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Development of biomaterials and their application to the preservation of fruit and vegetables

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To my family

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PART I

1. Introduction

Biobased food packaging materials have been defined as materials derived from renewable resources, thus excluding paper-based materials, since trees used for papermaking generally have renewal time of 25-65 years depending on species and country (Robertson, 2008). Biopolymers are largely used to produce biodegradable and edible films and coating that can improve product quality and/or reduce the problem of waste disposal due to the synthetic packaging (Krochta J.M. 2002). In recent years, in fact, the growing concern about the environmental impact of plastic waste, the increasing of the population and stress on limited resources have led to explore the uses of biopolymers. To harmonize national measures concerning the management of packaging and packaging waste and to prevent or reduce its impact on the environment Directive 94/62/EC was adopted; the aim was to provide a high level of environmental protection and to ensure the functioning of the internal market by avoiding obstacles to trade and distortion and restriction of competition. The latest revision of the Packaging and Packaging Waste Directive occurred on 29 April 2015 with the adoption of Directive (EU) 2015/720 of the European Parliament and of the Council amending Directive 94/62/EC as regards the consumption of lightweight plastic carrier bags.
The 94/62 EC Directive defined biodegradable materials as those that was adopted 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. As biodegradable materials, 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.
Edible films and coatings, such as was on fruit, have been largely used in the past to prevent loss of moisture and create a shiny fruit surface but the term edible film has been related to food application only in the past 50 years (Pavlath & Orts, 2009). Edible films and coatings offer potential to extend the shelf life of food; they can control the moisture, oxygen, carbon dioxide, flavour and aroma transfer between food components and the atmosphere sourrounding the product. (Avena-Bustillos & McHugh, 2012). The application of coatings to fruit and vegetables is a standard practice in Unites States and other countries with the aim to reduce water loss, slow senescence, impart shine and allow for better quality (Bai & Plotto, 2009).
In recent years there has been a large diffusion among the consumers of fresh cut fruit and vegetables; these products undergo to minimal processing that include all the operation such as trimming, peeling, washing and cutting; finally they are packed in order to offer consumers nutrition, convenience maintaining freshness (Torrieri et al., 2008). The influence of minimal processing unit operations causes disruptions of cells, and since the product remains biologically and physiologically active, there is a shifting of cellular processes and interactions in response to the damage, which induces an increase in respiration rate, transpiration and enzymatic activities after harvest (Lareo et al., 2009, Dea et al., 2011). During storage and distribution, the packaging of a fresh-cut produce plays a critical role in quality preservation and shelf-life extension.

1.1. Biomaterial for food packaging application

The polymers belong to the category of bio-based materials, in which organic carbon is derived exclusively from renewable biological resources. The class of biopolymers encompasses all the polymeric compounds produced by living organisms and are mainly represented by polysaccharides, proteins and nucleic acids. Generally proteins and polysaccharides have played a major role in the food industry, presenting a lot of application. Protein in food system could can be denaturated by heat, pH, whereas polysaccharides are branched or linear polymers of sugars; moreover polysaccharides may also be synthesized by microorganism such as exopolysaccharides (Telis, 2012). Among the many kind of polysaccharides, cellulose and chitin are the most important biomass resources, being the most abundant organic compounds on Earth. Biomaterial are not necessarily edible, not completely biodegradable according to official definitions, but their main advantage consists in the ease with which they can be eliminated (compostable) and in the low environmental impact. Biobased polymers can be classified in a number of ways, based on their chemical composition, origin, synthesis method, application etc. The traditional way of classifying biobased packaging materials has been to divide them into three categories based on their origin and production. Various naturally sources from which biopolymers can be extracted are shown in Figure 1.

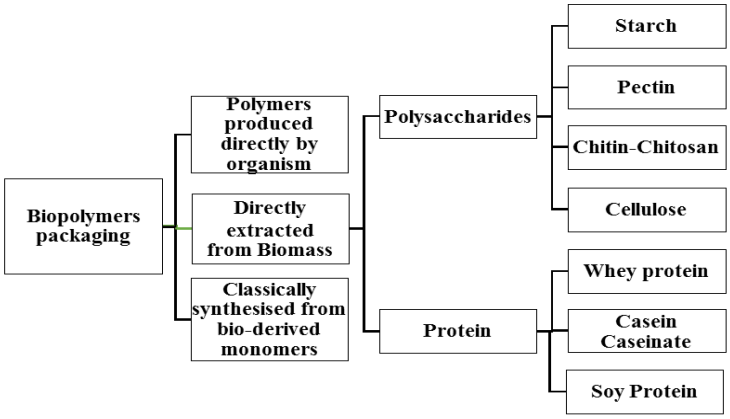

Figure 1. Different categories of bio-based material for edible and biodegradable films

- Polymers directly extracted/removed from biomass.

The polymers, most commonly available, are extracted from marine and agricultural animals and plants. Examples are polysaccharides such as cellulose, starch, and chitin and proteins such as casein, whey, collagen and soy. All these polymers are, by nature, hydrophilic and somewhat crystalline –factors causing processing and performance problems, especially in relation to packaging of moist products. On the other hand,
these polymers make materials with excellent gas barriers.

- Polymers produced by classical chemical synthesis using renewable biobased monomers. A good example is polylacticacid, a biopolyester polymerised from lactic acid monomers. The monomers themselves may be produced via fermentation of carbohydrate feedstock.
- Polymers produced by microorganisms or genetically modified bacteria. To date, this group of biobased polymers consists mainly of the polyhydroxyalkonoates, but developments with bacterial cellulose are in progress.

Edible films and coatings are produced from edible biopolymers and food-grade additives: hydrocolloids (such as proteins or polysaccharides), lipids (such as fatty acids, acylglycerols or waxes) and composites of them. The production of edible and biodegradable films by combining various polysaccharides, proteins and lipids is considered with the aim of taking advantages of the properties of each compound and the synergy between them (Falguera et al., 2011). Edible coatings and film are usually classified according to their structural material. They are a mixture of a high molecular weight polymer, solvent and a plasticizer but there are several ingredients such as antimicrobial compounds, antioxidants, flavours, colouring that can be incorporated (Krochta *et al.*, 1994).

1.1.1. Protein

Functional properties of proteins include the ability to form films and coatings. Proteins are polymers with specific amino acid sequences and molecular structure. Depending on the sequential order of the amino acids, the protein will assume different structures along the polymer chain which will determine the secondary, tertiary, and quaternary structures. The secondary, tertiary, and quaternary structures of proteins can be easily modified to optimize the protein configuration, protein interactions, and resulting film properties(Perez-Gago, 2012). Films and coatings based on proteins are edible or biodegradable, depending on formulation, formation method, and modification treatments (Krochta, 2002). 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 aminoacid functional groups, which can allow for property improvement and tailoring. Chemical changes can improve the stability of films and coatings (Dargaran et al., 2009). As long as food-grade proteins and additives are used and only protein changes due to heating, pH modification, salt addition, enzymatic modification, and water removal occur, the resulting film or coating is edible(Krochta and DeMulder-Johnston, 1997).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).
Generally, the protein used for fresh cut fruits and vegetables are whey protein (Yong Cho et al., 2002a; Anker et al., 2002; Shaw et al., 2002; Ferreira et al., 2009)), casein extracted from milk (Audic and Chaufer., 2005; Barreto et al., 2003; Belyamani et al., 2014; Pereda et al., 2008), soy protein (Pol et al., 2002; Yong Cho et al., 2002b; Yong Cho et al., 2007; Mariniello et al., 2003)fish protein (Bourtoom et a., 2006), egg protein (Gennadios et al., 1996; Wongsaulak et al., 2006; Di Pierro et al., 2006).

1.1.1.1. 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. (Perez-Gago, 2012). The primary structure of the four casein fractions contains many hydrophobic amino acid residues with non-polar sidechains (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, b-casein is the most hydrophobic, and αs2-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. 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 crosslinking ions, are examples of approaches used by investigators.

1.1.2. Polysaccharides

Polysaccharides are widely used as edible coatings and films; they 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 gelaqueous 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.
Polysaccharides can exhibit either a neutral charge (e.g., acetate esters, methylethers, 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 due to the hydrophilic nature 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. 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 (Dea et al., 2012; Gill & 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 (Tonget 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.,2008), agarose (Phan The et al., 2009a; Phan The et al., 2009b), arabinose (Phan The etal., 2009b) and alginate (Olivas eta l., 2008).

1.1.2.1. Chitosan

Chitosan is a naturally occurring polysaccharide whose commercial forms are essentially produced from N-deacetylation of chitin. Chitin is chemically composed of repeating units of 1,4-linked 2- deoxy-2-acetoamido-α-D-glucose, and chitosan refers to a family of partially N-acetylated 2-deoxy-2-amino-α-glucan polymers derived from chitin. The difference between chitin and chitosan is essentially related to the possibility to solubilize the polymer in dilute acidic media. Thus, when the structure can be dissolved in this kind of solvent, it corresponds to chitosan; in the

reverse case, to chitin. Therefore the degree of acetylation (DA), which is related to the balance between the two kinds of residues, is essential to define these two terms. When DA is below 60%, the polymer is soluble in dilute acidic solutions. This behaviour is related to the fact that the protonation of the amino groups of glucosamine residues contributes to the disruption of hydrogen bonding, the solvation of the cationic sites, and then to the solubilisation when the balance between solvent/polymer and polymer/polymer interactions becomes favourable. (Domard & Domard, 2002). When DA becomes over 60%, we enter the range of chitin and the chains become completely insoluble in water. This insolubility has to be related to the numerous hydrogen bonds occurring between the alcohol, amide, and ether functions distributed on the repeating units all along the polymer chains. They also correspond to hydrophobic interactions due to the presence of the methyl groups of the acetamide functions and to the -CH and $-CH_2$ of the glucosidic rings. Another important parameters to take into account is the molecular weight; for thermodynamic reasons, the solubility of neutral polymers is known to decrease with an increase of their molecular weight. The former behaviour has been identified in the case of high molecular weight chitosan, whose aggregation capacity increases with molecular weight.

Chitosan has been extensively studied for coating applications because of its film forming properties; by virtue of the attraction of oppositely charged molecules, chitosan, owing to its cationic polyelectrolyte nature, spontaneously forms water-insoluble complexes with anionic polyelectrolytes such as alginate, carrageenan, xanthan, various polyphosphates, and organic sulphates. This method is frequently used for enzyme immobilization into gel beads, which is achieved by adding an anionic polyelectrolyte solution containing the enzyme into an acidic chitosan solution (Soliva Fortuny et al.,2012). Polysaccharide films and coatings may be used to preserve the quality of several food commodities.

The oxygen and moisture barrier properties of these coatings can protect fresh fruit and vegetables from dehydration and, in some cases, even retard

their respiration rate. Polysaccharide coatings can also be used to prevent oxidation of lipid ingredients, or to reduce loss of food colours and flavours. Some of the most common applications of edible coatings for improving the quality and extending the shelf life of foods.

1.1.3. 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 non equilibrium (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 and 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).

