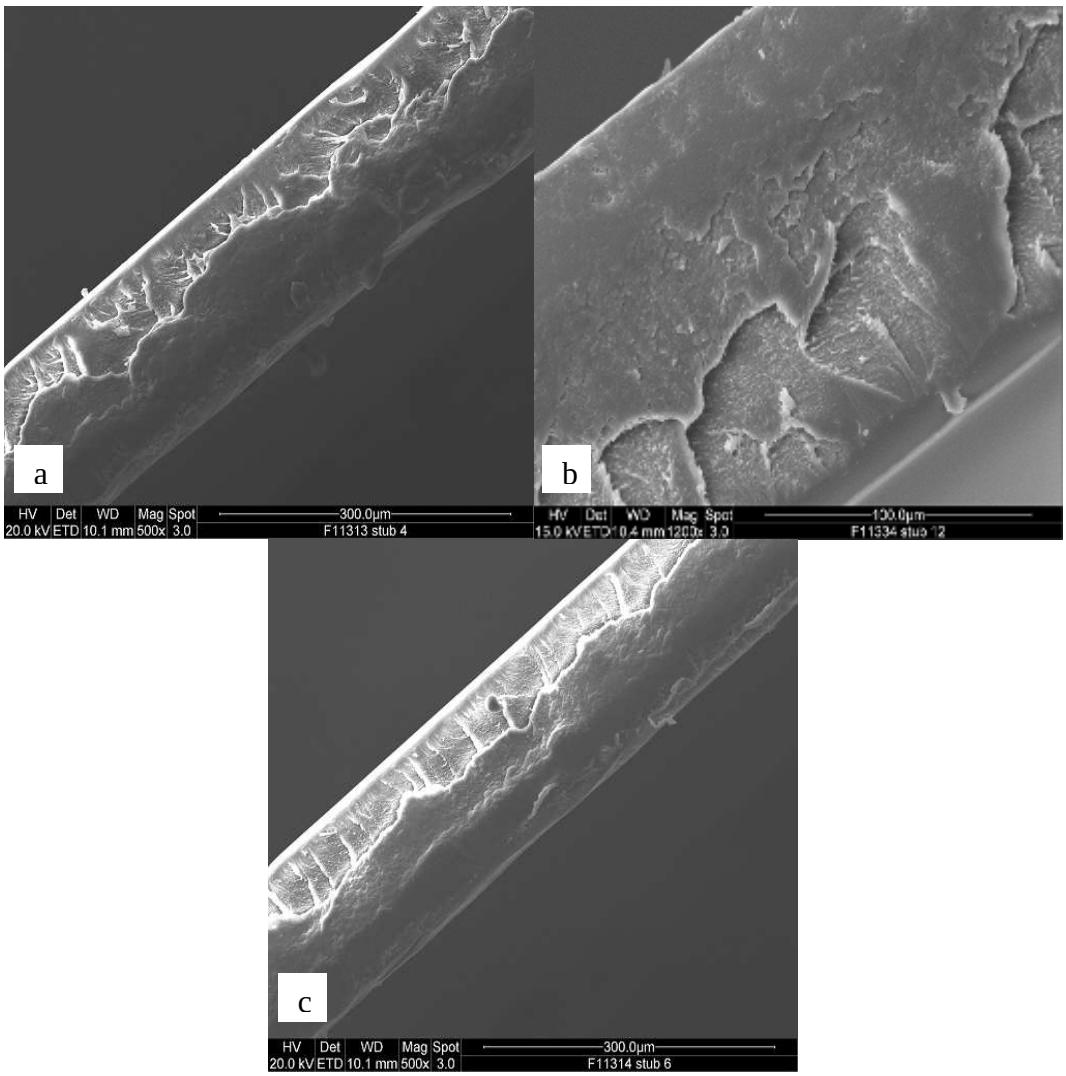


Figure 2. SEM micrographs of HPMC films with different HPMC content: (a) 2% magnification 500x (b) 4% magnification 1200x, (c) 6% magnification 500x.

Figure 3 and 4 show SEM micrographs of the cross-sections of the 2% and 6% HPMC films at different oil concentration.

The final structure after drying was influenced by the structural arrangement of the different components (HPMC, Tween 80 and rosemary oil) in the initial dispersion, and their development during the drying process when droplet flocculation, coalescence and creaming can occur (Vargas eta l., 2009; Sanchez Gonzales et al., 2009)

For 2% HPMC films at different concentration (Fig. 3), the presence of rosemary oil caused discontinuities due to lipid droplets, which appeared, in some cases, as voids due to the fact that droplets remain in the non-observed part of the film during fracture. One can observe that the droplets oil was not well embedded into the HPMC matrix and the creaming phenomena involved. Probably, most oil had migrated toward the evaporation surface. This phenomena was most evident to maximum oil concentration (figure 2d). These results was in accordance with Phan The et al., (2002) who reported that drying condition (temperature and air speed) influenced film structure and in particular air speed contributes to the destabilization of film-forming emulsion.

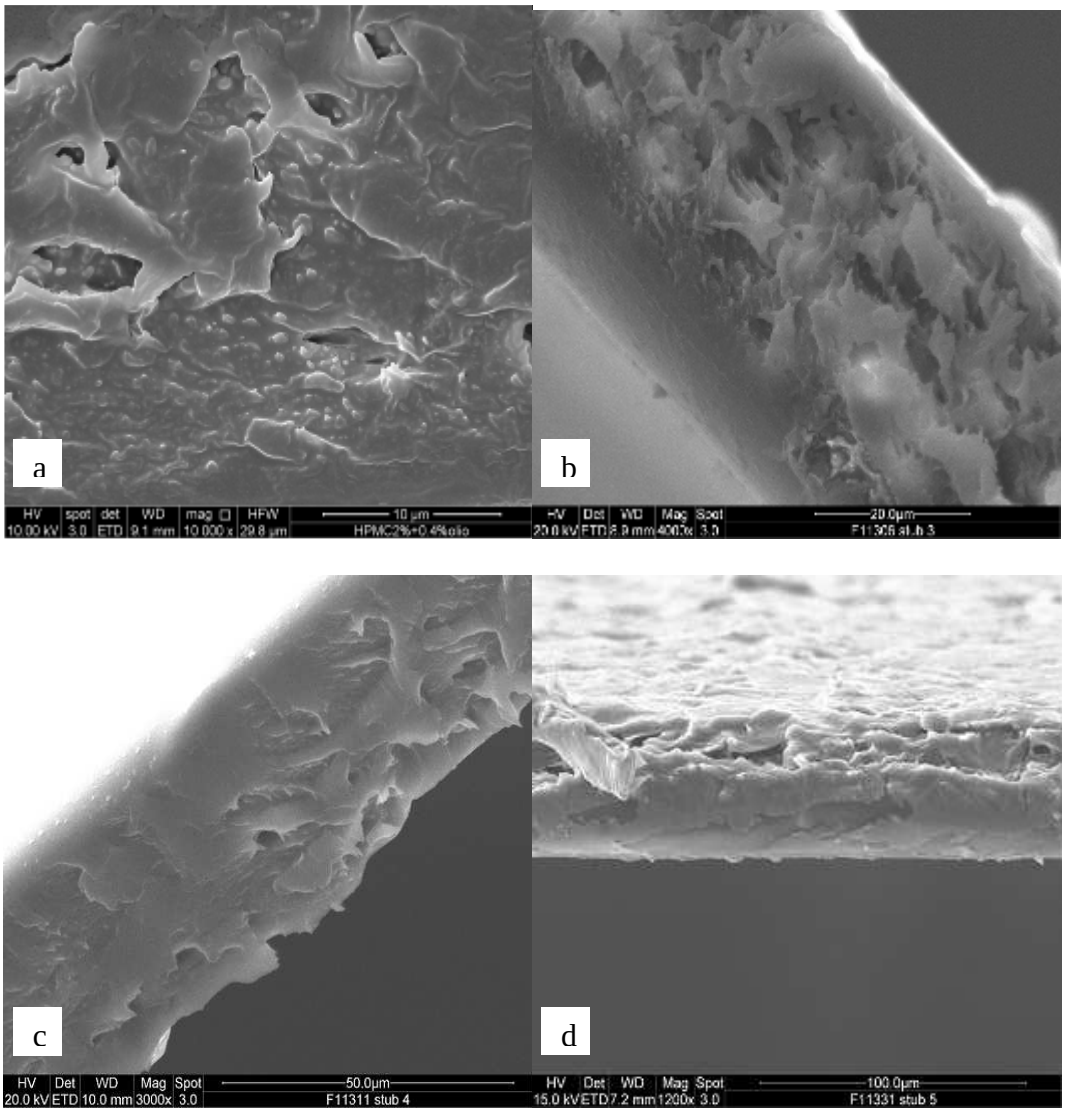


Figure 3. SEM micrographs of 2%HPMC film at 0.4% oil (a) magnification 10000x, 0.7% (b) magnification 4000x, 1% (c) magnification 3000x and 2% oil (d) magnification 1200x.

Figure 4 show 6% HPMC films cross section at different concentration of rosemary oil. In a different way respect 2%HPMC films, its seems that droplets oil were better homogenously distributed across the film. This can be an indication that destabilization phenomena, like creaming did not occurred or occurred slowly during the film drying, probably due to the increased viscosity of the solution of HPMC at 6% compared to 2%.

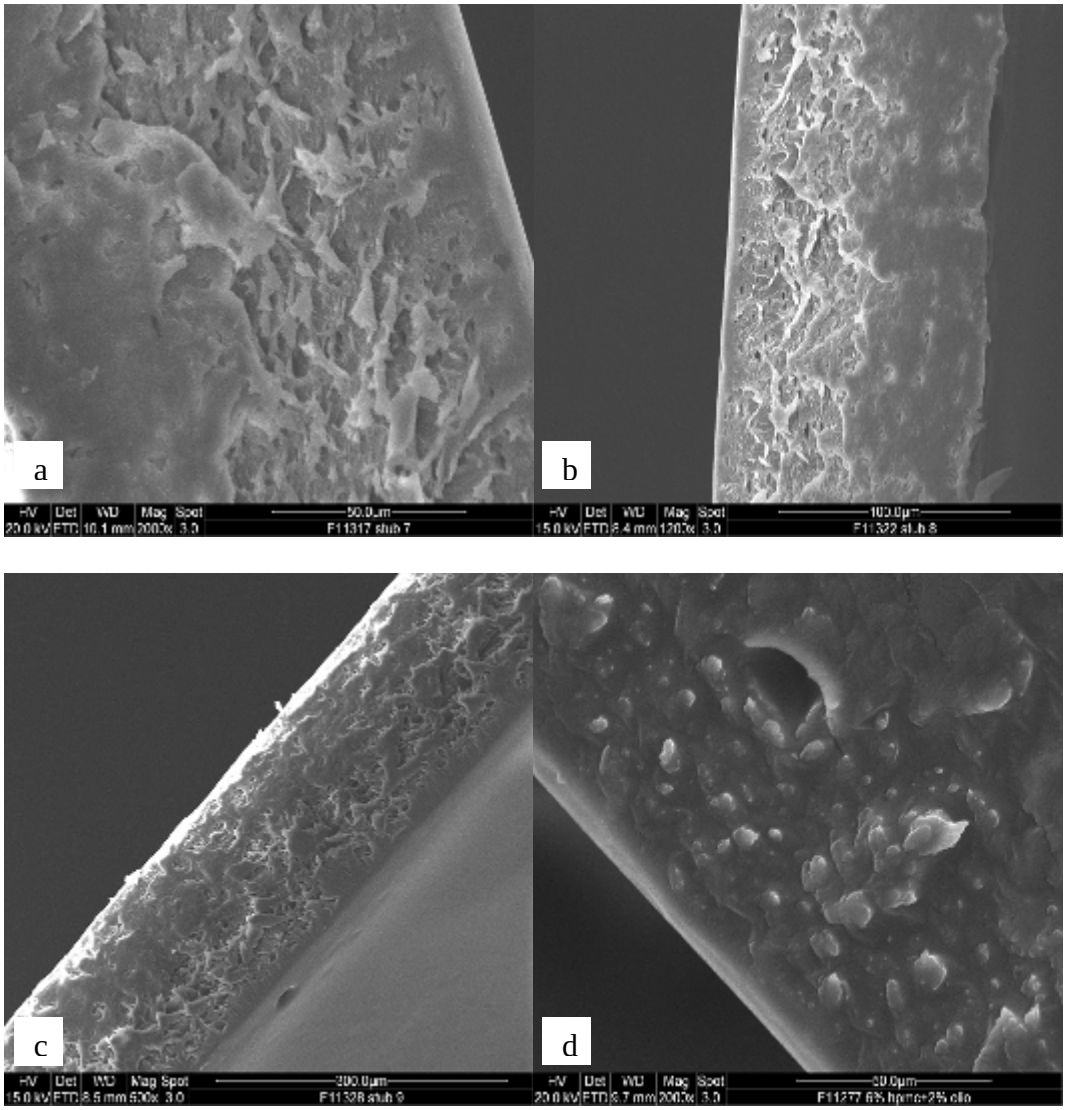


Figure 4. SEM micrographs of 6% HPMC film at 0.4% oil magnification 2000x (a), 0.7% magnification 1200x(b), 1% magnification 500x (c) and 2% oil magnification 2000x (d).