1.1.4. Polymer solution

Knowledge of the role of protein-polysaccharide interactions, in relation to their functionality in complex multiphase systems, such as food mixed solutions, biopolymeric films or coatings, emulsions or gels, is still rather limited. Functional properties of food proteins, such as solubility, surface activity, conformational stability, gel-forming ability, emulsifying and foaming properties, are affected by their interactions with polysaccharides. Interactions of these biopolymers with each other and their competitive interactions with other system components (water, lipids, surfactant, metal ions, etc.) determine structure-property relationships in a food system such as bio polymeric packaging that differ strongly from those of the macromolecular reactants. Thermodynamics provides valuable information as to the direction in which 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:

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix} < 0 \quad (1)$$

where $\Delta Gmix$ (or G mixture- G pure components) is the free energy of mixing, $\Delta Hmix$ 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), $\Delta Hmix = 0$ (a thermal mixing), so the sing of $\Delta Gmix$ depends only on the entropic term.
For the so-called *regular solutions*, $\Delta Hmix$ is finite, and the free energy of mixing
takes this form:

$$\frac{\Delta G_{mix}}{N} = \Delta H_{mix} + RT(x_1 ln x_1 + x_2 ln x_2) \quad (2)$$

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 $\Delta Gmix$. 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:

$$\frac{\Delta G_{mix}}{N} = RT(x_{12}\phi_1\phi_2 + \phi_1 ln\phi_1 + \frac{\phi_2}{x} ln\phi_2 \quad (3)$$

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 $x12$ is called the Flory-Huggins interaction parameter and is equal to:

$$x_{12} = \frac{\Delta H_{mix}}{RTN_1\phi_2} \quad (4)$$

where $\Delta Hmix$ is the excess energy involved in neighbouring interaction, $N1$ 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:

$$X_{12_c} = \frac{1}{2} + \frac{1}{2x} + \frac{1}{\sqrt{x}} \quad (5)$$

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 (χ) of components. For monomeric mixture (χ = 1), $\chi 12c$ =2 , whereas for large polymer ($\chi \to \infty$) in solution, it approaches 1/2 . So if $\chi 12 <$ 1/ 2 the polymer should be soluble if amorphous and linear. For a mixture of two long polymers, it can be shown that $\chi 12c$ 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).

1.1.5. Consequence of mixing

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 1.2. 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)

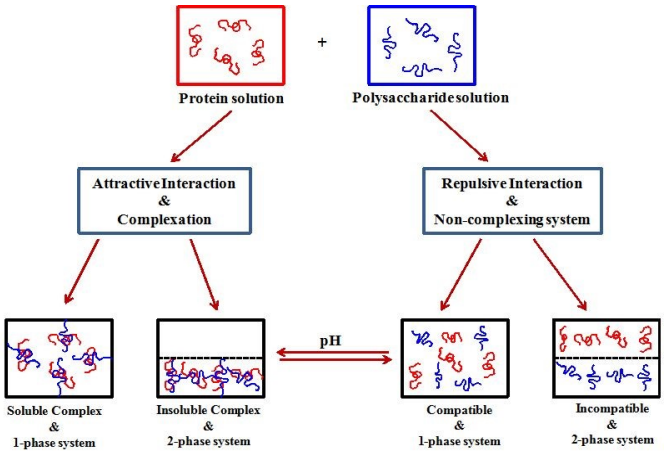

Figure 2. Main trends in the behaviour of protein/ polysaccharides mixture (from Gosh & Bandyopadhyay, 2012).

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 ($\Delta Gmix = \Delta Hmix - T\Delta S$) 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 inter biopolymer 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.

1.2. Definitions of edible films and coating

An edible coating is a thin layer of edible material applied in a liquid form and formed directly on food surface; *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).
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. Edible films and coatings should have several characteristic:

- To be non toxic and allergic
- Have good adhesion to the surface of food

- Control moisture loss
- Retarding senescence by maintaining internal equilibrium of gases involved in aerobic and anaerobic respiration
- Maintain or enhance aesthetics and sensory attributes of the product
- Not alter the taste or appearance of products
- Be easily manufactured and economically viable. (Pavlath and Orts, 2009).

1.3. Film forming technology

There are two technologies that can be used to make edible films and coatings: *wet* and *dry* (Han & Gennadios, 2005).
The *wet process* uses solvents for the dispersion of film-forming materials, followed by drying to remove the solvent and form a film structure . The choice of the solvent is very important; since the film-forming solution should be edible and biodegradable, only water, ethanol and their mixtures are appropriated as solvents (Han & Gennadios, 2005). With the exception of corn zein, wheat gluten, sorghum kafirin, and keratin, most film-forming proteins are soluble in water. Edible film and coating production that requires ethanol necessitates appropriate safety measures and attention to environmental release of solvent to the atmosphere (Krochta, 2002).
The *dry process* of edible film production does not use liquid solvent, such as water or alcohol. The heat is applied to the materials to increase the temperature to above the melting point of the film-forming materials, to cause them to flow; some of dry processes are molten casting, extrusion, and heat pressing. (Krochta, 2002).
Casting method is a technique used to produce industrial films, including nonedible 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).For formation of a film biopolymer powders are first dissolved in the solvent(water or other edible); Subsequently, the film forming solution may undergo heat treatment for or adjustments of pH a period of time. Degassing is an important step to eliminate bubble formation in the final film or coating. Then a controlled amount of solution is filtered and poured into a casting plate in order to allow to dry to a stand-alone film at a specific condition of temperature, relative humidity and air velocity in a specific time period (12 to 24 hours). Finally, the dried film is peeled from the plate and evaluated for various basic film properties. (Lee and Wan, 2006).
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 films. 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 flow ability of the film-forming material for compression molding can be low, while for extrusion, the material needs to have high flow ability. Because compression molding has very limited production amount, it is economical for small production (Lee and Wan, 2003).

1.3.1. Edible Coating formation

Coating application consists of applying a liquid or a powder ingredient onto a base product and can be obtained in different ways. The simplest way to apply a film is directly to form the solution; depending on concentration of coating solution, the product will absorb an appropriate amount of material necessary to form the desired layer, which when dried, forms a protective layer on food surface (Pavlath and Orts, 2009). Generally the application of coatings 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.
The *dipping method* lends itself to food products that require several applications of coating materials or require a uniform coating on a irregular surface. After dipping, excess coating material is allowed to drain from the product and it is then dried or allowed to solidify. Final formed coatings may be less uniform than coatings applied by other methods, and multiple dipping(with draining and drying steps between dipping operations) sometimes may be necessary to ensure full coverage (Krochta et al. 1994) .

Spraying method allows to obtain thinner, more uniform coating over a food surface than those applied by dipping. However, spray-coating requires that the bottom surface of the product be coated in a separate operation after application of the initial coating and drying. In this scenario, the product must then be turned to expose the bottom for subsequent coating application. Spray-coating is preferred for items possessing a large surface area (Dangaran et al., 2009). 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. The spraying nozzle plays acritical 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. Rheological properties

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

$$\sigma = \frac{F}{A} = E\varepsilon \tag{6}$$

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:

$$\tau = \eta \frac{d\gamma}{dt} \tag{7}$$

Where η is viscosity and dγ/dt is strain rate (ε).
Low molecular mass liquids and solution of low molar mass solutes are usually Newtonian, what means that the fluid viscosity is constant and independent of shear rate; a linear relationship is followed between shear stress (τ) and shear rate. 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 behaviour 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.
Aqueous solution of high-molecular mass polymers or polymer melts, and suspension of very fine particles are usually non-Newtonian; most dispersed systems, colloidal and non colloidal, show various non-Newtonian properties, such as shear thinning, shear-thickening, and time-dipendent viscosity changes. For non-Newtonian fluids, the relationship between shear stress (τ) and shear rate (γ) is not linear and a shear rate-dipendent viscosity is defined, which is called apparent viscosity, $\eta_{ap} = f(\gamma)$. Many food biopolymer suspensions present shear thinning (when η_{ap} decreases with increasing γ) or shear thickening behaviour (when η_{ap} increases with increasing γ). This kind of correlation between τ and γ can be satisfactorily model by a power low equation (8 and 9):

$$\tau = K\gamma^n \tag{8}$$

$$\eta = K\gamma^{n-1} \quad (9)$$

K is the consistent index and the exponent n is the behaviour index. When n<1 the fluid presents shear thinning behaviour, whereas when n>1 shear thickening is observed.
Most complex materials often yield stress fluids, that is, fluids that will not flow before the applied shear stress exceed a critical finite value characteristics of each product. This critical shear stress value is called yield stress τ_0 and these types of fluids are known as viscoplastics fluids; the model used to describe this behaviour is the Bingham plastic model (10) :

$$\tau = \tau_0 + \eta_p\gamma \quad (10)$$

this model describe the behaviour of a fluid with yield stress, but that presents a constant plastic viscosity (η_p) at $\tau > \tau_0$. Since most fluids that present yield stress are also shear-thinning, the Herchel-Bulkley model was proposed as an extension of power low to include yield stress:

$$\tau = \tau_0 + K\gamma^n \quad (11)$$

this resulted in a more general model, able to represent Newtonian, shear thinning, shear thickening and plastic behaviours, depending on the values of τ_0 and n.

1.4.1.1. Viscoelastic properties

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):

$$\varepsilon = \varepsilon_0 \sin(\omega t) \quad (12)$$

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:

$$\tau = G'\varepsilon_0 \sin(\omega t) \quad (13)$$

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 $\tau = \eta\varepsilon$, that is,

$$\tau = \eta\varepsilon_0\omega\cos(\omega t) \quad (14)$$

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:

$$\tau(t) = \varepsilon_0 G'(\omega)\sin(\omega t) + \varepsilon_0 G''(\omega)\cos(\omega t) \quad (15)$$

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).

1.4.2. 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 3.

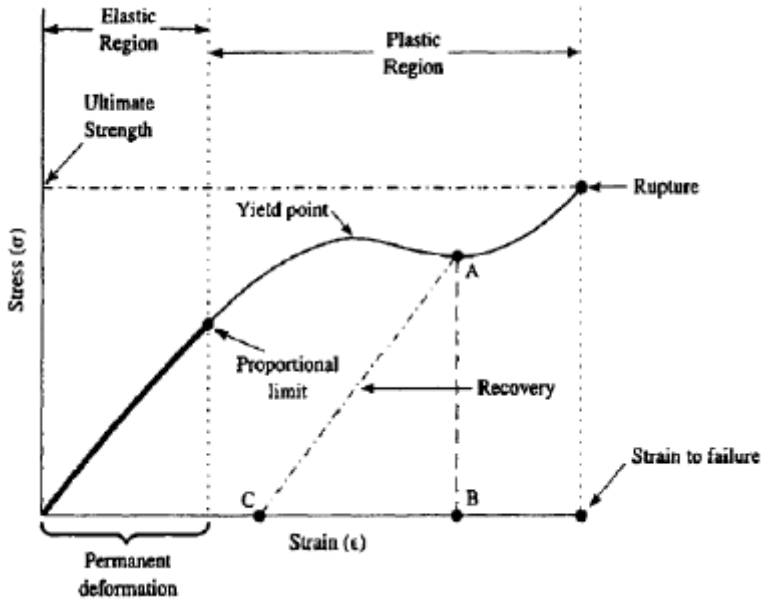

Figure 3. Stress strain behaviour of polymeric materials (from David and Mirsa 2001)

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.

1.4.3. 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 (C1) to the region where the concentration is lower (C2). 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:

$$J_{d=} - D\frac{\delta C}{\delta x} \quad (16)$$

where Jd , 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 analyse unsteady state diffusion with time t:

$$\frac{\delta C}{\delta t} = -D\frac{\delta^2 C}{\delta x^2} \quad (17)$$

When the steady state of diffusion has been reached, J is constant and eq. (16) 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 (16) for the case where C1 and C2 remain constant, the flux of the molecules in the steady state is given by equation:

$$J_d = \frac{Q}{A*t} = D\frac{(C_1 - C_2)}{L} \quad (18)$$

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 $m^{-2}s^{-1}$:

$$D = \frac{J_d * J}{\Delta C} = \frac{Q*L}{A*t*\Delta C} \quad (19)$$

Before gas diffuse through the packaging material from C1 to C2 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's law under conditions where the gas concentration is lower than its saturation concentration or maximum solubility:

$$p = \sigma X_s \quad (20)$$

Where p and Xs 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:

$$C_s = H^{-1}p_1 \quad (21)$$

Where p1 is the partial pressure of the gas on the high concentration (C1) 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:

$$Q = \frac{D*S(C_1 - C_2)At}{L} \quad (22)$$

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

$$\frac{Q}{t} = \frac{P}{L}A * (\Delta p) \quad (23)$$

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

1.5. Edible coatings for minimally processed fruit 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. The main factors that contribute to the deterioration of fruit and vegetables are respiration, transpiration and ethylene production.

Respiration. Any type of food undergoes continuously to biochemical, biological and physiological process; enzymatic reactions occur in fruits and vegetables during respiration where oxygen is consumed, carbon dioxide is produced, and heat and energy are released. Respiration is required to keep produce alive and to support any developmental changes. When oxygen composition is lowered, respiration decreases and hence, senescence does as well. However when oxygen concentration falls below threshold level anaerobic respiration occurs, which in turn causes ethanol production, off-flavour formation and loss of harvested produce (Kays & Paull 2004). Respiration activity of a product is influenced by storage temperature, type of processing, oxygen to carbon dioxide ratio, and absolute value of oxygen concentration itself (Pavlath & Ortis, 2009). Moreover some unit operation such as cutting and wounding can cause an increase in respiration, thereby increasing production of carbon dioxide and consumption of oxygen, further causing decrease in stored reserves.

Transpiration. One important factor, which is necessary to preserve quality of fruits and vegetables, is avoidance of water loss. Most of fresh produce contain from 65% to 85% of water when harvested. After harvested, the water supply is broken but loss of water continues; if weight loss exceed 5% of weight, produce will appear too shrunken to be saleable (Bai & Plotto, 2009). Cutting, slicing, and peeling, etc., of fruits and vegetables, will increase water transpiration rate due to exposure of tissue following removal of natural epidermal layer, and will also increase surface area of exposure (Toivonen & DeEll 2002). Water loss through transpiration cannot be replaced and is problematic, since loss of small amounts of water will severely impact produce quality. When water is lost, turgor of produce decreases, as does firmness; moreover water stress causes metabolic alterations changes in enzyme activation causing accelerated senescence, reduced flavour and aroma, decline in nutritional value, and increased susceptibility to chilling injury and pathogen invasion (Olivas & Barbosa-Canova, 2009). Respiration rate is affected by the difference between the water vapour pressure inside and outside the tissue, air movement, packaging or coating and surface area; the RH in the produce is almost 100% versus 90% to 95% in a well-maintained storage room and 30% to 80% on the market shelves; thus, to prevent the water loss, is important to keep the produce in a moist atmosphere as much as possible. Temperature plays an important role because higher temperatures raise water activity of molecules, increasing water loss. Moreover the faster the surrounding air moves, the quicker water is lost. Thus, the ideal storage condition should be as low temperature as possible (above chilling injury temperature) and relative humidity of 90%or above (Bai & Plotto, 2009). The water loss rate varies with the type of produce: generally, leafy green vegetables such as spinach and leaf lettuce, lose water quickly because they have a high surface-area to volume-ratio and a thin waxy cuticle with many pores; other multi-layered product, such as onion or iceberg lettuce, are carrying an inherent protection because water transport between leaves occurs via the dwarf stem.

Ethylene production. Ethylene is a hormone produced when vegetable or fruit undergoes stress. Ethylene triggers ripening and senescence, and is partially responsible for changes in flavour, colour and texture of fruits and vegetables (Kays & Paull 2004). On the other hand, removal of ethylene will slow ripening and senescence. Controlled atmosphere (CA) or modified atmosphere (MA) storage of produce can reduce ethylene production/action, preserving quality of fruits and vegetables for longer periods.

Edible coating improve the quality and extend the shelf life of minimally processed fruit and vegetables. Edible films or coatings can reduce respiration and, hence increase shelf life of a commodity. In selection of a coating, several considerations should be addressed to avoid extremely low oxygen concentration inside the commodity. Low oxygen concentration in the product could lead to anaerobic respiration, which can result in deterioration of product due to production of off-flavours and accelerated senescence (Kays and Paull 2004). Respiration and ripening of fruits and vegetables involve gas exchange with the environment. Carbon dioxide, oxygen, water and other metabolic by products, such as ethylene and other volatile compounds, are main substances exchanged during storage. Surface coatings modify gas exchange rate between environment and fresh produce, and thus control transpiration, respiration and other metabolic processes that lead to loss of quality.

Moreover edible films and coatings decrease water vapour transmission rate by forming a barrier on the fruit or vegetable surface; in case of minimally-processed fruits, there is usually very high water activity present at the surface, making it difficult to develop a coating that delays water loss, since capacity of films to work as barriers to water vapour decreases as water activity increases in the commodity (or relative humidity of the environment) (Hagenmaier and Shaw 1990).

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., 2009).

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3. Aim of the thesis

Recently the concept of using edible film and coating to extend the shelf-life of food has been increased. This success 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. Films and coatings are mainly made by polysaccharides, proteins and lipids; they can improve the quality and extend the shelf life of minimally processed fruit and vegetables by acting as a barrier to water loss and gas exchange, creating a micromodified atmosphere around the product.
This work, was organized in three different parts:

I Study case: **Protein-polysaccharides interaction: Characterization of Chitosan-Caseinate edible film**
The properties on sodium caseinate/chitosan based films at different ratio have been studied; film forming dispersion and film structure have been studied by rheological, dynamical mechanical analysis, and microstructure characterization. The properties of the film have been investigated by moisture sorption adsorption behaviour, water vapour permeability and mechanical analysis.

II Study case: **Condensation and Moisture Regulation in Fresh-cut Packaged Lettuce**
The second part aimed to understand the transpiration behaviour of a minimally processed product (iceberg lettuce) under different storage conditions and to quantify the amount of condensation and use it for designing a package to regulate moisture in headspace.

III Study case: Application of edible biopolymers coating on minimally processed fruit
The objective of the last part was to study an application on minimally processed fruit of the studied edible film. The effect of coatings (1% chitosan, chitosan 2%, sodium caseinate/chitosan blend) in combination with anti-browning agent (1% citric acid, 1% l-ascorbic acid agents, 1% $CaCl_2$) on minimally processed apple slices was studied during storage. The effect on respiration rate, moisture loss and changes in colour were studied.

PART II

I STUDY CASE

Protein-polysaccharides interaction: Characterization of Chitosan-Caseinate edible film

ABSTRACT

In this work aqueous sodium caseinate/chitosan (SC/CH) mixture were studied and used for edible film making The aim was to investigate the effect of SC/CH ratio on engineering properties and microstructure of the film.
Solutions of SC and CH, at different concentration, were mixed in a ratio of 1:1 to obtain 16 different mixture. Rheological behaviour of homogeneous mixture and of their film have been studied. Moreover microstructure, moisture sorption adsorption behaviour, water vapour permeability and mechanical properties of film have been also investigated. .
The stability test highlighted that at SC/CH ratio >2 a non-homogeneous mixtures were obtained. From viscosity curves of homogeneous mixtures, at increased SC/CH ratio from 0 to 2, it has been possible hypothesize the formation of polysaccharide-protein complexes, with different molecular structure.
Moreover film thickness, yellowness and greenness increased while the moisture content decreased with SC/CH ratio. Dynamic mechanical analysis showed that adding sodium caseinate to chitosan reduced film rigidity and increased the order of the relaxation function. Mechanical properties confirmed the positive role of SC on elastic modulus, tensile strength and elongation at break; the different ratio of SC/CH film water vapour permeability was negatively affected .

1.1. Introduction

Biopolymers, such as proteins and polysaccharides and their mixtures, have been extensively studied to prepare edible coatings or films to preserve quality and extend shelf life of food product (Andrade, et al., 2012; Bruno et al., 2008; Gennadios, 2002; Giancone et al., 2008; Giancone et al., 2009; Malmiri et al., 2012; Moreira et al., 2011; Poverenov et al., 2014). Nowaday, the performance of packaging materials obtained from biopolymers are still far from that based on common polymer. Nevertheless, they show interesting properties, such us CO2/O2 permeo selectivity, that for fruit and vegetable is as important as the gases permeability factors per se(Mahajan et al., 2007; Mensitieri et al., 2011). Thus, further research is needed in order to understand the basic structural features that underlie functional properties: knowledge on of the effect of composition on film structure and properties is valuable in the modulation of the functional properties of edible film required for food packaging.

In this work the structure and functional properties of sodium caseinate/chitosan mixtures were investigated.

Chitosan is a copolymer of d-glucosamine and N-acetyl-d-glucosamine with β-(1–4) linkage; it derived from chitin by alkaline or enzymatic deacetylation, which is the structural element in the exoskeleton of crustaceans as well as a cell wall constituent of fungi and green algae and after cellulose, is the most abundant polymer in the world (Arvanitoyannis, 2008).

Chitosan has been extensively studied for coating applications because of its film-forming properties, biodegradability, selective permeability to gases, good mechanical properties but quite high water vapour permeability (Elsabee and Abdou, 2013). Moreover several studies have been showed that chitosan is characterized by an antimicrobial activity alone or in combination with other biopolimers (Beverlya et al., 2008; Lago et al., 2014; Leceta et al., 2013; Liu et al., 2009; Martinez-Camacho et al., 2010; Moreira et al., 2011; Shen et al., 2010;Vasconez et al., 2009). In an acid environment, the amino group $NH_2$of chitosan can be protonated to NH3+ forming electrostatic interactions with anionic groups of other biopolymers; this property has been applied to improve the functional properties of chitosan by combining it with other biopolymers, such as polysaccharides and proteins (Xu et al., 2005; Moreira et al., 2011; Pereda et al., 2008, 2009; Pinotti el al., 2007; Kurek et al., 2014).