1.3.3. Dynamical mechanical measurement

Figure 5 (a-b) shows the dependence of storage Young modulus (E') and loss tangent (tanδ) on angular frequency (ω) for HPMC film at different HPMC concentrations (% w/v).

As ω was increased from 0.01 to 1000 rad s^{-1}, E' was found to be practically constant for HPMC at 6%, whereas a slight dependence from ω was observed for HPMC films at 2% and 4% (figure 5a).

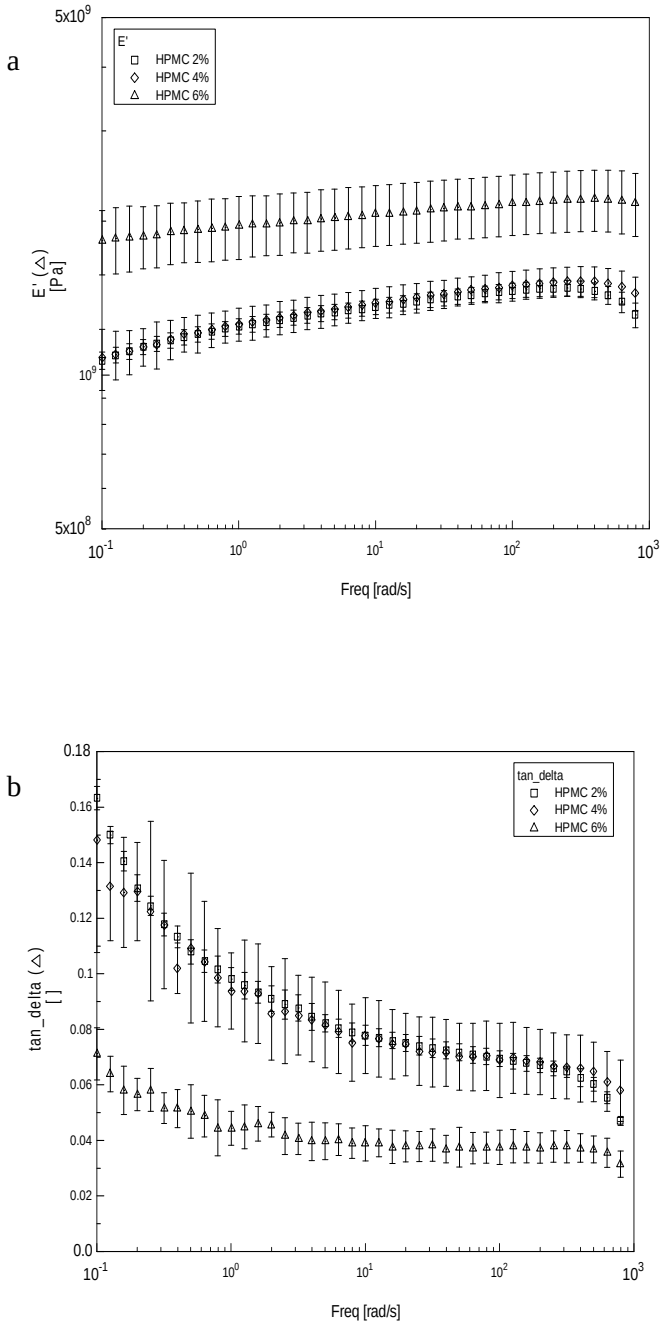


Figure 5. Storage tensile modulus (E') (a) and loss tangent (tanδ) (b) versus angular frequency (ω) for HPMC films at different HPMC concentrations.

The low dependence of E' on ω showed that HPMC films acted as solid-like materials. All over the frequency range explored, the loss Young modulus (E'') was always lower that E' (data not shown).

Decreasing HPMC from 6% to 4%, E' decreased approximately by two order of magnitude, but no differences were observed between films at 4% and 2%. Thus, results revealed that mechanical properties depend upon HPMC concentration, but not linearly. Different results were reported by pectin film at different pectin surface content for which it was found that E' Young modulus was nearly independent of pectin surface content (Giancone et al., 2008).

The loss tangent values, being a measure of the ratio between the energy lost and that stored throughout any deformation cycle, were less than one and decrease as ω increased from 0.01 to 1000 rad s^{-1}. It dependence of ω was higher for film at 2% and 4% of HPMC (figure 2b).

Due to the dependence of the mechanical behavior from HPMC concentration, the influence of oil concentration was studied on HPMC films at 2% and 6% (Figg.6 and 7).

For films at 2% of HPMC, in presence of oil at concentration up to 1%, E' modulus showed a slight lower dependence on ω at low frequency and an higher value of E' as the oil concentration increased (figure 3a). However, film at 2% of oil behaved as the control samples obtained in absence of oil. Moreover, statistical analysis performed on E' at 1 rad s^{-1} highlighted that the oil concentration did not have a statistically effect on E' modulus (p<0-05).

In terms of loss tangent, it can be observed that the films at 2% of oil showed a higher values all over the ω range tested (Figure 6b). From the above results it is not possible to exclude an effect of the oil concentration on the mechanical behavior of the films, but its effect depends on oil concentration.

Different results were obtained for films at 6% of HPMC. First, it must be highlighted that for these films an higher variability of the data was measured respect to film at 2% of HPMC, thus from a statistically point of view not significant differences can be highlighted among samples at different oil concentration. However, for HPMC at 6%, the presence of the oil caused a decrease of the E' of almost one order of magnitude, with the only exception of the samples at 0.7% of oil that showed a behavior similar to control samples (figure 7a). Whereas, loss tangent was slight affected by the presence of oil (figure 7b).

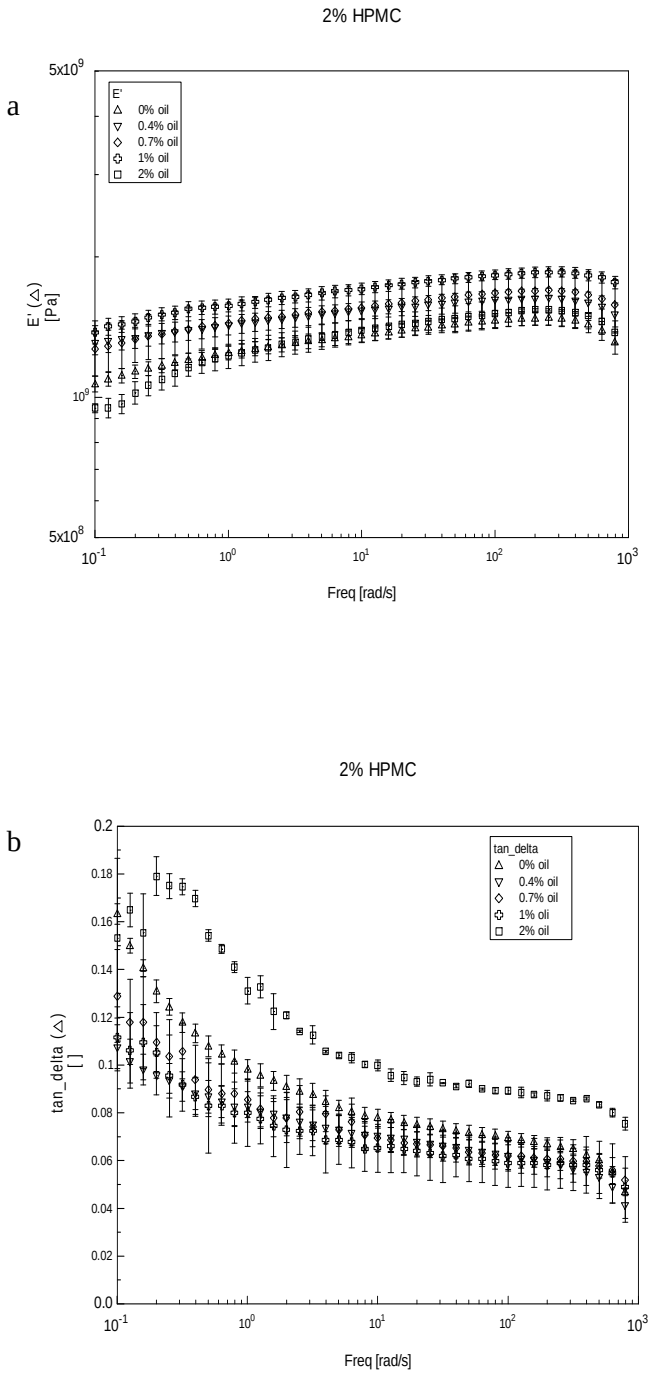
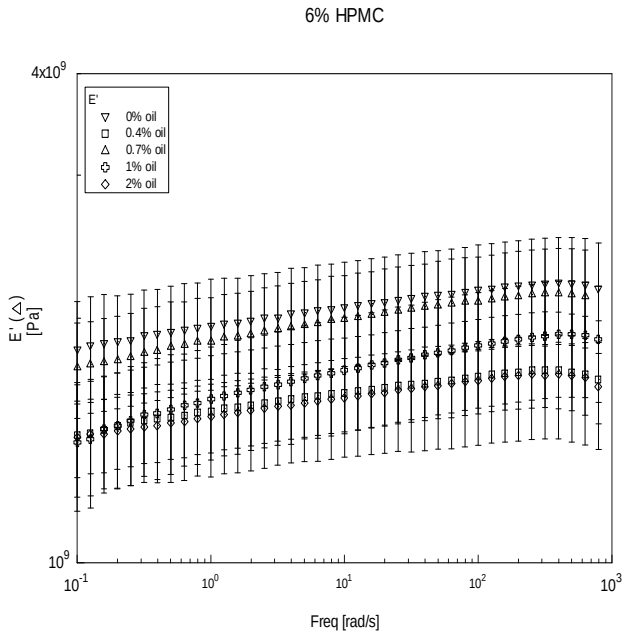


Figure 6. Storage tensile modulus (E') (a) and loss tangent (tanδ) (b) versus angular frequency (ω) for 2% HPMC films at different oil concentrations.

a



b

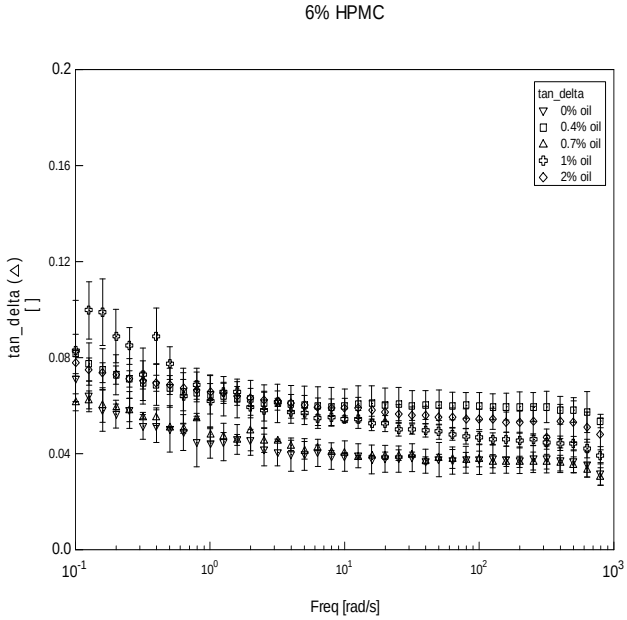


Figure 7. Storage tensile modulus (E') (A) and loss tangent (tanδ) (B) versus angular frequency (ω) for 6% HPMC films at different oil concentrations.

1.3.4.Mechanical properties

The typical mechanical behaviour of the films at high deformation is shown in figure 8 in terms of true stress- Hencky strain curves. From these curves, mechanical parameters: elastic modulus (E), tensile strength (TS) and elongation to break (ε%) were obtained (figure 9).

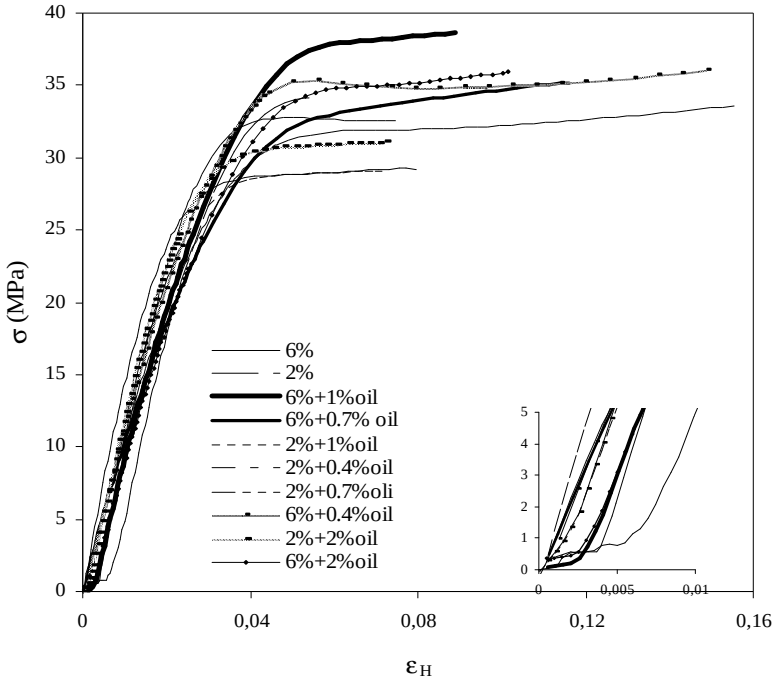


Figure 8. Typical true stress (σ) vs. Hencky strain (ε_H) curves obtained in tensile test carried out on HPMC film at different oil concentration. (2% HPMC: dotted lines; 6% HPMC: solids lines).