Milk proteins, such as caseinates, have special properties which make them highly suitable for biopolymer films. Sodium caseinate films are colourless, tasteless, odourless, transparent, flexible, highly impermeable to oil and oxygen and resistant to thermal denaturation (Schou et al., 2005).Recently, Belyamani, et al. (2014) showed that by using twin-screw extrusion, it was possible to produce thermoplastic pellets from plasticized sodium caseinate to make caseinate based edible films.

Although the properties of composite films based on caseinate or chitosan have been studied in several publications, there are few published data on caseinate-chitosan films. Pereda et al. (2007)have characterized edible film made from sodium caseinate and chitosan, at one concentration and at ratio 1:1, by chemical FT-IR, mechanical and thermogravimetric analysis and showed that polyelectrolyte complex between the cationic chitosan ($NH3^+$ groups) and the anionic caseinate (COO^- groups) generating a strong interaction between two main component with a positive effect on the mechanical film properties. Moreover, the same authors studied the water vapour absorption and the permeability of films and showed that chitosan films showed the lowest rate of moisture sorption but reached a higher equilibrium moisture content, whereas the chitosan/caseinate ones exhibited the opposite behaviour due to the strong interactions developed in the polyelectrolyte complex which lead to fewer sites in the polymer matrix at which water can be held(Pereda et al., 2008).

Khwaldia et al. (2014) studied caseinate/chitosan film to develop a coating for improve the mechanical and barrier properties of a paper packaging. By studying the chemical and thermal properties of the blend film, the authors showed that the complexation occurred via carboxylate and phosphate groups with removal of Na^+ ions and that the ratio between the two biopolymer have an effect on their interaction.

The critical role of nature of constituents and ratio between constituents on the structure and physical properties of the blend film has also been highlighted in previous work on pectin-soy protein and HPMC-sodium caseinate blend film (Bruno et al., 2008; Giancone et al., 2008, 2009; Perone et al., 2014).

The objective of the present work was to investigate how different ratio between caseinate and chitosan have an effect on biopolymer interaction and in turn on structure and physical propertiesof the obtained blend films.To this end, film forming dispersion and film structure have been studied by rheological, dynamical mechanical analysis and microstructure characterization. The properties of the film have been investigated by moisture sorption adsorption behaviour, water vapour permeability and mechanical analysis.

1.2. Materials and Methods

1.2.1. Materials

Chitosan (MMW, deacetylation degree75-85%), sodium caseinate, glycerol, sodium hydroxide, TRIS were purchased from Sigma-Aldrich (Milan, Italy); acetic acid (99-100% glacial) was obtain from J.T, Backer (USA).

1.2.2. Preparation of film forming solution

Sodium caseinate (SC) solutions with protein concentration of 0.5%, 1.0%, 1.5% and 2.0% (g/100mL),were obtained by dispersing SC powder in a buffer solution (TRIS 0,02M, pH=8) and stirring continuously for 4 h at room temperature; glycerol (GLY) was added as plasticizer to obtain GLY/SC weight ratio of 0.1.
Chitosan (CH) solutions with a concentration of 0,25%, 0.50%, 0,75%, 1.0% (g/100mL) were prepared by dissolving the powder in acid acetic solution (1% v/v, pH=3,3) and stirring over night at room temperature to obtain complete dispersion of chitosan. An exact amount of glycerol was added to achieve GLY/CH weight ratio of 0.1. In order to eliminate solid impurities, the dispersion was centrifuged at 3500 rpm for 10 min. Preparation of the polyelectrolyte complex between SC and CH required a careful control of the solution pH. SC is remarkably heat-stable at pH = 6.5 and highly insoluble at the isoelectric point, pH between 3.8 and 4.0. CH shows best solubility in a 1% (v/v) acetic acid solution (pH = 4.4), but remains soluble at pH lower than 6.4 (Moreira et al., 2011). In this work, the pH of the CH dispersion was adjusted to 5.0 using NaOH 1M.
Film forming solution were obtained by mixing all solutions of SC and CH, in a 1:1 ratio for a total of 16 samples (table 1). All mixtures were stirred for 1h, then were degassed under vacuum for 15 min to remove air bubbles.

1.2.3. Film forming solution (FFS) stability test

SC/CH mixture were placed in corning tubes to observe any sedimentation, flocculation or phase separation, during 30 days. Subsequently,10 mL of each samples were centrifuged at 3500 rpm for 10 min, to accelerate any separation phenomena. Only stable samples (see paragraph 1.3.1.) were used for the further investigations.

1.2.4. Rheological properties of FFS

The FFS viscosity was measured using a strain controlled rheometer (RFS II, Rheometrics Inc. Piscataway, NY) equipped with coaxial cylinders (34mm o.d.; 32mm i.d.). Measurements were performed at 25°C, by increasing the shear rate from 0.01 to 100 s^{-1}. All measurement were replicated three times. In addition, the viscosity measurement of a protein solution, at 3%, and a chitosan solution, at 1%, was carried out. The viscosity curves were modelled according to the power law:

$$\tau = K\gamma^n \quad (1.1)$$

K ($Pa*s^n$) is a consistent index, and n (dimensionless) is a flow behaviour index suggesting if the liquid is Newtonian (n=1), pseudoplastic (n<1) or dilatant (n>1) (Steffe, 1992).

1.2.5. Film-making Procedure

Films were obtained by casting, as reported in a previous work (Perone et al., 2014). 20 ml of FFS were poured into Petri dishes (Area=56,7 cm^2) and allowed to dry at 40°C and 50% relative humidity (RH) for 16 h in circulating air. The dried films were peeled from the Petri dishes and stored at 20°C and 50% RH prior to testing.

1.2.6. Film Colour, Thickness and Moisture Content

The colour of the film was determined using a colorimeter (Minolta Chroma Meter, CR 300, Japan). The Hunter parameters L* (from 0=black to 100=white), a* (- a*= greenness to + a*= redness), and b* (-b*=blueness to +b*= yellowness) were measured and averaged from three randomly position for each sample. The total colour difference (ΔE) was calculated according to the following equation (Ghorpade, Li, Gennadios, & Hanna, 1995):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1.2)$$

Where ΔL, Δa*,Δb* are the difference between a sample colour parameter and the colour parameter of a standard (L = 96.94, a= +0.23, b=+1.85) used as background.
Film thickness were measured using a micrometer model HO62 (Metacontrol Srl, Napoli, Italia) with a sensitivity of ±2μm. Film strips were placed between the jaws of the micrometer and the gap reduced until the first indication of contact. Mean thickness (μm) of films was determined by averaging 8 measurements at different locations.
The moisture content of films at the end of drying process was determined gravimetrically by drying film samples (0.4g) at 105 °C in an oven for an adequate time to achieve a constant weight. Moisture value was expressed as a percentage of water compared to the total weight of the sample.

1.2.7. Microstructure

The fracture surfaces (transversal area), obtained after immersing film samples in liquid nitrogen (fragile fracture), were observed using a scanning electron microscope (LEO EVO40, Zeiss, Oberkochen, Germany). Film were mounted on bronze stubs using a double-sided tape and then coated on specimen stubs with the cross-section oriented upward, with a thin layer of gold by a DC sputter coater (AGAR B7340, Agar Scientific Ltd, Stansted, UK). Digital images of film cross-sections were collected at a tilt angle of 0° to the electron beam using an acceleration voltage of 20 kV.

1.2.8. Mechanical Analyses

Tensile properties of the films were measured by Dynamic mechanical analyses and by tensile test according to Standard Test Method for Tensile Properties of Thin Plastic Sheeting (Method D882, ASTM (1991)).
Dynamic mechanical analyses were performed using a DMTA V (Rheometrics Inc., Piscataway, USA) on rectangular film strips (20x7mm). Each sample was cut with scissors and mounted on grips so that its effective length was 10 mm. 3 minutes delay was assumed to relax the stress induced during sample loading. All the samples were submitted to a strain sweep test at a given frequency (ω) of 1 rad s^{-1} to determine the linear viscoelastic region. Then, a frequency sweep test was carried out applying a strain amplitude (ε) of 0,01% (within the linear viscoelastic region) and increasing the frequency from 10^{-2} a 10^{3} rad s^{-1}. the storage modulus (E'), and tangent delta (tanδ) were monitored All measurements were replicated 5 times.
Storage and tangent delta were modeled by using the reduced form of the Friedich and Heimann model (1988) as reported by Giancone et al. (2008):

$$E' = E_{\infty,\alpha} + \sqrt{\frac{2}{\pi}}\, E_{\alpha}\, \cos\left(\frac{\pi}{2}\alpha\right)\omega^{\alpha} \tag{1.3}$$

$$\tan(\delta) = \frac{E''}{E'} = \tan\left(\frac{\pi}{2}\alpha\right) \tag{1.4}$$

where α is the order of the relaxation function, $E_{\infty,\alpha}$, and E_{α} are, respectively, the equilibrium modulus and a material shear parameter pertaining to α.
Tensile test were carried out at room temperature using an Instron Universal Model No 4301 (Instron Engineering Corp., Canton, MA) equipped with a 1000N load cell. Film samples were cut into 25 wide and 100-mm long strips using a sharp razor blade. Young modulus (EM), tensile strength (TS) and elongation at break (ε%) were calculated. Results are reported as the average of ten replications for each sample.

1.2.9. Adsorption isotherm

The water vapour sorption isotherm was determined at 20°C by using a standard gravimetric methodology; films were cut in pieces of 3 cm x 1 cm and dried in a desiccator containing silica gel for two weeks. Rectangular samples (in triplicate) were placed into desiccators containing saturated solution of Lithium Chloride, Potassium Carbonate, Magnesium Nitrate, Sodium Chloride, Potassium Chloride and Potassium Sulfate, in order to reach known water activities equal to 0.11, 0.43, 0.52, 0.74, 0.85, 0.97, respectively (Greenspan, 1977). The films were weighted periodically until constant weight was achieved. The moisture content of the equilibrated samples was calculated after drying at 105 ºC during 24 hours.
In this study the recognized Guggenhiem-Anderson- deBoer (GAB) (Eq. 1.5), and Peleg (Eq. 1.6), equations were employed to fit the experimental data. These models were explained and rearranged as given below:

$$X = \frac{x_m C a_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \tag{1.5}$$

$$X = k_1 aw^{n1} + k_2 aw^{n2} \tag{1.6}$$

Where x_m is the monolayer moisture content, aw is the water activity, C, K, k_1, k_2, n_1 and n_2 are model parameters.

1.2.10. Water vapour permeability

Water Vapour Permeability (WVP) of films was evaluated by the gravimetric test according to ASTM E96 (1993) by means of a Payne Permeability cup (Carlo Erba, Milan, Italy). Eight grams of silica gel were introduced into each cup. A film sample with a 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 surface exposed to vapour transmission was 9.89 cm^2. The cups were weighed and then placed in desiccators containing a saturated KCl solution (aw= 0.85±0.003). The desiccator was stored in a

Heareus thermostated incubator (binder KBF240, Turin, Italy) at 20°C.Cups were weighed at scheduled times, and the 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 function of the time. It was assumed that the steady state was reached once the regression analysis based on the last four data points resulted in $R^2 \geq 0.998$.Adopting the method proposed by McHugh, et al., (1993), the experimental WVTR data were used to determine the vapour pressure on the inner surface of the film (p2) to correct the effect of concentration gradients established in the stagnant air gap inside the cup:

$$WVTR = \frac{P \cdot D \cdot Ln\left[\frac{P - P_2}{P - P_1}\right]}{R \cdot T \cdot \Delta z} \tag{1.7}$$

where P is the total pressure, D is the diffusivity of water through air at 20°C (the D value was 2.4 x $10^{-5}m^2/s$ (Crank, 1976)), 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 and P_2 is the corrected water vapour pressure on the film's inner surface in the cup. The permeance was then calculated as follows:

$$Permeance = \frac{WVTR}{(P_2 - P_3)} \tag{1.8}$$

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 the average of three replications of each sample.

1.2.11. Data analysis

To assess the effect of the SC/CH ratio on structural and functional properties of SC/CH films, four SC/CH ratios (0; 0.5; 1; 2) were tested, each sample level being replicated three times. To assess the effect of solid surface content on film structural and functional properties, the ratio SC/CH equal to 2 was obtained at two different biopolymer concentration (0.75g/100mL SC and 1,5 g/100mL CH; 1 g/100mL SC and g/100mL CH). Data were submitted to analysis of variance by means of SPSS v17.0 for Windows (SPSS, Milan, Italy). Duncan's test was carried out to find the source of the significant differences within the samples examined; Significance of difference was defined at $p<0.05$. The root-mean-square error (RMSE) has been used as the indicator for the accuracy of the fit of the models reported in the equations 1, 3, 4, 6 and 7:

$$RMSE = \sqrt{\frac{\sum(Me - Mp)^2}{n}} \tag{1.9}$$

where M_e is the experimental value, M_p is the predicted value, and n is the number of data points.

1.3. Results and discussion

1.3.1. Stability test

All the film forming solution obtained are shown in Table 1.

Table 1. Film forming solution. (CH=chitosan; SC=sodium caseinate; Gly=glycerol)

Mixture	Concentration, g/100ml			Phase separation		Ratio
	CH	SC	Gly	Day 0	Day 7	SC/CH
A1	0,25	0,5	0,075	✓		2.00
A2	0,25	1	0,125	✓		4
A3	0,25	1,5	0,175	✓		6
A4	0,25	2	0,225	✓		8
B1	0,5	0,5	0,1			1
B2	0,5	1	0,15		✓	2
B3	0,5	1,5	0,2	✓		3
B4	0,5	2	0,25	✓		4
C1	0,75	0,5	0,125			0,67
C2	0,75	1	0,175			1,33
C3	0,75	1,5	0,225			2
C4	0,75	2	0,275		✓	2,67
D1	1	0,5	0,15			0,5
D2	1	1	0,2			1
D3	1	1,5	0,25			1,5
D4	1	2	0,3			2

The film forming solution (FFS) with a SC/CH ratio >2 gave rise to non-homogeneous solutions due to opaque and white flocs and a transparent liquid phase, characterized by a soft yellow colour. Samples A1, A2, A3, A4, B3 and B4 were not stable at room temperature immediately after mixing; samples B2 and C4 showed phase separation after 7 days whereas samples B1, C1, C2, C3, D1, D2, D3, D4 were stable (table 1).Chitosan concentration played an important role in bringing about stability, in fact although SC/CH ratio was 2 for A1, B2, C3 and D4,phase separation didn't occur only for C3 and D4, in which chitosan concentration was higher than 0.5 g/100 ml. According to Anal et al. (2008), solutions with a controlled pH values equal to 5.3 to 5.4 and with a ratio SC/CH ranging from 0.5 to 2 were stable and after 7 days these solutions were opaques and milk-white without flocs, sedimentation or phase separation.
All the solutions, subjected to centrifugation, presented a more or less pronounced precipitate depending on the amount of dissolved solids; it outlines that the solutions were metastable.
Among metastable films, it has been chosen to study blend film at chitosan concentration equal to 1 and SC/CH ratio of 0.5, 1 and 2 (samples D1, D2, and D4). Moreover, it has also been chosen to study the samples C3 to investigate the effect of biopolymer concentration of film structure and functional properties...

1.3.2. Rheological properties

The viscosity curves of SC/CH blend are reported in figure 1

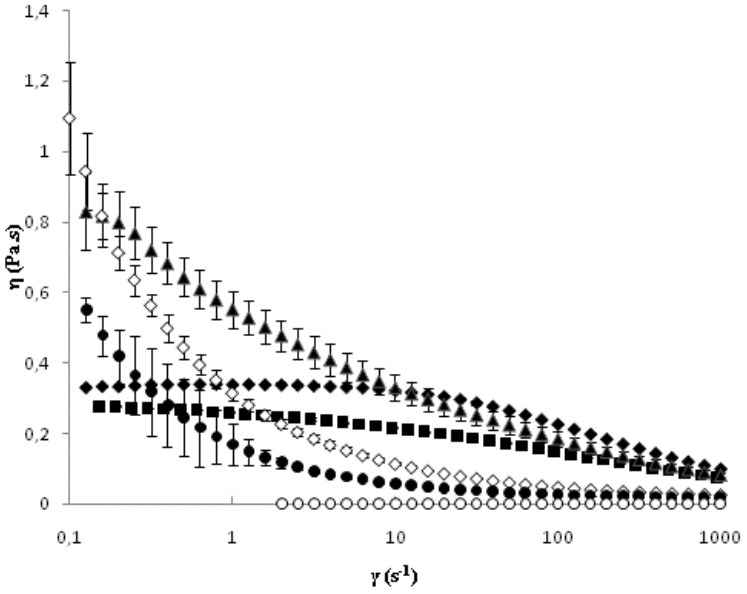


Figure 1. Viscosity curves of SC/CH blend at different ratio: CH 1% (♦), D1 (■), D2 (▲), D4 (◊), C3 (●), Cas 3% (○)

Stock solutions of CH1%, and SC3% had different rheological behaviours: SC solution exhibited a Newtonian behaviour , with a low viscosity, than CH solution behaves as a shear thinning liquid.
The protein-polysaccharide solutions presented a shear thinning behaviour. The curve with the lowest SC/CH ratio (D1) had a trend similar to CH1% but with lower viscosity values. D2 sample did not present the first Newtonian plateau and, for shear rate below 10 s^{-1},the viscosity was higher than the other samples. Instead, the viscosity curve of C3 and D4 samples showed a descending portion and the second Newtonian plateau. Moreover, the viscosity increased as increased the surface solid content: D4 and C3 had the same SC/CH ratio equal to 2 but D4 had higher viscosity than C3. Protein-polysaccharide complex formation could justify the different rheological behaviour reported. ANOVA showed that the SC/CH ratio have a significant effect on both rheological parameters, k and n. A part for the sample D2, increasing the SC/CH ratio, decreased the k value and increased the n index (Table 2).
Rheological behaviour is reported in Figure 1 .

Table 2. Consistent index (k), flow behaviour index (n) of SC/CH film forming solution at different ratio

Sample	K(Pa.s^n)	n	RMSE	η(100s^{-1})
CH1%	1,073±0,012^e	0,659±0,001^b	1,218±0,007	0,227±0,002
D1	0,58±0,010^c	0,705±0,004^e	0,620±0,053	0,002±0,001
D2	0,890±0,115^d	0,657±0,009^b	0,528±0,087	0,066±0,001
D4	0,163±0,005^b	0,736±0,004^c	0,202±0,011	0,184±0,017
C3	0,071±0,012^a	0,789±0,029^d	0,127±0,045	0,048
Cas3%	0,002^a	1,078±0,032^f	0,008	0,026

^{a,b,c,d,e} Different letters indicate significant differences ($p<0.05$)

It is known that the molecular mass of biopolymers affects the dependence of viscosity on shear rate (Williams & Phillips, 2000), so this result could be attributed to the formation of complexes between CH and SC which may break by increasing shear rate, reducing the viscosity. Thus, it's probably that due to the low presence of SC in D1, didn't occur a formation of a considerable soluble complex and the sample viscosity depended on the CH concentration available in solution. Similar results were reported by Zinoviadou et al. (2012) for Oil-in-water emulsions (10%, w/w, oil) stabilized by SC/CH complexes; in absence of chitosan, sodium caseinate showed a Newtonian behaviour with low apparent viscosity, but all SC/CH containing emulsions exhibited a slight shear thinning behaviour. Moreover, above a

concentration of 2% (w/w) a significant increase of viscosity over entire range of shear rate was observed. At rate over 0.4 s^{-1}, a pseudoplastic behaviour was observed for chitosan and chitosan-corn starch film forming solution with and without polyphenol-rich aqueous extract from murta (Silva Weiss et al., 2013). Furthermore, film forming solution with chitosan and tapioca starch showed a non–Newtonian behaviour and in particular a pseudoplastic behaviour (n<1), with an apparent viscosity highly dependent on the shear rate (Chillo et al., 2008). Moreover Speiciene et al. (2007), studied rheological properties of oil–in-water emulsion (40% of rapeseed oil) containing whey protein isolate (4%) and chitosan (0-0.5%) showing that in absence of chitosan, the emulsion exhibited close to Newtonian behaviour; the flow behaviour indicated that at increasing chitosan concentration, pseudoplasticity has proved, according with our results.

1.3.3. Film colour, thickness and moisture content

The chitosan film resulted homogenous and transparent whereas the blend presented a light yellow hue; the colour parameters are summarized in Table 3.