By increasing the HPMC concentration from 2% to 6% a reduction of the EM (MPa) and an increment of the **ε** (%) was observed. On the contrary, the addition of the rosemary essential oil did not affected the mechanical properties of the film, with the exception of the ε% of film at 6% of HPMC which is reduced at high oil concentration.

This result was in accordance with (Sanchez Gonzales et al., 2009; Pranato et al., 2005; Mahdi Ojagh et al., 2010; Du et al., 2009; Du et al., 20010; Seydim et al., 2006). However, literature provides evidence of very diverse effects of lipid addition on mechanical parameters. In some of this studies (Bonilla et al., 2012; Fabra et al., 2008; Vargas et al., 2009),when a essential oil was added a decreased of tensile to break (TS), elastic modulus (EM) and increase of elongation to break (ε%) was observed, depending of oil content.

When a hydrophobic substance is added, it can cause the interrupt of the polymer matrix with a reduce cohesion matrix (Vargas et al., 2011; Fabra et al., 2008; Sanchez Gonzales et al., 2009; Bonilla et al., 2012; Atares et al., 2010a; Atares et al., 2010b; .Benavides et al., 2012; Hosseini et al., 2009).

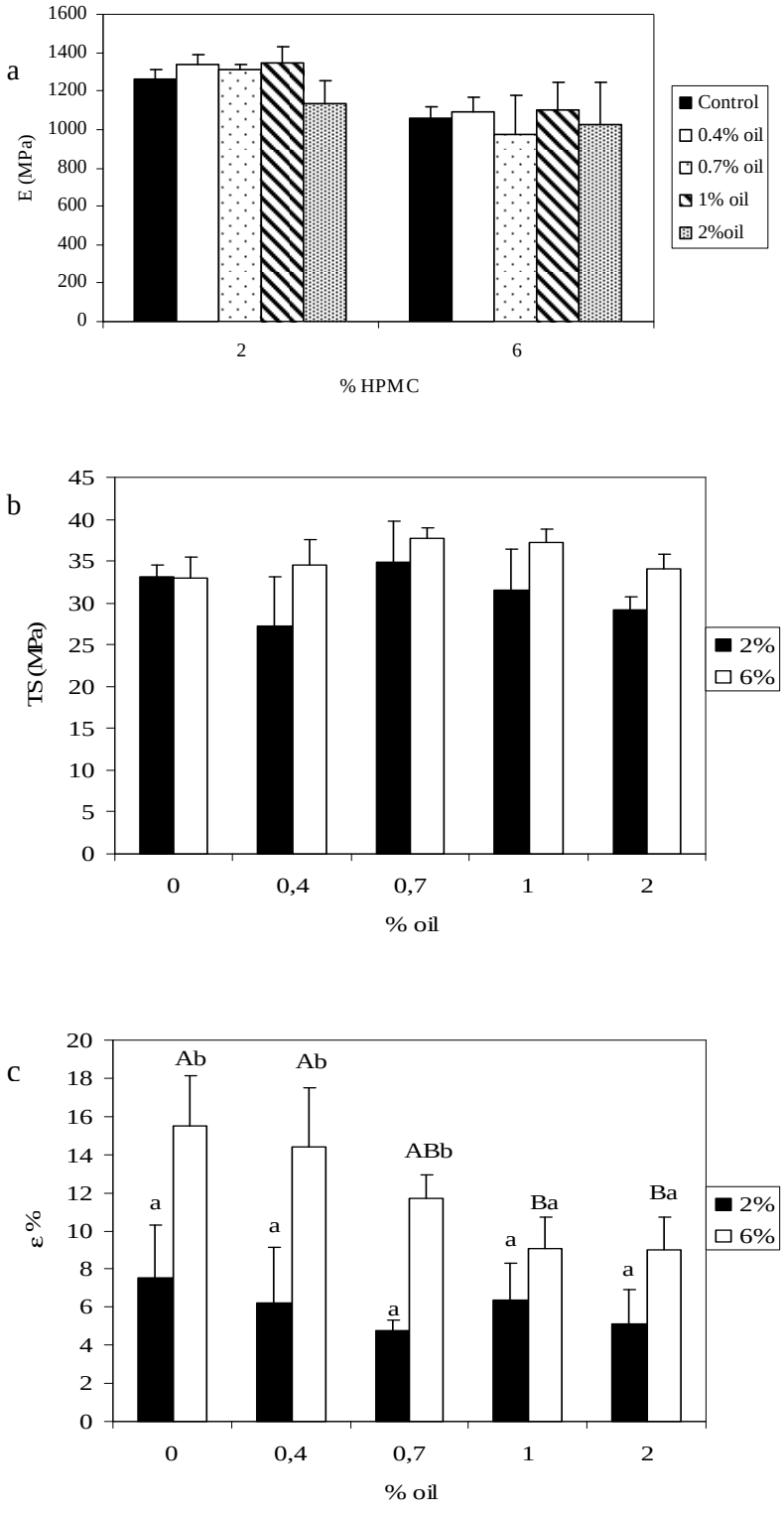


Figure 9. (a): Elastic Modulus (E), (b):Tensile Strength (TS) and (c) Elongation to break (ε%) of HPMC films at different oil concentration (%). Means with different letters are significantly different (capital letters refer to HPMC effect and small letters to oil concentration effect).

1.3.5. Water vapour permeability

One of primary function of an edible film is to restrict to moisture transfer between the food and surrounding atmosphere or between two compounds of heterogeneous food product.

Figure 6a shows the WVP value of HPMC film at different concentration, measured at 25°C and 87% RH gradient. Results showed that WVP was influenced by HPMC concentration, and in particular it increased from $0.89x10^{-10}$ g m^{-1} s^{-1} Pa to $2.68x10^{-10}$ g m^{-1} s^{-1} Pa, as HPMC concentration increased from 2% to 6 %· ($p < 0.05$). This effect can be attributed to the higher number of polar group, enhancing interaction with water and favoring water transmission through film (Miller and krochta, 1997). Similar results was reported for pectin film (Giancone et al., 2011).

Hernandez (1994) suggested that water vapour transfer generally occurs through the hydrophilic portion of the film and depends on the hydrophilic–hydrophobic ratio of the film components. Thus, it is generally accepted that the addition of hydrophobic lipids to hydrophilic polymer films improves water vapour barrier properties.

The addition of rosemary oil had a significant effect only on WVP of film at 6% of HPMC which decreased from $2.68x10^{-10}$ g m^{-1} s^{-1} Pa to a value of $1.2x10^{-10}$ g m^{-1} s^{-1} when 0.4% of rosemary oil has been added to the film (figure 10b). Rosemary oil concentration of 0.7% and 1% also caused a decrease of the WVP to a value slightly higher than at 0.4%. In contrast, the addition of rosemary oil at 2% had detrimental effect on the WVP that increased up to $5.3x10^{-10}$ g m^{-1} s^{-1}.

Similar result were obtain by Bonilla et al. (2012), who reported that 0.5% of basile and thyme oil reduce WVP of chitosan film, whereas 1% of both oil have a detrimental effect on WVP. It is recognized that a not optimal distribution of the oil particles into the film structure can have a negative impact on the film barrier properties. In particular this effect could occur because hydrophobic substance decrease the cohesion forces of the polymer network. This aspect could enhance transport phenomena though the film, despite the increase in the hydrophobic character of the matrix when oils were dispersed (Atares et al., 2010; Bonilla et al., 2012, Benavides et al., 2011). However, many studies reported different effect of EOs on WVP. For sodium caseinate, alginate, whey protein films no effect of the incorporation of essential oils and natural extracts on WVP have been reported (Atarés et al., 2010b; Pranoto et al., 2005; Zinoviadou et al., 2009). Whereas, for chitosan films (Hosseini et al., 2009; Zivanovic et al., 2005), starch–chitosan films (Pellissari et al., 2009), HPMC films (Sanchez Gonzales et al., 2009), and *Geladiuim corneum* edible film (Lim et al., 2010) containing essential oils it was reported that essential oil improve WVP.

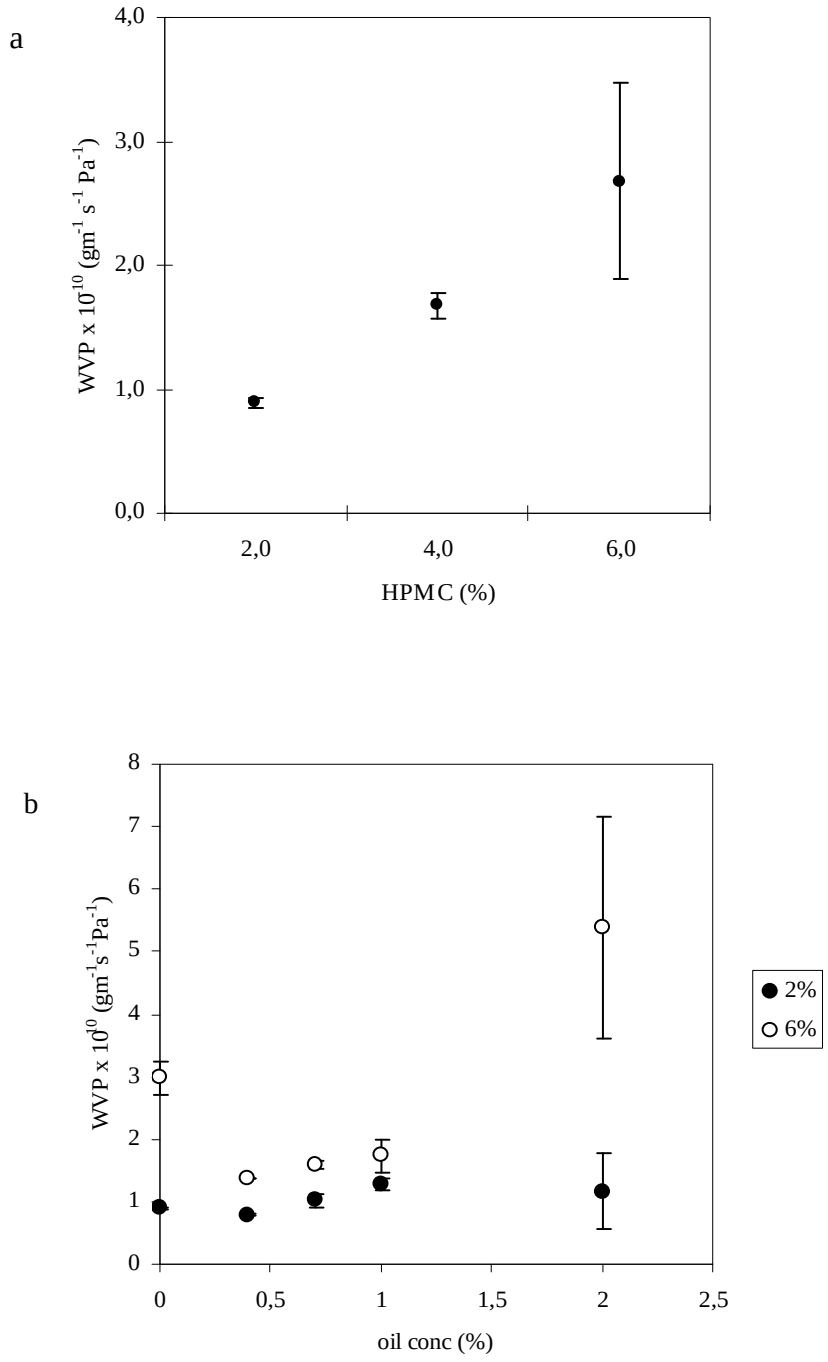


Figure 10. Water vapour permeability (WVP) of HPMC film at different concentration (a) and of HPMC film (● 2%, ○ 6%) as function of essential oil (b) (87% RH gradient at 20°C)

Moreover, Atarés et al. (2010) reported that lipids with similar size distribution in film forming emulsion may exhibit different water barrier effectiveness at specific lipid to protein ratios, depending on the interactions between components and on the destabilizing phenomena taking place during drying. So a determinant factor that affects the water vapour barrier is the impact of the lipid addition on the microstructure of the emulsified film.

1.4. Conclusions

The structure of HPMC film was affected by rosemary oil concentration. Probably, most of the oil migrated toward the evaporation surface and migration was function of HPMC concentration. In fact, this phenomena was most evident for 2%HPMC film at maximum oil concentration, maybe for the low viscosity of HPMC solution. For 6%HPMC film, no destabilization phenomena occurred or occurred slowly during the film drying.