Table 3. Colour prameters of the SC/CH film at different ratio

Sample	SC/CH	L*	a*	b*	ΔE
CH1%	0	99.84 ± 0.62^{d}	0.74 ± 0.11^{c}	0.21 ± 0.61^{a}	3.41 ± 0.70^{a}
C3	2	95.01 ± 0.41^{a}	$(-0.75)\pm0.05^{ab}$	7.43 ± 0.48^{bc}	6.00 ± 0.47^{cd}
D1	0,5	96.48 ± 0.70^{bc}	$(-0.43)\pm0.48^{b}$	6.06 ± 0.36^{b}	4.35 ± 0.47^{ab}
D2	1	96.28 ± 0.81^{b}	$(-0.79)\pm0.10^{ab}$	6.90 ± 0.61^{bc}	5.24 ± 0.73^{bc}
D4	2	97.38 ± 1.44^{c}	$(-0.98)\pm0.49^{d}$	8.31 ± 2.40^{c}	6 ± 2^{d}

^{a,b,c,d} Different letters indicate significant differences ($p<0.05$)

The lightness value (L*) of chitosan films is 99.8±0.6. In presence of SC, the lightness decreased to an average value of 95±0.4. The colorimetric parameter a* and b* decreased by increasing SC/CH ratio, indicating that the protein increased the yellowness and the greenness. ΔE, a global indicator of colour changes, varied from a minimum of 3.4±0.7 for pure chitosan to a maximum of 6±2 for D4. ANOVA analysis showed that SC/CH ratios ≥ 1 caused a significant increase of ΔE.
The thickness of film is reported in table 4:

Table 4. Thickness of the SC/CH film at different ratio

Sample	SC/CH	Δx (μm)
CH1%	0	24.1 ± 1.9^{a}
C3	2	71.9 ± 4.5^{e}
D1	0,5	43.7 ± 5.9^{b}
D2	1	59.3 ± 4.4^{c}
D4	2	76.2 ± 5.5^{d}

^{a,b,c,d,e} Different letters indicate significant differences ($p<0.05$)

Our results showed that by increasing the SC/CH ratio, the thickness of the film increased ($p<0.05$).Our results are in accordance with Giancone et al., 2008, who reported that increasing the solid surface density a denser structure is obtained due to the higher solid concentration. Only for sample D4 the increment of thickness did not followed a linearity with the solid surface density and this can be justified by the electrostatic interaction between the cationic groups of CH and anionic groups of SC, that might form matrices with more complexes arranging in less space taking up a smaller volume than expected in absence of polyelectrolyte complex.
The SC/CH ratio has a significant effect on the moisture content of the films, as shown in Table 5:

Table 5. Moisture content of the SC/CH film at different ratio

Sample	SC/CH	RH (%)
CH1%	0	15.7[b]
C3	2	18,7[c]
D1	0,5	20,15[d]
D2	1	15,73[b]
D4	2	13,15[a]

[a,b,c,d] Different letters indicate significant differences ($p<0.05$)

The moisture content changed from a minimum of 13% to a maximum of 20%. Sample D4 showed the lowest value of moisture content. Differences in moisture content could be justified by the formation of protein-polysaccharide complexes with different binding water capacity.

1.3.4. Microstructure

Figure 2 shows the cross-section micrographs of CH and SC/CH based film at different SC/CH ratios.

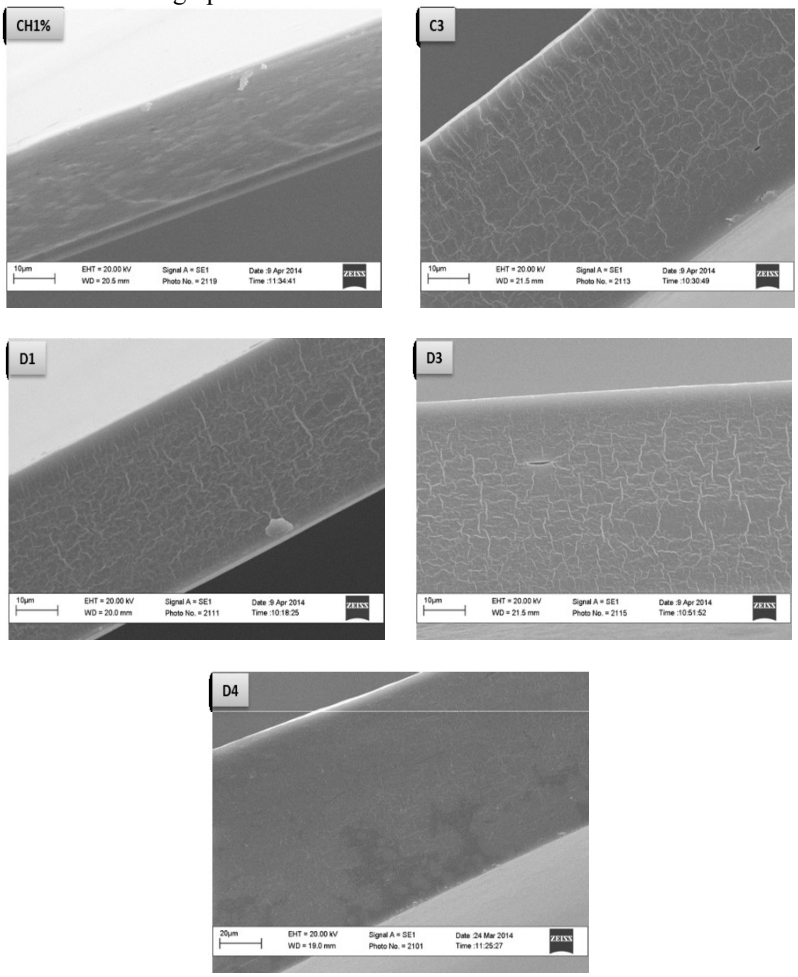


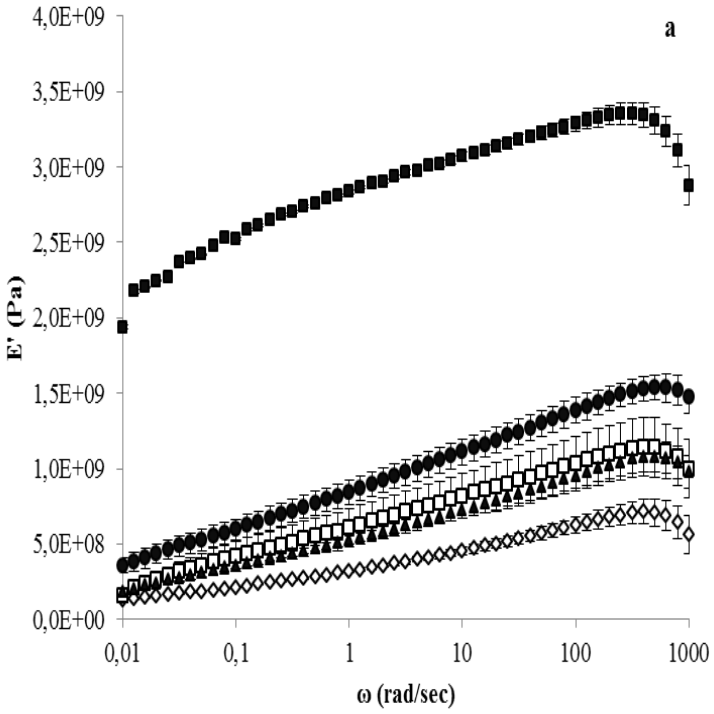
Figure 2. SEM micrographs of SC/CH films at different ratio. Magnification 4000x (Ch1%, C3, D1, D3), 2000x (D4)

CH and SC/CH blend cross sections were characterized by a compact, uniform and homogeneous structure, although some particles inside the films are presented; this might attributed to some impurities present in powder material or to non-dissolved powder grain. The samples D4 showed the more compact structure with low surface imperfection. Similar results were obtained for chitosan/gelatin film (Rivero et al., 2009), chitosan/whey protein film (Kurek et al., 2014) and hydroxypropyl methyl cellulose/sodium caseinate film (Perone et al., 2014).

1.3.5. Mechanical properties

1.3.5.1 Frequency sweep testing

Figure 3 shows the dependence of storage modulus (E') and loss tangent (tan δ) on ω of CH and SC/CH blend.



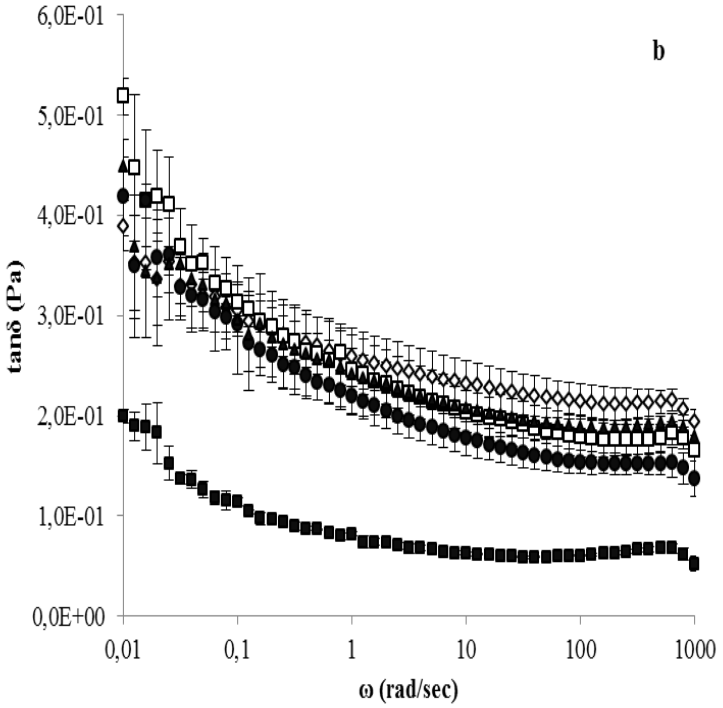


Figure 3. Elastic modulus (E') (a) and loss tangent (tan δ)(b) versus angular frequency (ω) of SC/CH films at different ratio: CH 1% (■), D1 (□), D2 (▲), D4 (●), C3 (◊).

By increasing ω from 10^{-2} to 10^{3} rad s^{-1} E' increased for all the films; CH1% had the highest E' value, ranged from 1.94×10^{9} to 2.88×10^{9}, whereas all the blend had a lower E' value. Comparing C3 and D4 an evident reduction of E' was observed for C3 due to the different solid surface content even if they had the same ratio SC/CH equal to 2. The loss tangent values were less than one and decreased as ω increased from 10^{-2} to 10^{3} rad s^{-1}; no great differences were observed for D1 and D2 whereas the dependence of tan δ by ω was higher for D4. These results suggest that the structure of the films changes according to the composition, due to the interaction that can occur between CH and SC in different ratio and solid concentration; in fact the higher the presence of SC a strongest network is obtained. To obtain more information on the film networks, the curves were described by Friederich and Heymann model (1988). Based on the model, the three-dimensional film structure may be described in terms of the order of relaxation function (α) and the elasticity coefficient E_α (Giancone et al., 2008); α could be related to the number of interactions within the network, whereas E_α is related to the rigidity network. Fig 4 A shows the α parameter of the model as function of SC/CH ratio.

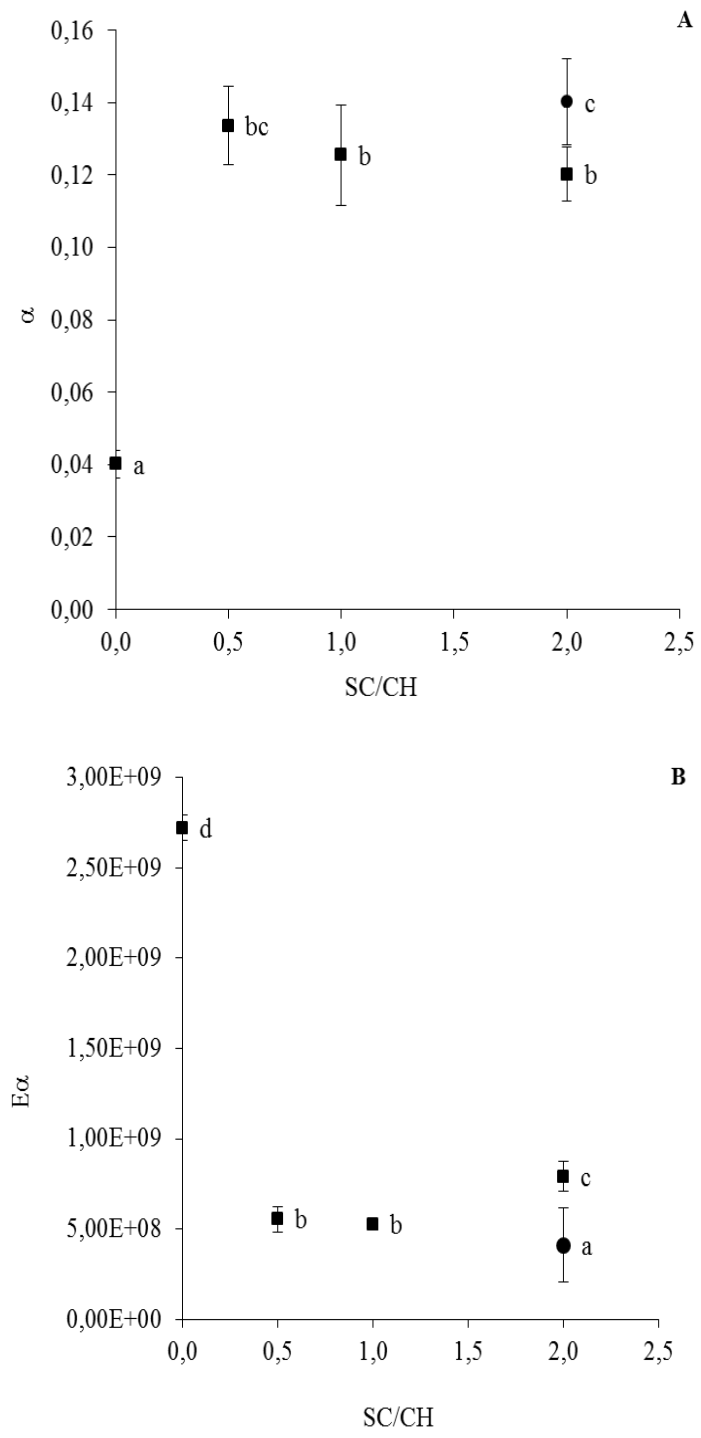


Figure 4. Effect of the SC/CH ratio on the relaxation function (A) and on the elasticity coefficient (B). Samples with SC/CH ratio equal to 0 (CH1%), 0.5 (D1), 1 (D2), 2 (D4) (■), C3(●)). Different letters indicate significant differences ($p<0.05$)

All the blend had an αvalue higher than CH1% suggesting an higher number of interactions into the blends; these interactions are entanglements between polysaccharides and protein that determined a tridimensional network that can relaxes easier than the CH film. Films obtained with the same SC/CH ratio but higher biopolymer concentration showed a lower α value; this can be explained if we consider that the film formed a more compact structure with a lower free volume and thus less mobility. Also E_α is influenced by the increase of SC/CH ratio, in fact as showed in Fig 4B, CH1% had higher value compared to the blend; however there were no great differences between the blend in different SC/CH ratio.

1.3.5.2. Tensile properties

Tensile properties are reported in Table 6.

Table 6. Tensile properties of the SC/CH film at different ratio (EM=elastic modulus; TS=tensile straight; ε= elongation at break)

Sample	SC/CH	EM (MPa)	TS(MPa)	ε (%)
CH1%	0	8,96±6,06[a]	0,56±0,15[a]	36,95±10,65[a]
C3	2	77,54±13,01[c]	14,61±3,17[b]	52,28±8,95[b]
D1	0,5	49,01±10,76[b]	15,93±5,02[bc]	48,76±8,30[b]
D2	1	124,9±45,18[d]	19,18±2,38[c]	52,09±2,81[b]
D4	2	135,79±31,55[d]	19,57±1,38[c]	62,28±3,55[c]

As a general trend, by SC/CH ratio, the elastic modulus of the blend increased; the film with the highest concentration of SC showed the highest elastic modulus equal to 136±32 MPa, but there were no significant differences between samples D2 and D4; Also ε% and the TS were positively influenced by the increase in SC/CH ratio: TS reaching values of 20±1 MPa, leading to stronger films than the control chitosan film, whereas e% varied from 37±10 % to 62±3 % leading to a more flexible films. Mathew et al. (2006) have shown that the starch–chitosan blend films exhibited a significantly higher elongation values compared to films made from starch or chitosan alone. Hosseini et al. (2013) observed a different behaviour for chitosan and fish gelatin based films; tensile strength and elastic modulus decreased when fish gelatin were added to chitosan. A previous study (Xu et al., 2005) has shown that the TS values of chitosan/starch composite films first increased with the addition of starch and the maximum occurred at the starch to chitosan ratio of 1:1 but the TS then decreased with further increase in the starch to chitosan ratio up to 2. Furthermore the higher ratio starch to chitosan was responsible for a negligible effect of the chitosan on the elongation at break; in this study the increasing TS values can be attributable to the increasing surface solid content and to the formation of inter molecular hydrogen bonds between NH3+ of the chitosan backbone and OH− of the starch. In our case, it's probable that at higher concentration of SC, the anionic group of protein (COO-) might establish interaction with the cationic group of chitosan, leading to the formation of a stronger complex.

Moreover several studies reported a different behaviour in mechanical properties when proteins are added to chitosan; Ferreira et al. (2009) have shown that TS and ε% decreased with increasing whey protein isolate proportion in the film, whereas EM slightly decreased only for protein proportion of 50%, maybe due to the less cohesive structure compared with the pure polymer. Di Pierro et al. (2006) studied the influence of the addition of different amounts of whey proteins on the maximum elongation at break of the chitosan films prepared in the absence or presence of microbial transglutaminase as cross-linking agent; a marked decrease of flexibility dependent on the increase in protein amount was determined for both the films even though the effect resulted significantly higher in the films obtained in the presence of the enzyme. Also bilayer films with a thicker chitosan layer respect to why protein layer, had the highest TS, with also high values in EM and ε% , leading to stronger films (Kurek et al., 2014). Our study showed that the mechanical properties of the blend are better than that of pure chitosan; in fact, the combined materials contributed to have a synergic effect that improved strength, according to Pereda et al.(2008). However the comparison of the data is difficult due to different powder composition such as purity, viscosity, deacetylation degree, molecular weight and polymorphous (Ziani et al., 2008), solubilisation method, glycerol concentration and drying methods (Thakhiew et al., 2010).

1.3.6. Moisture sorption isotherm

The moisture adsorption isotherm of chitosan film and SC/CH blend film are reported in figure 5.

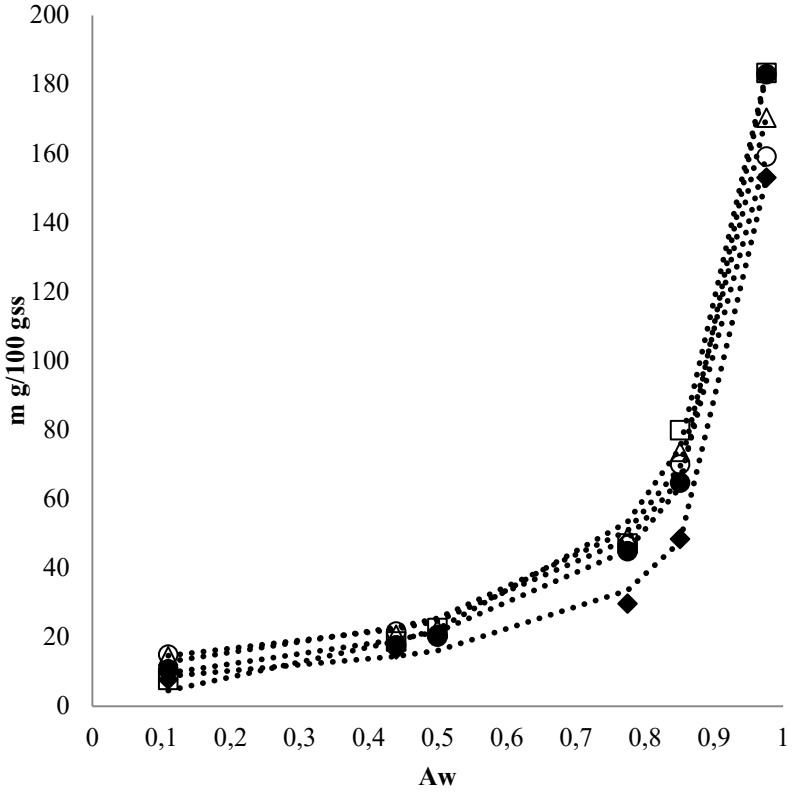


Figure 5. Equilibrium moisture sorption isotherm (CH 1% (♦), D1 (□), D2 (Δ), D4 (●), C3(○)); dotted line are from the equation obtained by fitting the experimental data to GAB equation).

All samples film can uptake up to 64% moisture when conditioned at 0,976 Aw. The adsorption isotherm showed the typical sigmoidal curve of this kind of material. A aw<0.2 the adsorption of water in the monolayer zone takes place, in the region of aw between 0.2 and 0.65, the water is absorbed at multilayer whereas at aw>0,65 the water absorbed corresponds to the condensation of water in the pores of the film. In table 7 are reported the GAB parameters x_m, C and K for all the samples.

Table 7. Estimated model constants for SC/CH film and CH1%

		CH1%	D1	D2	D4	C3
GAB	x_m	0,082948	0,167189	0,13649	0,117609	0,134488
	C	85,95712	2,801599	34,5035	23,29568	141,5666
	K	0,969059	0,934366	0,942914	0,95893	0,938154
Peleg	k1	25,90961	31,12011	22,23493	29,23358	27,9386
	k2	171,1192	186,4909	177,6451	195,3945	159,6397
	n1	0,544167	0,618461	0,1981	0,506719	0,295711
	n2	12,1359	8,197418	7,505845	9,85768	8,060434

According to the GAB model, the value of x_m corresponds to the moisture content in the monolayer. By increasing the SC content in the blend, x_m increased, but at SC/CH ratio of 2 the value decreased again. This behaviour can be explained by the different electrostatic interaction between chitosan and casein at different ratio. The minor value of x_m for sample D4 (ratio 2) respect to samples D2 can be justified by a strong interactions between caseinate and chitosan in forming polyelectrolyte complex films lead to fewer sites in the polymer matrix at which water can be held, and they are responsible for the reduced final water content (in comparison with sample D2). The parameter C and K are related

to the quantity of water at multilayer and to the energy need to cut the interaction. Our data pointed out that there is not a significant effect of SC/CH on C and K, maybe for the high variability of the estimated parameters.
Based on the Peleg model, the lower k1, the higher the initial water adsorption rate whereas the lower k2 the higher the adsorption capacity. The SC/CH ratio had a significant effect on the Peleg parameter (table 7). In particular, sample D2 showed the lower value of K1 and thus the higher initial adsorption rate whereas samples D1 and D4 showed the higher value of K2 and thus the lower sorption capacity. Our results are in agreement with Pereda at al., 2008.