Dynamical mechanical analysis results highlighted that mechanical properties depend upon HPMC concentration: decreasing HPMC from 6% to 4%, E' decreased approximately by two order of magnitude, but no differences were observed between films at 4% and 2%.For both samples, the oil concentration did not have a statistically effect on E' modulus and loss tangent.

Tensile strength, elongation to break and elastic modulus were evaluated. The addition of the rosemary essential oil did not affected the mechanical properties of the film, with the exception of the ε% of film at 6% of HPMC which is reduced at high oil concentration.

WVP of HPMC films increased as increased HPMC concentration for increased of hydrophilic groups. Rosemary oil improve water barrier properties only to 0.4% concentration oil of 6% HPMC films. This results was explain by decrease of the cohesion forces of the polymer network due to presence of hydrophobic substance, as confirmed by microscopy analysis.

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2. II STUDY CASE

Structure and properties of Hydroxy propyl methyl cellulose-sodium caseinate film cross-linked by Transglutaminase

ABSTRACT

The objective of the present work was to study the structure and the functional properties of Hydroxypropil methyl cellulose (HPMC)-sodium caseinate (SC) edible films cross linked with TGase. SDS-PAGE, scanning electronic microscopy and dynamical mechanical analysis were performed to investigate the structure of the films. Film performance were studied by means of solubility test, thickness, mechanical properties and water vapour permeability. Results show that structure and functional properties of HPMC/CS films were affected by the film composition. In particular, the number of cross-links within the blend edible films is function of HPMC/SC ratio and increased by increasing the protein concentration, but only for HPMC concentration less than 50%. The cross linked structure in presence of protein can enhance blend film solubility but only at specific ratio HPMC/SC, maybe due to immiscibility lacunas.

Mechanical properties confirmed the positive role of polysaccharides on the film stiffness, but the negative effect on film extensibility. In contrast, the different ratio of HPMC/CS film did not affect the permeability to water vapour of the films, showing that it is the hydrophilic nature of the polymer that play the major role in determine the barrier properties of the films.

2.1. Introduction

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, non-toxicity, etc.). These properties are dependent on the type of material used, its formation and application (Guilbert et al., 1996).

Protein films are brittle and susceptible to cracking due to the strong cohesive energy density of then polymers. Thus they require addition of a plasticizer to achieve adequate tensile properties, especially flexibility, to avoid cracking. Plasticized protein films are good oxygen, carbon dioxide and lipid barriers. However, their predominantly hydrophilic nature results in poor water barrier characteristics (Lim et al., 2002; Lacroix and Cooksey, 2005). Polysaccharides are generally suitable for the production of edible films without addition of a plasticizer. Like proteins, they are good barriers to oxygen,

carbon dioxide and lipids but have limited control of water vapour migration and can be soluble in water (Sothornvit and Krochta, 2005, Gennadios, 2002).

However, in general, individual proteins and polysaccharides lack the combination of structural integrity and barrier functionality to make them broadly useful in food systems.

Blend biopolymer films, which contain both protein and polysaccharide ingredients, may advantageously use the distinct functional characteristics of each film-forming ingredient . Blend edible film can be formed by protein/polysaccharide, protein/protein, polysaccharide/ polysaccharide, while lipid were added for their hydrophobic characteristic, to improve barrier properties. 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 (Han and Gennadios, 2005).

Generally, blend systems consist of separated phases, where domains of one polymer are dispersed in a matrix of the other polymer and, rarely, there is an attractive electrostatic interaction between chemically dissimilar polymers within a single-phase material (Tolstoguzov, 1997).

Another way to improve properties of edible film is the modification of protein enzymatic or chemically. The chemical or enzymatic introduction of new covalent bonds into protein systems is expected to affect film structure and, in turn, film performances, such as water permeability and/or mechanical properties (Gennadios,2002). Among the numerous cross-linking agents, transglutaminase (TGase, protein-glutamineç glutamyltransferase, EC 2.3.2.13) is an enzyme capable of catalyzing acyl-transfer reactions, resulting in the formation of -(ç-glutaminyl)lysine intra- or intermolecular cross-links in proteins (Nielsen, 1995).

Milk proteins, such as caseinates, have special properties which make them highly suitable for obtaining edible films. Their excellent nutritional value and their numerous functional properties such as their solubility in water and ability to act as emulsifiers are important factors for the formation of edible films. Casein-based films can be cast from aqueous solutions without further treatment, due to the random coil nature of the proteins and their ability to hydrogen bond extensively and engage in electrostatic interactions. Caseinate films are transparent, flavourless, flexible and highly soluble. Finally, considerable interest exists in finding new uses for milk proteins due to their surplus availability industrially (Arvanitoyannis et al., 1996; Chen, 2002; Khwaldia et al., 2004).

Casein is an ideal substrate for TGase due to its less ordered conformation and relatively high content of glutamine and lysine. The susceptibility of all components of sodium caseinate for mTGase-induced reaction decreased in the order of β-casein > α-casein > κ-casein (Chen, 2002).

Hydroxypropylmethylcellulose (HPMC) is a readily available nonionic edible plant derivative shown to form transparent, odorless, tasteless, oil-resistant, water-soluble films with moderate moisture, oxygen, and aroma barrier properties, high TS, and low flexibility (Nisperos-Carriedo 1994; Kroch and De Mulder-Johnston 1997). Little research has been published on protein–HPMC blends (Perez-Gago and al., 2005; Brindle and Kroctha, 2008).

Based on results present in literature on use of cellulose polysaccharide edible film, we hypothesized that HPMC matrix can act as a filler on sodium caseinate cross linked matrix. Thus, the objective of the present work was to study how different concentration of a filler (the polysaccharides) obtained making films at different ration between protein and polysaccharides can have an effect on the structure of the blend films and thus its functional properties.

2.2. Materials and Methods

2.2.1. Materials

Sodium caseinate (SC), hydroxypropylmethylcellulose (HPMC) (1.8-2.0 methyl substitution (DS); 0.20-0.30 hydroxypropyl substitution (MS)) and glycerol was purchased from Sigma-Aldrich (Milan, Italy). Other reagents used were analytical grade.

The enzyme transglutaminase (TGase), excreted by Streptoverticillium mobaraense, *(*Ca^{2+}-independent transglutaminase (Activa EB)) was supplied by Ajinomoto Co. (Tokyo, Japan), with a nominal activity of 34-65 U/g (1 U of TGase being defined as the amount of enzyme that releases 1 μmol of hydroxamic acid in 1 min at 37 °C according to the hydroximate test by Folk and Cole 1965), a maximum reaction temperature of 55 °C, and an optimal pH range of 6–7. Microbial transglutaminase (mTGase) was used without further purification (http://www.ajinomoto.de/).

2.2.2. Film making procedure

HPMC film forming solution was prepared dissolving 4 g of powder in 100 ml of deionised water (4%) at 65°C for 90 minutes and then cooling it at 25°C.

SC film forming solution was prepared dissolving 4 g of powder in 100 ml of citrate buffer solution (100mM and pH 7,0) and stirring for 4h at 25°C. During the stirring, 0.3g of glycerol were added.

The two film forming solution were mixed together at different ratio (100:0; 75: 25; 50:50; 25:75; 0:100) for all night. After mixing, with the exception of samples HPMC/SC 100:0, the enzyme was added to the blend solution into which the enzyme was easily solubilised. Reaction was started by storage the blend solutions in a environmental chamber at 40°C. Once reached the set temperature, the samples were stored for 4h. Then, in order to stop the reaction, samples were stored for few minutes in a environmental chamber at 80°C.

Before casting, solutions were de-aerated under vacuum to prevent pinhole formation. 20 ml of film forming solutions were poured onto levelled 56.7 cm^2 polystyrene Petri dishes and allowed to dry at 40 °C and 50% relative humidity (RH) for 16h under air circulation. The dried films were peeled from the Petri dishes and stored at 20°C and 50% relative humidity prior to testing.

2.2.3. SDS-PAGE analysis

The sodium caseinate (SC) enzymatically modified was evaluated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions as described by Laemmli (1970). A discontinuous system consisting of a 4% (w/v) acrylamide stacking gel and a 12% (w/v) acrylamide running gel was used. A total of 10 mg of protein samples was dissolved in 1 ml of sample buffer (0.5 M Tris–HCl, pH 6.8) containing 10% (w/v) SDS, 10% (v/v) glycerol, 5% β-mercaptoethanol and 0.1% (w/v) bromophenol blue and heated at 100°C for 5 min. Every sample (10 μL) was applied to each lane of gel. The gel electrophoresis was carried out at 120 V constant voltage. The gel was stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 10% (v/v) acetic acid, 40% (v/v) methanol and destained with 10% (v/v) acetic acid containing 40% (v/v) methanol.

2.2.4. Scanning electron microscopy analysis

Microstructural characteristics of film samples were examined using an LEO EVO 40 scanning electron microscope (Zeiss, Oberkochen, Germany). All film samples

were dried in a desiccator containing lithium chloride (a_w=0.113±0.003) and then manually fragmented. Dried strip fragments of films were mounted on specimen stubs with the cross-section oriented up and coated with a thin layer of gold by a DC sputter coater (AGAR B7340, Agar Scientific Ltd, Stansted, UK). Digital images of film cross-section were collected at a tilt angle of 0° to the electron beam using an acceleration voltage of 20 kV.

2.2.5. Dynamical mechanical properties

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^{-1}. Then, the frequency sweep tests were conducted by applying an oscillation amplitude of 0.01% (within the linear region) over a frequency range between 0.1 to 1000 rad s^{-1}. The parameters used for this study were the storage modulus (E') ,the loss modulus (E") and tan(δ).

2.2.5.1. Modelling of rheometrical determinations

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):

$$G'(\omega) = G_0 + \omega \int_0^\infty [G(t) - G_0] \sin(\omega t) dt \qquad (2.1)$$

$$G'(\omega) = \omega \int_0^\infty [G(t) - G_0] \cos(\omega t) dt \qquad (2.2)$$

To solve the equations (2.1) and (2.2), G(t) was assumed to coincide with the Friedrich and Heymann model (1988):

$$G(t) = G_{0,\alpha} + \frac{S_\alpha^*}{\Gamma(1-\alpha)} t^{-\alpha} e^{\frac{-t}{\lambda\alpha}} \qquad (2.3)$$

where α is the order of the relaxation function, Γ(1−α) 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 (2.1) and (2.2), the analytical dependence of *G*'(ω) and *G*"(ω) on frequency was expressed as (Friedrich and Heymann, 1988):

$$G'(\omega) = G_{\infty,\alpha} + \sqrt{\frac{2}{\pi}} S_\alpha^* \lambda_\alpha^{-\alpha} (\omega\lambda_\alpha) \frac{\sin[(1-\alpha)\arctan(\omega\lambda_\alpha)]}{\left[1-(\omega\lambda_\alpha)^2\right]^{\frac{1-\alpha}{2}}} \quad (2.4)$$

$$G''(\omega) = \sqrt{\frac{2}{\pi}} S_\alpha^* \lambda_\alpha^{-\alpha} (\omega\lambda_\alpha) \frac{\cos[(1-\alpha)\arctan(\omega\lambda_\alpha)]}{\left[1-(\omega\lambda\alpha_\alpha)^2\right]^{\frac{1-\alpha}{2}}} \quad (2.5)$$

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. $\rightarrow\infty$ $\omega\lambda_\alpha$) equations (2.4) and (2.5) can be reduced to:

$$G'(\omega) = G_{\infty,\alpha} + \sqrt{\frac{2}{\pi}} S_\alpha^* \cos\left(\frac{\pi}{2}\alpha\right)\omega^2 \quad (2.6)$$

$$G''(\omega) = \sqrt{\frac{2}{\pi}} S_\alpha^* \sin\left(\frac{\pi}{2}\alpha\right)\omega^2 \quad (2.7)$$

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

$$\tan\delta = \frac{G''}{G'} = \tan\left(\frac{\pi}{2}\alpha\right) \quad (2.8)$$

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).

2.2.6. Film thickness measurement

Film thickness was measured using a micrometer model HO62 with a 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 instrument feel in contact with the film. Mean thickness (µm) of films was determined by averaging 10 measurements at different locations.

2.2.7. 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 6.67 kPa in a vacuum oven for 24 h and then weighed to the nearest 0.0001g to determine the initial dry weight of the film. Each film piece was incubated at 25°C for 24 h in a screw-top tube (150 x 15 mm) with 10 mL of deionised water. At the end of the incubation the samples were poured onto Whatman #1 qualitative filter paper. The non-dissolved material, removed from the filter by using 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 total soluble matter (TSM) was calculated as follows:

$$TSM(\%) = \frac{dm_i - dm_f}{dm_i} \cdot 100 \qquad (2.9)$$

where *dm* is the dry matter and subscripts *i* and *f* correspond to the initial and final dry matter. Tests were carried out in triplicate and averages are reported.

2.2.8. Mechanical properties

Mechanical analysis was carried out at room temperature using an Instron Universal Testing Instrument Model No 4301 (Instron Engineering Corp., Canton, MA) equipped with a 1,000 N load cell. Film samples were cut into 25 wide and 100mm length strips using a sharp razor blade. The strips were equilibrated overnight at 50±5% RH and 23±2 °C in an environmental chamber. Ten samples of each film type were tested. Tensile properties of the films were measured according to the ASTM (1991) Standard Method D882 using Test Method A, the Static Weighing, Constant Rate-of-Grip separation test. The initial grip separation was 50 mm and crosshead speed was 15 mm/min in a Tension Mode. Tensile strength (TS) and percent elongation at break (ε%), Young modulus (EM) were calculated. Results are reported as average of ten replications of each sample.

2.2.9. Water vapour permeability

Water vapour permeability (WVP) of films was evaluated by gravimetric test according to ASTM E96 (1993) by means of a Fisher/Payne permeability Cup (Carlo Erba, Milan, Italy). Three grams of silica gel were introduced in each cup. Film sample having diameter of about 6 cm was placed 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 desiccators containing a saturated distilled water. The desiccator was stored in a Heareus thermostated incubator (Binder KBF240, Turin, Italy) at 25.0 (± 0.1 °C). Cups were weighed at scheduled times, and the amount of water vapour transmission rate (WVTR) 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 $R^2 \geq 0.998$.

From WVTR data, the value of vapour pressure on film's inner surface (p_2) was obtained taking into account the method proposed by Mc Hugh et al. (1993) to correct the effect of concentration gradients established in the stagnant air gap inside the cup.

$$WVTR = \frac{P \cdot D \cdot Ln\left[\frac{P - P_2}{P - P_1}\right]}{R \cdot T \cdot \Delta z} \qquad (2.10)$$

Where, P is the total pressure; D is the diffusivity of water through air at 25°C; R is the gas law constant; T is the absolute temperature; Δz is the mean stagnant air gap height, considering the initial and final z value; P_1 is the water vapour pressure on the solution

surface; P_2 is the corrected water vapour pressure on the film's inner surface in the cup. Then permeance was calculates as followed:

$$Permeance = \frac{WVTR}{(P_2 - P_3)} \qquad (2.11)$$

Where P_3 is the water vapour partial pressure at the film outer surface. Permeability was obtained by multiplying the permeance by the average film thickness. Results are reported as average of three replications of each sample.

2.2.10. Statistical analysis

Data were submitted to analysis of variance by means of SPSS v13.1 for window (SPSS, Milan, Italy). Duncan's test was carried out to find the source of the significant differences within the samples examined. Significance of differences was defined at $p \leq 0.05$.

2.3. Results and discussions

2.3.1. SDS PAGE analysis

The extent of cross-linking of the proteins incubated with mTGase was evaluated by SDS-PAGE analysis. The SDS-PAGE patterns of the polymerization process of the SC incubated with the mTGase at 40°C at different times in comparison with molecular mass markers (lane 8) were showed in Fig. 1.

The disappearance of proteins bands with the concomitant appearance of new bands at higher molecular weight and/or the accumulation of protein polymers at the stacking and running gel origin was observed.

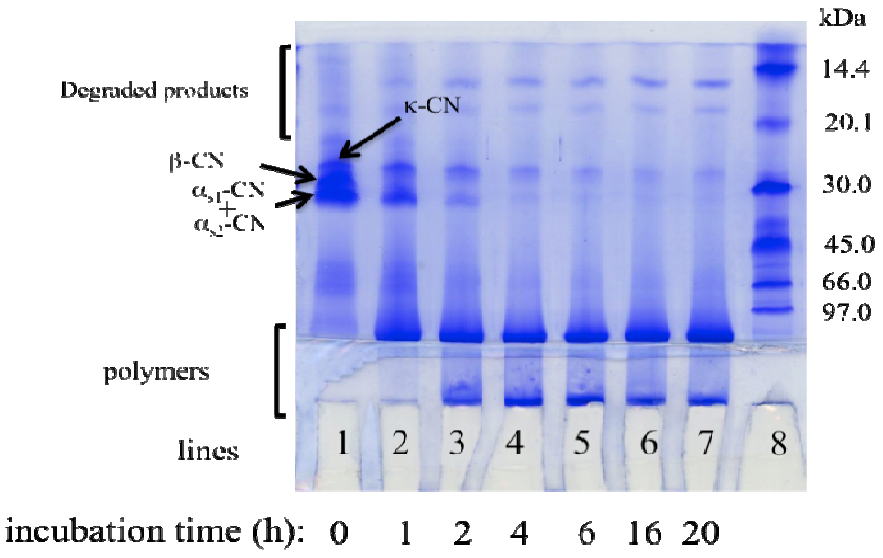


Figure 1. SDS-PAGE analysis (reducing conditions) of bovine SC cross-linked with microbial transglutaminase (mTGase) at 40°C. Lanes 1-7: SC samples incubated respectively for 0, 1, 2, 4, 6, 16 and 20 hours; lane 8: molecular mass standards. (caseinate fraction: (α_{s1}+ α_{s2}), β- and k-CN).

The unpolymerized SC (lane 1), showed three major bands, rappresenting the caseinate fraction (CN): (α_{s1}+ α_{s2}), β- and k-CN with apparent molecular weight about 30.0 kDa. The incubation of sodium caseinate with mTGase at 40°C for 1 hour (lane 2) resulted in a high decrease of the relative intensity of β-, α_{s1}- and α_{s2}-CN bands and a concomitant appearance of new high molecular weight polymers (> 97.0 kDa) on the top of separating gel. After four hours of the incubation (lane 4) α_{s1}-, α_{s2}-, and β-CN were completely polymerized. After 20 hours of the incubation (lane 7), there was still a small amount of κ-CN unpolymerized. The intensity of new high molecular weight bands increased with incubation time ranging from 1 to 20 hours, while other polymers heavier did not enter in the stacking gel.

By SDS-PAGE experiments carried out under reducing conditions (containing β-mercaptoethanol), it can be confirmed that the new polymers were originated by covalent cross-linkages throughout mTGase treatment. The mTGase polymerization reactivity, observed by SDS-PAGE analysis, of individual caseins was different, and the polymerization rate was in decreasing order: β- > α_s- > κ-CN.

This data was in accordance with Kuraishi et al. (2001) results on milk powder or skim milk powder, incubated with mTGase at 25°C for 2 h, by SDS-PAGE analysis where mainly β-casein was cross-linked; for α_s-casein, only a minor decrease in the intensity of the monomer band was observed, and no visible decrease occurred for κ-casein.

2.3.2. Scanning electron microscopy

Scanning electron microscopic examination was performed to understand better the structure of the different films examined.

Figure 2 shows the cross section micrographs of HPMC/CS-based films at different HPMC/CS ratio. HPMC cross section present a homogeneous structure, but some irregular particles of the films can be observed, which can be attributed to some amount of non-dissolved polymer which remains non integrated in the matrix due to a not perfect control of the temperature during gelling. In fact, at temperature higher than 65°C, it is possible that same HPMC polymer as function of DS and MS can precipitate (Morris, 2007).

SC films cross linked with mTGase exhibited quite a compact and dense structure as expected for a homogeneous material (figure 2 b). Similar result was obtain by Bruno et al. (2008).

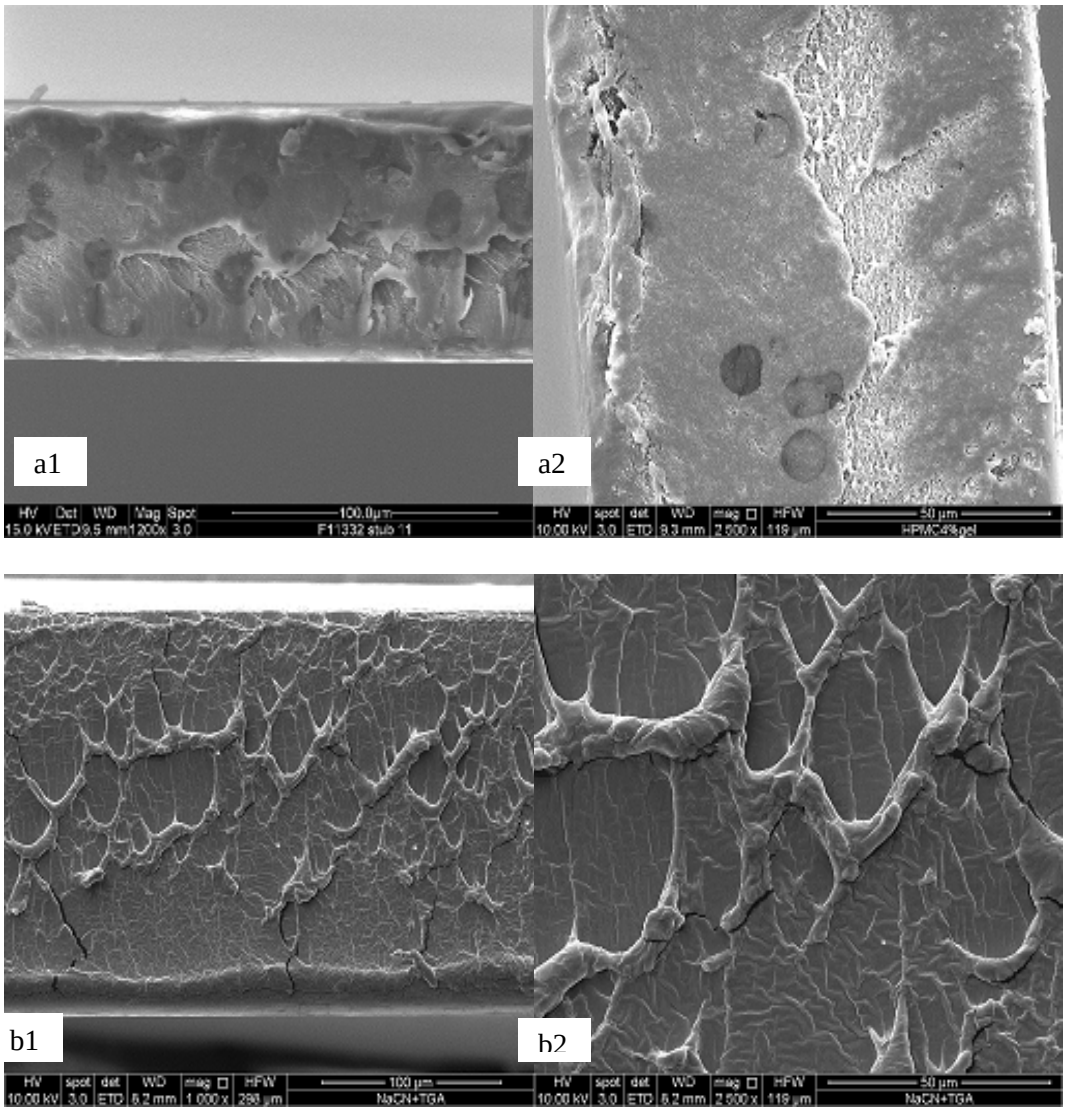


Figure 2. (a) SEM micrographs of HPMC film at magnification 1200x (a1) and 2500x (a2) and (b2) SC based film crosslinked with mTGase at magnification 1000x (b1) and 2500x (b2)

Figure 3 show the cross-section micrographs of HPMC/SC-based filmsat different ratio. By blending HPMC and SC at 75:25 and 50:50 ratio leads to a rough surface, with increasing density of crack deflection sites that results in increasing amount of ripples and ridges due to high concentration of HPMC (figure 3, a-b).

A more dense and compact structure was found when protein concentration was 75% (figure 3c). These results can be justified by an increase in link density with increasing of protein amount. On the other hand, when the HPMC content was overwhelming (that is, greater than 50% w/w), the microstructure was less compact and seems to be not affected by the cross-linking activity of TGase.

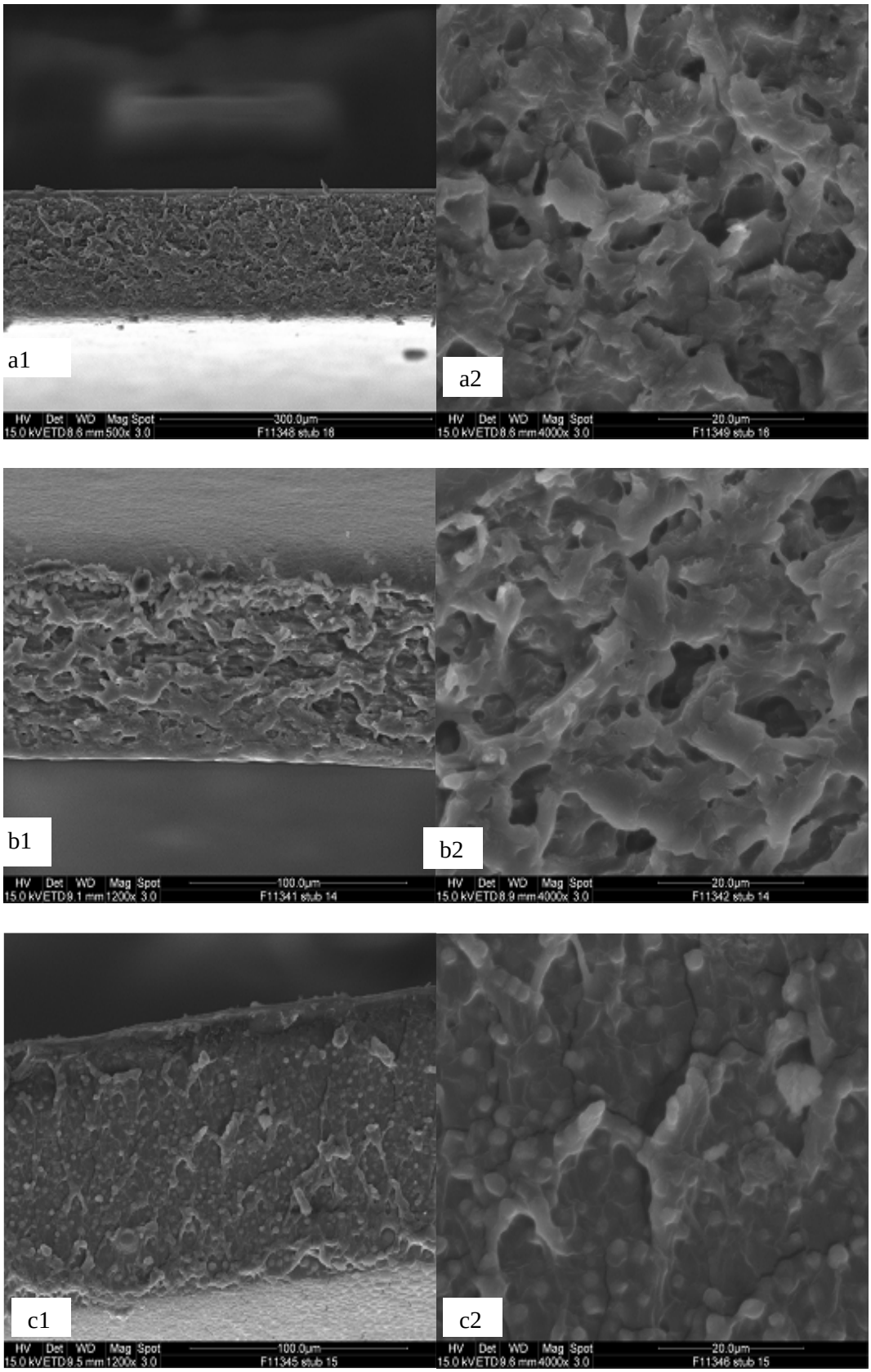


Figure 3. SEM micrographs of HPMC/SC based films at three different ratios: 75: 25 (b1-2), 50:50 (a1-2), 25:75 (c1-2).

2.3.3. Dynamical mechanical analysis

Dynamic mechanical measurements at small-deformation represent an investigation technique not destructive, that by following the variations of E' and tan (δ) with oscillatory frequency (ω) allow a qualitative determination of material nature (Ferry, 1980).

Figure 4a shows the dependence of storage modulus (E') and loss tangent (tanδ) on ω of SC based films prepared in either the presence or absence of TGase.

As ω was varied from 1 to 1000 rad s^{-1}, E' increased almost linearly with E' one order of magnitude greater than E" (data not shown). The loss tangent values, being a measure of the ratio between the energy lost and that stored throughout any deformation cycle, were less than one and decrease as ω increased from 0.01 to 1000 rad s^{-1}(figure 4b). The low dependence of E' on ω and a loss tangent being less than one showed that SC films acted as solid-like materials.

TGase cross-linked films apparently behaved as the corresponding films produced in absence of such a cross-linking agent, except for the higher values of E' and a slight higher frequency dependence. Moreover, films obtained in absence of TGase showed an higher values of loss tangent all over the frequency range.

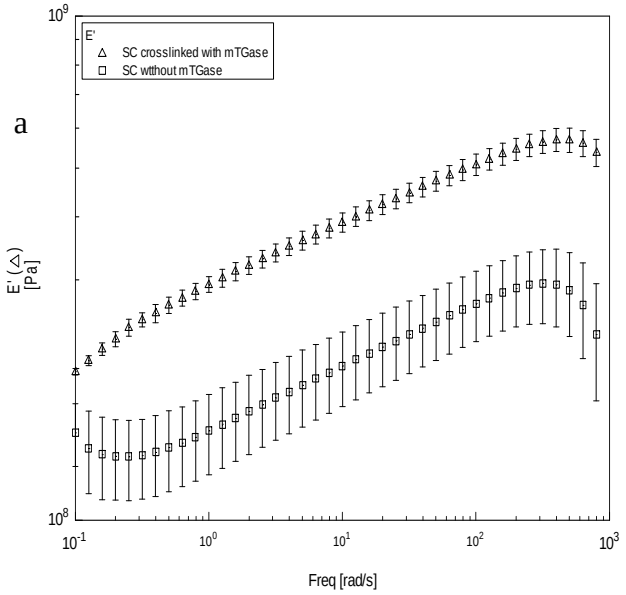
Figure 5 (a, b) shows the dependence of E' and tanδ on ω of HPMC/SC films obtained at different ratio, in presence of TGase.

As ω was increased from 0.01 to 1000 rad s^{-1}, E' was found to be not only practically constant, but also nearly independent of HPMC/SC ratio for films at ration HPMC/SC 100/0, 75/25 and 50/50. On the contrary, for films obtained with 100% of SC, as showed before, and for films at ratio HPMC:SC (25:75) E' increased almost linearly with ω.

The loss tangent values were less than one and decrease as ω increased from 0.01 to 1000 rad s^{-1}. It dependence of ω was higher for film HPMC/SC at ratio 25: 75 (figure 5b).

The mechanical spectra revealed that mechanical properties depend upon film polysaccharides content, in agreement with Giancone et al. (2008). Nevertheless, due to the nature of HPMC, it is most probably that polysaccharides act as filler of the protein network, affecting the mechanical spectra of the blends film as function of the ratio HPMC/SC films. In presence of high concentration of HPMC, it is possible that protein are unable to form a continuous network and in turn the mechanical spectra of the blends are similar to HPMC film.

In order to obtain information on structural organization of film network, the frequency sweep curves were described by the Friedrich-Heymann model (1988). Based upon the model, the three-dimensional film structure might be described in terms of the order of relaxation function (α) of the film network (Giancone et al., 2008). Parameter α may be related to the number of cross-links within the polymer, since macromolecule relaxation broadens with increasing cross-link density (Ferry, 1980).



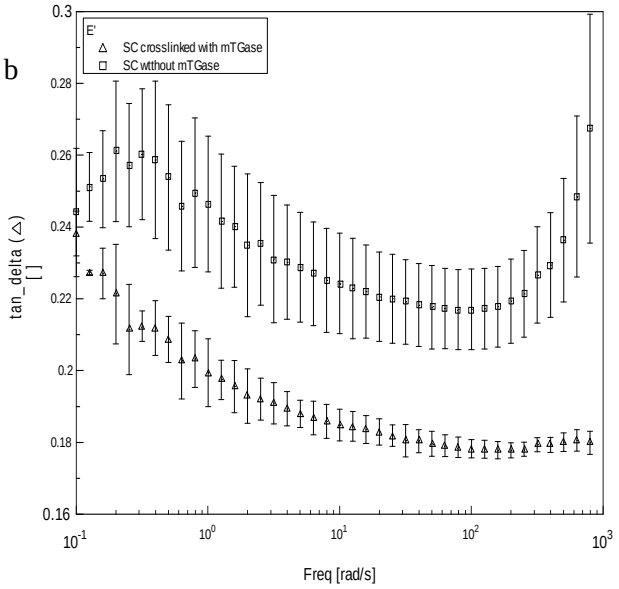


Figure 4. Storage tensile modulus (E') (a) and loss tangent (tanδ) (b) versus angular frequency (ω) for sodium caseinate (SC) film cross linked and not cross linked with mTGase

a

b

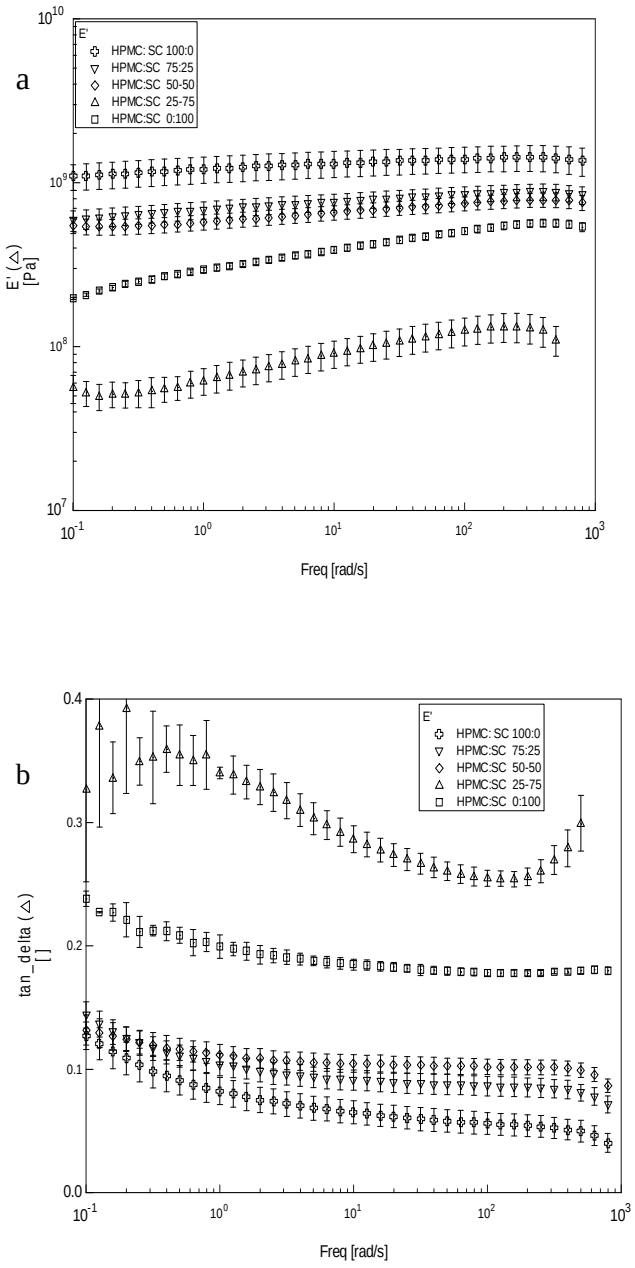


Figure 5. E' (Pa) (a) and loss tangent (tanδ) (b) versus angular frequency (ω) as for different HPMC/SC films cross linked with TGase

Figure 6a shows the α parameter of the model as a function of HMPC/SC ratio. The α parameter the HPMC/SC blend was not influenced by SC up to a concentration of 50%. For higher concentration of SC, that is for HPMC/CS ratio 25/75 and 0/100, α considerably increase for increased from 0.0464±0.002 to 0.111±0.002. The increase in α implies an enhancement in the link density within the polymer network, thus proving that HPMC macromolecules were a limiting factor for protein-protein interaction, because by acting as filler into the protein network, at high concentration reduce the probability that protein can cross-links.

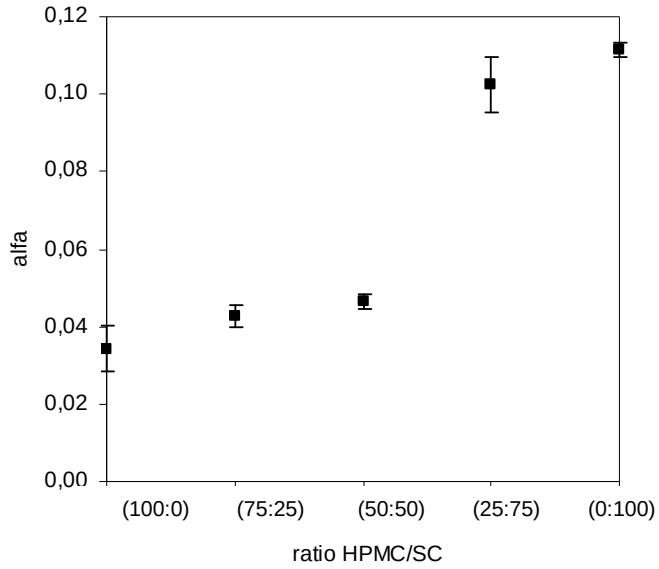


Figure 6. Effect of HPMC/SC ratio on the order of the relaxation function α

2.3.4. Optical properties and thickness

Pure HPMC film was colourless and transparent. SC film cross-linked with mTGase show good appearance and a homogeneous texture. HPMC/SC blended films were translucent. Translucent films have been reported in chitosan–gelatin (Lopez-Caballero and others 2005), konjac gluccomannan (KGM)–gelatin (Li and others 2006), corn starch–casein, corn starch–gelatin and corn starch–albumin film blends (Jagannath and others 2003).

Generally, transparency of films is an auxiliary criterion to judge the miscibility of two or more polymer mixed films (Li et al., 2006). A translucent appearance has been attributed to the less viscous phase forming a continuous matrix, with the more viscous phase forming dispersed domains. Light refracts from these domains, causing the film to appear translucent. Typically, translucence indicates incomplete miscibility between two or more components (Brindle and Krochta 2008).

The thickness of the film samples is reported in figure 7. The HPMC/SC ratio had a significant effect on thickness ($p<0.05$). The thickness of films decreased by increasing the protein content.

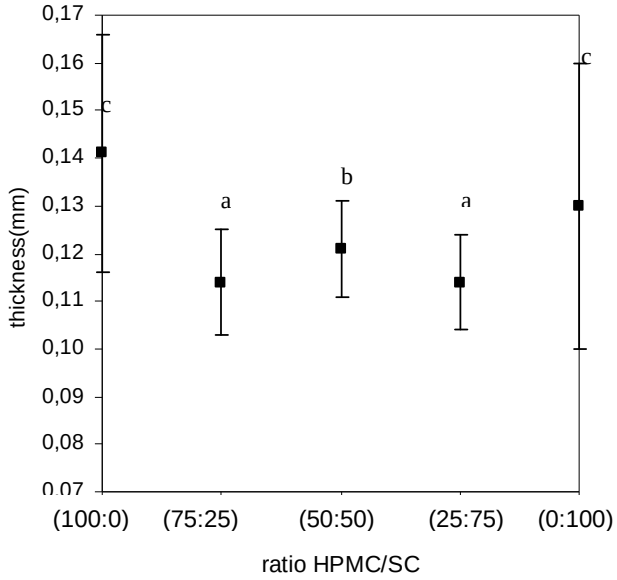


Figure 7. Thickness of HPMC/SC films at different ratio.

2.3.5. Solubility

Solubility is an important characteristic of edible films and coatings. A film with low water solubility aids in maintaining film and food integrity and will have different applications than a film with high water solubility. Some applications require solubility or disintegration in water prior to product consumption such as pouches for instant soups or drinks. However, in the most of food application a low solubility is needed.

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 bio polymeric packaging that differ strongly from those of the macromolecular reactants (Aguilera, 1999).

In figure 8 was reported water solubility of HPMC/SC edible film cross linked with mTGase, at different ratio.

The composition of blend had a significant effect on blend film solubility ($p<0.05$). Considering that the chemical structure of HPMC contains many hydrophilic hydroxyl groups, the HPMC content in the films was expected to dissolve in less than 1 minute in water. Also pure SC film is a hydrophilic for nature and in absence of mTGase, SC was totally soluble in water (data not shown). In contrast, SC edible film in presence of mTGase have a solubility of 26% at 20°C and 31% at 70°C, due to the formation covalent bonds (figure 8). The improvement of solubility in presence of mTGase was reported for chitosan-ovoalbumin filnm (Di Pierro et al., 2007), fish skin-gelatine films (Piotrowaska et al., 2008), fish gelatine-chitosan (Kolodziejska et al., 2007) and chitosan- whey protein film, too (Di Pierro et al., 2006).

Thus, film solubility was chosen as measurement to explore the interaction between SC and HPMC.

HPMC and glycerol were expected to dissolve, leaving the SC matrix almost intact. Results at 75/25 and 50/50 ratio let suppose that when HPMC and SC are

blended same miscibility lacunas can affected the solubility results. In particular, the blend at 50:50 HMPC/SC+TGase ratio was completely soluble than as expected.

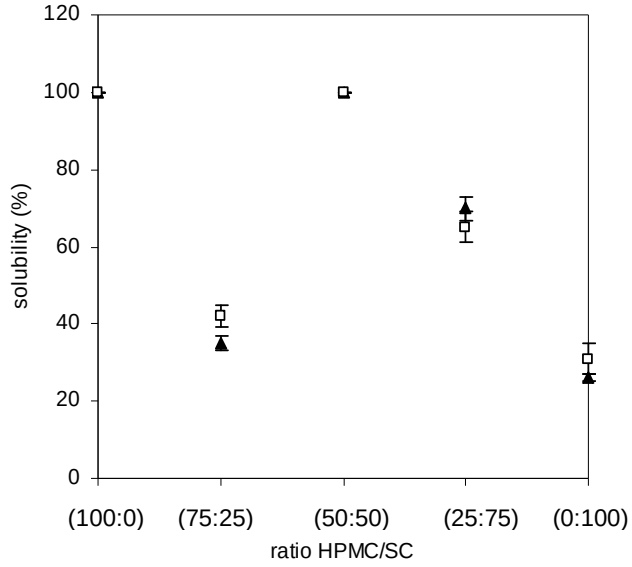


Figure 8. Solubility in water, at 20°C (▲) and 70°C (□), of HPMC/ SC-cross linked with TGase

2.3.6. Mechanical properties

The mechanical properties of edible films and coatings depend on the type of film-forming material and especially on its structural cohesion. Cohesion is the result of a polymer's ability to form strong and/or numerous molecular bonds between polymeric chains, thus hindering their separation. This ability depends on the structure of the polymer and especially its molecular strength, geometry, molecular weight distribution and the type of position of its lateral groups. The mechanical properties are also linked with the film-forming conditions, e.g. type of process and solvent, cooling or evaporation rate, etc., and the coating technique (spraying, spreading, etc.) (Guilbert et al.,1996)

Blend properties may be linearly interpolated between the individual component values (for the case of blending 2 polymers) or may be higher or lower than the individual components, depending upon the interactions in the system (Ehrenstein 2001). Polymer blends may exhibit more than 1 phase, depending on how the individual components interact with each other (that is, component solubility and/or miscibility) (Peters 2003).

Thus, the tensile properties of the composite films were evaluated and the results are presented in figure 9. As a general trends, it is noticed that the elastic modulus of composite film increases as the concentration of HPMC increases (figure 9a). Thus, confirming the positive role of polysaccharides on the film stiffness.

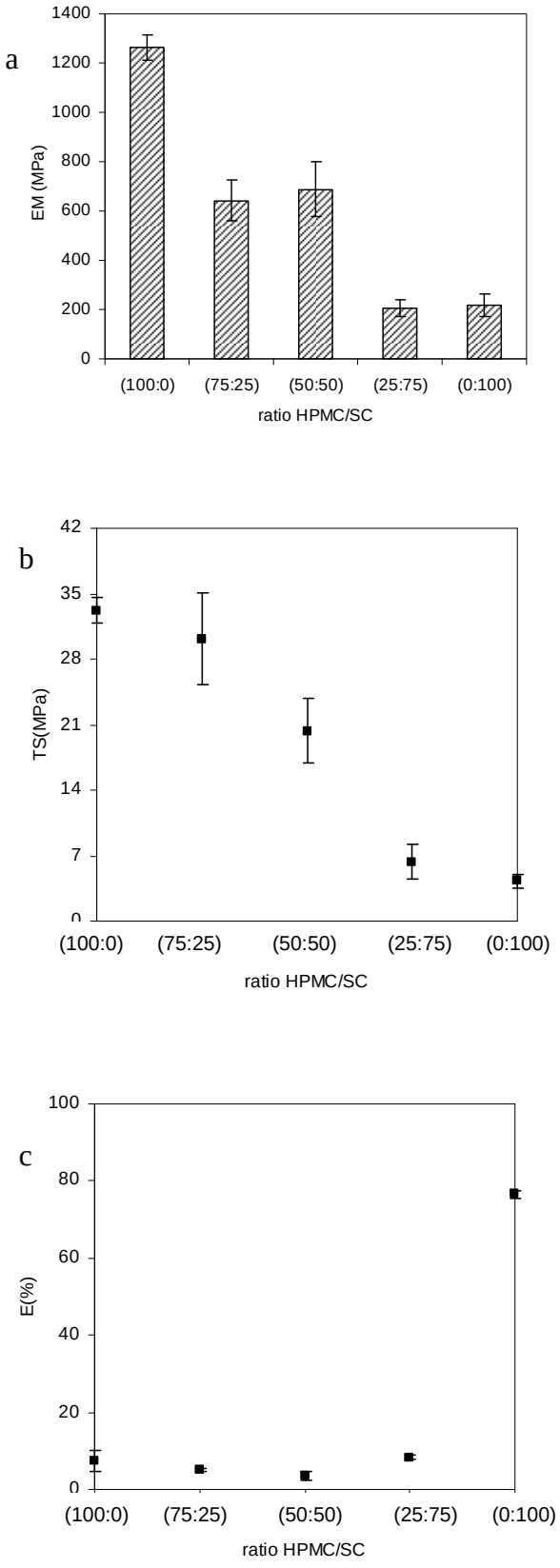


Figure 9. Elastic Modulus (EM) (MPa) (a), tensile strength (TS) (b) and elongation at break (ε%) (c) of HPMC-SC cross linked with mTGase at different ratio.

This result was in accordance with Brindle and Krochta (2008). Moreover, Pereda et al., (2011) showed that at the maximum filler concentration (3 wt % fibers), the tensile modulus is 80% higher than that of pure caseinate film, which is, however, lower than the two-fold increase observed when cellulose fibers, instead cellulose derivatives, were used as reinforcement (Pereda et al., 2011).

On the other hand, the tensile strength increases and the elongation at break decreases as the concentration of modified polysaccharides increases (figure 9 b and c). The addition of cellulose derivatives harden the protein structure, decreasing the molecular mobility of the matrix. As a result of this, the reinforced materials become more stiff, more resistant to break and less stretchable than the pure protein film. Similar results (increase in tensile strength and decrease in elongation at break as carbohydrate concentration increases) were reported by Arvanitoyannis et al., (1996), Brindle and Krochta (2008), Pereda et al., (2011). Moreover, this behaviour can be ascribed to adequate interfacial interactions (good compatibility) between filler and matrix due to their chemical similarities, as was also reported by other researchers (Chang et al., 2010).

2.3.7. Water vapour permeability

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, 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).

In figure 10 was reported a WVP of blend edible film. There were no clear trends with respect to content of both polymers. Pure HPMC edible film had low barrier properties to water for its hydrophilic nature. Also, SC is an hydrophilic protein. Thus, pure SC edible film even if cross linked with mTGase showed WVP of the same order of magnitude of HPMC, even if slight higher barrier against water.

In preliminary water vapour test, it was showed that mTGase did not affect water vapour permeability of sodium caseinate edible film. This result was in accordance with Giancone (2006) phd thesis results.

As reported by Di Pierro et al., (2006) TGase catalyse the formation of covalent bonds between lysine and glutamine in a protein, as consequence with the disappearance of primary amino and amide groups and the formation of less hydrophilic secondary amide linkages should be less available hydrophilic and therefore should reduce the water permeability. However, the positive or negative effect of cross-linking on film porosity, and thus, on tensile and water barrier properties, is still contradictory. Oh et al. (2004) observed that TGase had no practical effect on their water vapour permeability (WVP) of casein, whey protein, zein-casein, zein- whey protein and Kolodziejska et al., (2007) reported that TGase did not had effect on fish gelatine-chitosan film. Also Lim et al. (1998) and Yildirim and Hettiarachchy (1998) showed that the enzyme had a negative effect on their WVP. Whereas, Chambi et al., (2006) reported that TGase treatement increased WVP of Casein, gelatine and mixture of casein- gelatine.

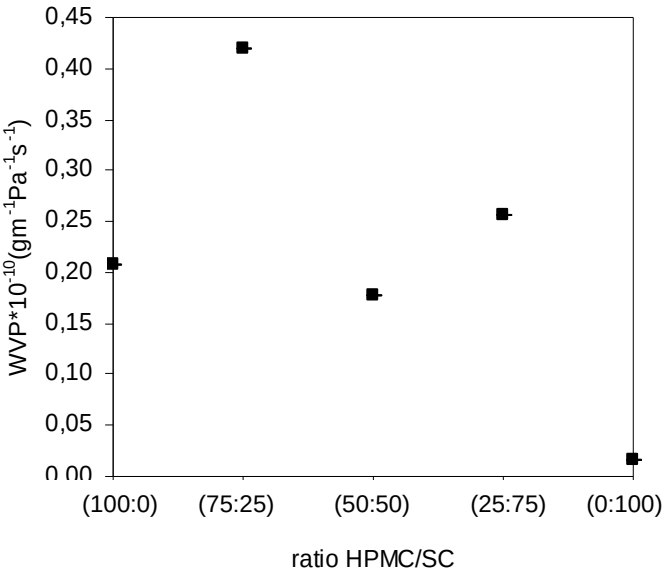


Figure 10. Water vapour permeability (WVP) of– Na-CN-HPMC cross linked with mTGase using desiccant method (ASTM 1993).

2.4. Conclusions

Functional properties of HPMC/CS films were affected by the film composition. In particular, solubility results highlighted that protein can enhance blend film solubility but only at specific ratio between polysaccharides and protein, maybe due to immiscibility lacunas.

Dynamical mechanical results showed that the number of cross links in protein based films increased in presence of TGase. Moreover, in blend films an enhancement in the link density within the polymer network was observed as protein concentration increased, but only for value higher that 50%. This means that, HPMC macromolecules were a limiting factor for protein-protein interaction, most probably because they act as filler into the protein network, and thus can reduce the probability that protein can cross-links.

These results were confirmed by mechanical characterizations of the blends films. In fact, as the HPMC concentration inside the blends increased, increased the stiffness of the films and decreased the elongation at break.

In contrast, the different ratio of HPMC/CS film did not affected the permeability to water vapour of the films, showing that the density of cross links has not an effect on diffusion of the gas through the film, and that it is the hydrophilic nature of the polymer that play the major role in determine the barrier properties of the films.

These results were also confirmed by microscopy analysis that proved to be a useful tool for characterizing the structure of the films under study.

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3. III STUDY CASE

Food application of sodium caseinate cross linked with trasglutaminase edible film: oil absorption reduction on French fried potatoes

ABSTRACT

The objective of the present work was to assess the oil barrier properties of Sodium caseinate (SC) cross linked with TGase coating on potato surface. Sodium caseinate cross linked with TGase coating at two 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. Results showed that the sodium caseinate coating at concentration tested did not reduce oil absorption during frying process. Because oil absorption depends by several factor including formulation coating and the homogeneity of the coated surface, an optimization of the coating formulation and of the process must be performed before to exclude a protective role of the coating again oil absorption.

3. 1. Introduction

Frying is a cooking method that enhances the flavour, texture and appearance of food products and thus resulted globally appreciated. One of the main problems associated with fried food is its high oil content owing to its association with the high incidence of diseases such as obesity, high cholesterol levels or high blood pressure. Health authorities and the media advise of the desirability of reducing the proportion of fat in the average diet.

The deep-fat frying process has been extensively studied. 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 by convection. Only if the amount of added food exceeds a critical value, will the temperature of the oil be significantly affected.

In deep-fat-frying, water in the crust evaporates and some water migrates from the core to the crust. Since this water leaves voids that allow the fat to enter, moisture loss and fat uptake are closely related. The microstructure of the crust is the main determining factor for oil uptake, which takes place by a capillary mechanism. Several physicochemical changes take place (starch retrogradation, Maillard reactions, glass transitions). These 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. Thus, during the frying process 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.

The concern to develop healthier products that contain less fat, absorbed during industrial pre-frying and frying processes, is one of the dominant factors in the latest research trends in this area, prompting studies of ways to lower the oil content of fried food. Different ingredients have been proved to be effective in reducing the amount of oil absorbed by fried food. Among these, hydrocolloids play a leading role (Mellema et al., 2003; Varela and Fiszman, 2011).

Coatings make the surface stronger and more brittle, with fewer small voids, which reduces evaporation and leads to less oil uptake; also, coatings alter the water holding capacity by trapping moisture inside and preventing the replacement of water by oil (Mellema, 2003; Garcia et al., 2008; Singthong and Thongkaew, 2009).

In fact, some edible coatings, particularly those based on hydrophilic polymers, are poor barrier to water and a good barrier to fats and oils. When frying coated food pieces, the film hinders absorption of the oil, improving its nutritional qualities and reducing the fat content and calories of the final product (Balasubramaniam et al., 1997).

Edible films are usually applied in liquid form (by dipping or spraying the food item or piece), using a solution or dispersion of the agent (a high molecular weight polymer). The solvent is then eliminated by evaporating or solidifying the material through thermal treatment, irradiation, the use of crosslinking agents, etc. A key requirement is to achieve a continuous layer of film. The film-forming solvent system and the conditions during film formation influence the final characteristics of the film. Cohesive strength and flexibility are critical to the porosity, permeability and uniformity of thickness of the barrier and to the support it provides. The mechanism by which gas and vapour flow through a uniformly applied film (with no cracks) is activated diffusion (solution in the coating matrix, diffusion driven by concentration gradient and evaporation on the other surface).

Many factors have been reported as affecting oil uptake, including oil quality, frying temperature and duration, the product's shape, its moisture, solids, fat or protein contents and porosity, pre-frying treatments (drying, blanching) and coating, among others. The coating has to be designed to minimize water loss, thus preventing oil from entering. The crust may act as a diffusion barrier that limits mass transfer, but inner moisture converted to steam may find selective channels in the structure and escape through open capillaries, pores, and crevasses, and oil may enter the voids left by the water (Pinthus, Weinberg, & Saguy, 1993).

Due to their hydrophilic nature, protein films can function as lipid barriers (Krochta and De Mulder-Johnston, 1997). Protein-based coatings also have been investigated for their potential to reduce oil absorption by coated foods during frying and, secondarily, to retain natural juices and flavors, enhance texture and appearance, and reduce moisture loss (Antonova et al., 2002). Protein less intensive studied as coating to reduce the oil uptake. Albert and Mittal et al., (2002) investigated ten hydrocolloids coating, including sodium caseinate, soy protein, whey protein and wheat gluten. Whey protein costing solution reduce the uptake oil of chicken strips of 30% (Dragich and Krochta, 2010).

The aim of this study was to study the effect of a sodium caseinate coating on the oil absorption of potato french fries during frying.

3.2. Materials and Methods

3.2.1. Materials

Sodium caseinate was purchased from Sigma Aldrich (Italy). The enzyme TGase excreted by Streptoverticillium, Ca^{++} independent, manufactured by Ajinomoto (Tokyo, Japan).Potatoes (variety Bintje) were purchased from local former.

3.2.2. Coating making procedure

Sodium caseinate (6g/100ml and 8g/ml) was dispersed in deionised water by stirring slowly until complete dissolution. The enzyme were added and the reaction was started as reported in the II case study. The solutions were de-aerated under vacuum in order to remove small bubbles.

3.2.3. Sample preparation and frying conditions

Potato chips (10mm diameter and 3mm thickness) 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 2min (potato-to-water ratio 1:6 w/w).

Samples of potato strips were dipped in the coating suspensions for 5 minutes. In order to allow the film formation on potato surface, the potato strips were dried under air circulation, thought a desiccators tunnel (UOP 8 tray dried, Armfield, UK).at controlled air velocity was used.

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 (Tefal, 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 30 minutes prior to frying, and was replaced by fresh oil after four frying batches. Experiments for oil uptake were run in triplicate.

3.2.4. 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 %.

3.2.5. 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 1 mg.

3.2.6. 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 discussions

3.3.1. Lipid content determination

A limited range of coating solution concentrations has been studied due to the excessive polymerization of sodium caseinate as a result of transglutaminase which caused an agglomeration of the polymer.

The film formation on potatoes was obtained 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: 45 minutes for samples coated with sodium caseinate suspension at concentration of 6% and 60 minutes for samples coated with sodium caseinate suspension at concentration of 8%.

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 70% (after drying in tunnel) for sample coated with film solution at caseinate concentration of 8%.

Figure 1 reports the effect of coating formulation on oil uptake of French fried potato in absence and in presence of coating. As can note, during thermal process the potato oil absorption was of 11%, in presence of coating oil absorption seems to increase, even if from statistical point of view no differences were highlighted between samples.

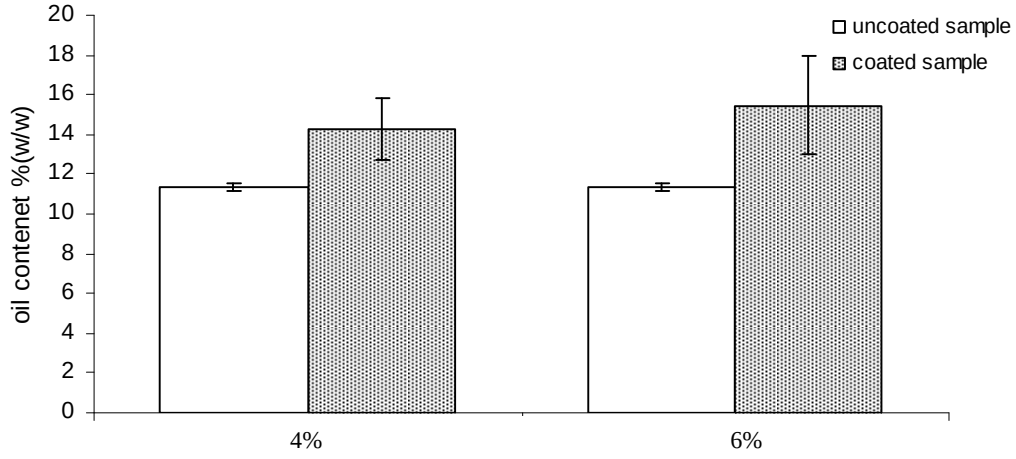

Figure 1. Effect of coating formulation on oil uptake of uncoated and coated fried potatoes.

As noted, because the coating acts as a barrier to the absorption of oil during frying is necessary that a continuous and homogeneous layer on the surface food is formed. In addition to the factors already considered, the formulation coating, such as concentration, viscosity (Albert and Mittal., 2002, Annapure et al., 1999) and temperature (Sanz et al., 2004), to which the coating was applied to the food can affect the absorption of oil during frying.

A possible reason to our result was a low concentration of coating solution and a consequent low viscosity. In this way, Albert and Mittal, (2002) investigated the effects of ten hydrocolloid materials, including gelatine, gellan gum, k-carrageenan-konjac-blend, locust bean gum, methyl cellulose (MC), microcrystalline cellulose, pectin (three types), sodium caseinate, soy protein isolate (SPI), vital wheat gluten, and whey protein isolate. The first part of work involved the selection of coating solutions on the basis of the formulation (concentration, pH, temperature) and of foods. The results showed that

only some formulations are effective in reducing the absorption of oil during frying, in comparison to blank samples. Garmakhany et al., (2008) reported that carboxymethyl cellulose coating solution was most effective at 1% than 0.5% and 1% for xantan respect 0.5%. These differences in adequate concentrations of coating formulations could be attributed, among other causes, to differences in adhesion between substrate and coating suspension, surface characteristics of the sample and frying conditions.

3.4. Conclusions

Preliminary results of this work show that the solutions of caseinate used are not adequate to reduce the absorption of oil during frying of potatoes. For this reason it is necessary to optimize the formulation of the coating in order to encourage the formation of a continuous film on the surface layer of potato.

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4. General conclusions

The results of this work have shown that dynamical mechanical analysis and scanning electronic microscopy together with mechanical and physical characterization are useful tools to study the relations between structure and properties of a edible film.

The film composition and the interaction among constituent play a crucial role on structure formation and thus on functional properties of the edible film.

In particular, in the first study case the role of an active additive on structure and functional properties of HPMC were studied. Results pointed out that the structure of HPMC film was affected by rosemary oil concentration and its effect was function of HPMC and oil concentration. On the base of preliminary results it was assessed that film forming solution in presence of essential oil were stable emulsion with similar rheological properties. Thus the difference among films at different HPMC and oil concentration can be related to a different behaviour of the film forming solution during the casting process. In fact, from the micrographs of the cross section of the film it was possible to suppose that for film at 2% of HPMC most of the oil migrated toward the evaporation surface and that the phenomena was most evident for 2%HPMC film at maximum oil concentration. In contrast, maybe for the high viscosity of HPMC solution, for 6%HPMC film, no destabilization phenomena occurred or occurred slowly during the film drying. Although these evident differences among samples, dynamical mechanical analysis results highlighted that mechanical properties depend only upon HPMC concentration: decreasing HPMC from 6% to 4%, E' decreased approximately by two order of magnitude, but no differences were observed between films at 4% and 2%. Whereas, the oil concentration did not have a statistically effect on E' modulus and loss tangent. Samples with different structures showed different barrier properties against water. The WVP of HPMC films increased as increased HPMC concentration for increased of hydrophilic groups. Rosemary oil improve water barrier properties only to 0.4% concentration oil of 6% HPMC films.

In the second study cases the interaction between polysaccharides (HPMC) and protein (CS) in presence of a cross linked agent (mTGase) were investigated. Results showed that the number of cross links in protein based films increased in presence of mTGase. Moreover, in blend films an enhancement in the link density within the polymer network was observed as protein concentration increased, but only for value higher that 50%. This means that, HPMC macromolecules were a limiting factor for protein-protein interaction, most probably because they act as filler into the protein network, and thus can reduce the probability that protein can cross-links.

These results were confirmed by mechanical characterizations of the blends films and solubility results. The solubility results highlighted that protein can enhance blend film solubility but only at specific ratio between polysaccharides and protein, maybe due to immiscibility lacunas. Mechanical results highlighted that the HPMC concentration inside the blends increased, increased the stiffness of the films and decreased the elongation at break.

In contrast, the different ratio of HPMC/CS film did not affected the permeability to water vapour of the films, showing that the density of cross links has not an effect on diffusion of the gas through the film, and that it is the hydrophilic nature of the polymer that play the major role in determine the barrier properties of the films.

These results were also confirmed by microscopy analysis that proved to be a useful tool for characterizing the structure of the films under study.

In the last study cases an application of one of the edible film studied was investigated. The objective was to assess the oil barrier properties of Sodium caseinate

(SC) cross linked with TGase coating on potato surface. The results obtained have to be considered preliminary and showed that the solutions of caseinate used are not adequate to reduce the absorption of oil during frying of potatoes. For this reason it is necessary to optimize the formulation of the coating in order to encourage the formation of a continuous film on the surface layer of potato.

In conclusion same reflection can be done concerning a future works:
film casting process parameter play an important role on the final structure of an edible films, mainly for emulsion film forming solution. Thus, a study on the influence of casting parameter (air velocity, temperature, relative humidity) as function of film composition on structural and functional properties of film can improve the knowledge on the relation between structure and functional properties of edible film.

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