1.3.7. Water vapour permeability

The water vapour permeability values are showed in Fig.6

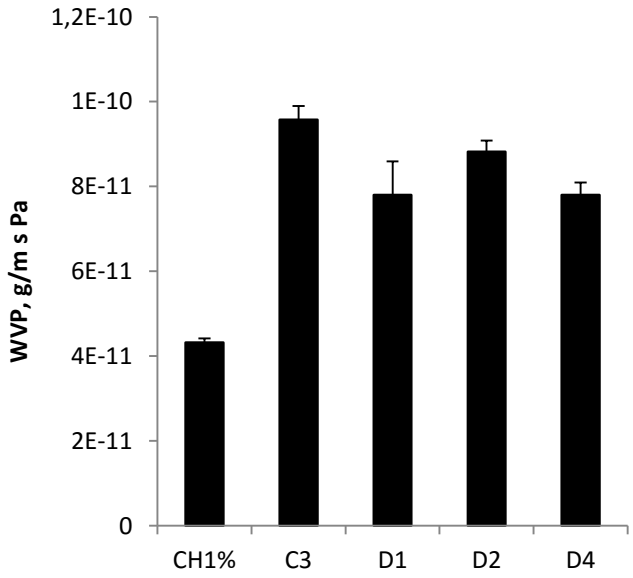


Figure 6. Water vapour permeability (g/m s Pa) of SC/CH film at different ratio

CH exhibited better water vapour barrier than blends (4.32 x 10^{-11} g/m s Pa); with the exception of sample D4, by increasing the SC/CH ratio, permeability increased. The increment of WVP as function of SC/CH ratio can be explained by the increased solid content. In fact due to its hydrophilic nature, blend film can link more water than CH film, in accordance with moisture content results. Moreover, in accordance with dynamical mechanical analysis results, the polyelectrolyte complex have a structure with an higher relaxation index in comparing with CH film and this can negatively affect the barrier properties. In fact, as reported by Pereda et al. (2008), the diffusion of low molecular weight compounds in polymers is generally governed by two simultaneously occurring phenomena, Fickian mass transport and a relaxation phenomenon. The only exception for sample D4 that showed a lower permeability than sample C3 although had the same ratio (7.80 x 10^{-11} vs. 8.82 x 10^{-11} g/m s Pa) and the highest solid content; this result could be explained considering that, at high proteins and polysaccharides concentration, the polyelectrolyte complexes were able to neutralize the charges, reducing the hydrophilic nature of the film. In addition, the high solid concentration reduce the relaxation phenomena, as showed in figure 3a. Similar results were reported by Ferreira et al. (2009) for chitosan and whey protein based edible films; for higher amounts of protein, WVP tended to increase with increasing protein content, except for the film with the highest WheyProtein/CH ratio, due to the polymer-polymer interactions in each phase domain. Overall, the WVP of chitosan-sodium caseinate based edible film was of the same order of magnitude of chitosan-gelatin (Arvanitoyannis et al., 1998; Hosseini et al., 2013) and chitosan-gliadin films (Li et al., 2010).Nevertheless the WVP of CH film was one order of magnitude lower than that of other chitosan based edible film (Pereda et al., 2009; Chillo et al., 2008; Kurek et al.,2014) and HPMC- caseinate films (Perone et al., 2014).

1.4. Conclusions

In this work rheological and mechanical analysis, moisture content, moisture adsorption isotherm and water vapour permeability were useful methods to better understand the structure of SC/CH film ad different ratio.
Our results showed that there is a formation of electrostatic interactions between the cationic group of CH and anionic of SC at particular range of pH and SC/CH ratio; moreover the blend structure is affected by the solid surface content and the ratio. The higher number of interaction into the blend allows to obtain films with better mechanical properties compared to those made with pure chitosan; in particular D4 showed the highest EM, TS and ε% values. Furthermore the sample D4 exhibited the lowest value of permeability and moisture content in monolayer compared to the other blend; these results indicate that at this ratio and solid surface content, a partial neutralization of charges between SC and CH occurs and it is possible to obtain a stronger network and reduce the hydrophilicity.

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II STUDY CASE

Condensation and Moisture Regulation in Fresh-cut Packaged Lettuce

ABSTRACT

During postharvest handling and storage, fresh fruits and vegetables continue to lose water through the process of transpiration which is a physiological process of water loss from fresh products. This process is influenced by factors such as temperature, relative humidity, respiration rate, surface area and air movement; an excessive weight loss causes change in texture, a favourable environment for microbial growth and leads to unsalable loss of market value, during retail marketing and to a direct financial loss. Iceberg lettuce is a highly perishable product with a short shelf life (7 days at 7°C) due to the influence of minimal processing unit operations, such as cutting and shredding that causes disruptions of cells, which induces an increase in respiration rate, transpiration and enzymatic activities after harvest. Iceberg lettuce is currently packaged in polypropylene and stored under refrigeration temperature; however the excess condensed water accumulated inside the package is still unresolved problem. The aim of this work was to quantify the transpiration (TR) of fresh cut lettuce, the amount of condensation and finally design a proper package to regulate moisture in headspace. Experiments were conducted by conditioning the sample at 2, 6 and 10°C and 76, 86, 96 and 100% RH; TR was recorded during 7 days of storage. The results showed that both temperature and relative humidity affected transpiration rate: TR increased by increasing temperature and by decreasing RH; TR ranged from 0.04 to 2.36 g/(kg.h) over all the combinations of temperature and RH tested. A mathematical model was developed to predict TR as a function of temperature-humidity. The models were further used to predict amount of moisture condensation inside a typical size package containing 150 g of fresh-cut lettuce and finally select a suitable packaging material with required water vapour transmission rate (WVTR) to alleviate this condensation. The estimated range of water transmitted per unit time was found to be 0.073 to 0.135 (g/h) to store lettuce at 6°C. Lastly, experimental validation was performed using films of different WVTR to package fresh-cut lettuce and quality analysis was performed.

2.1. Introduction:

Iceberg lettuce (*Lactuca sativa* L.) is a highly perishable product and it is considered one of the most popular of fresh-cut vegetables, that has been successful in recent years, especially for convenience. (Ragaert et al., 2014). However ,commercially available iceberg lettuce has a short shelf-life of 7 days at 7°C. This is due to the influence of minimal processing unit operations, such as cutting and shredding that causes disruptions of cells, which induces an increase in respiration rate, transpiration and enzymatic activities after harvest (Lareo et al.,2009).Lettuce is packed in modified atmosphere with low O_2 concentration (below 2%) and 10-13% CO_2 in order to reduce tissue browning and maintain the overall visual quality (Hamza et al., 2006; Lopez-Galvez et al.,1996). However, the excess condensed water inside the package due to temperature fluctuations and the transpiration, is not acceptable because of the loss of texture and favourable conditions for microbial growth. During postharvest handling and storage, fresh fruits and vegetables continue to lose water through the process of transpiration and respiration rates. Excessive weight loss causes change in texture, leads to unsalable loss of market value, during retail marketing and to a direct financial loss. A weight loss of 5-10% is not acceptable because the product rots rapidly, and this implies that the weight loss should be as low as possible (Mahajan et al., 2008). On the other hand, it creates favourable environment for microbial growth if excessive moisture accumulation is not controlled in in the package/storage environment (Aindongo et al., 2014; Caleb et al., 2013).The formation of water condensation is characterized by variations in heat and mass transfer process; in fact during the condensation of water vapour, the heat is released causing increased temperatures at the point of condensation (Linke and Geyer, 2013). The cooling can help to maintain the quality and extend shelf life of fruit and vegetables; this process involves the convection at the product surface, conduction within the product, internal heat generation due to respiration, moisture loss due to transpiration, and the evaporative cooling effect due to transpiration (Chau and Gaffney, 1990). So, even minor changes in temperature might influence the formation of drop water underneath the film packaging leading to deterioration of packaged product. Linke and Geyer (2013) studied dynamic condensation and intensity at fluctuating external temperature within plastic film packaging containing plumps in order to describe the condensate formation and its intensity in a package. They found that the package air volume mainly influenced the retention time of condensation water, followed by the flow conditions and external temperature; on the other hand, the condensation on fruit surface is caused primarily by temperature amplitude and cycle time. Transpiration rate (TR) is the moisture loss due to difference in water vapour pressure between the produce surface and the surrounding conditions and it can be easily measured by weighing the sample at regular intervals. The water vapour pressure gradient depends on temperature, relative humidity (RH), surface area and air movement (Mahajan et al., 2008). TR increases with the increase of temperature but decreases with increasing RH; this was experimentally proved for several products such as lettuce, mushrooms, pomegranate arils, strawberry and tomatoes. Lareo et al. (2009) found that the weight loss in lettuce stored in cool room (73-77% RH) at different temperature (5 to 15°C) significantly increased with the increase of temperature, reached 5% after 30 days of storage at 5°C; this percentage was reached in less than 10 and 5 days when lettuces were stored at 10 and 15°C, respectively. Mahajan et al., (2008) found that RH was the variable with greatest effect on transmission rate of mushrooms; by increasing RH from 76% to 96% TR decreased by 87% at 4°C; moreover decreasing the temperature from 16°C to 4°C, TR decreased by 61% at 96% RH. Sousa-Gallagher et al., (2013) found that the rate of weight loss of strawberry was higher at 76% RH and 15°C while at 96% and 4°C was least; further analysis showed that the effect of RH was more pronounced than that of temperature and that there was an interactive effects between temperature and RH. Similar results were reported by Aindongo et al. (2014) investigating the effect of temperature (5 to 22 °C) and RH (76 to 96%) on TR of pomegranate aril-sacs and arils; the TR increased with an increase in temperature and decrease in RH, with the fruit fraction stored at 5 °C and 96% RH showing the lowest TR in comparison to other storage conditions. Xantopulos et al. (2014) studied the water loss in grape tomatoes at different temperatures (10 to 20°C) and RH (70 to 92%); in agreement with other studies found that TR increased with temperature, especially from 15 to 20°C while TR decreased with RH in particular from 80 to 92%. Diaz-Perez (1998) measured the TR on eggplants of a wide range size; TR decreased as fruit increased in size because both of a reduction in the surface area/mass ratio of eggplants. Several models for estimating transpiration rate have been proposed but most of them describe the moisture transfer through the skin as a function of the biophysical and thermophysical properties such as surface cellular structure, skin thickness, pore fraction in the skin, geometry and thermal diffusivity of produce, which are not easily measured or determined. Kang and Lee (1998) proposed a simple transpiration model based on heat and mass balances between produce and storage atmosphere was developed and tested experimentally on apple and minimally processed cut vegetables to predict moisture loss in normal air and in a controlled atmosphere. A respiration-transpiration model was developed by Song et al. (2002) by applying simultaneous heat and mass transfer principles along with known physiological behaviour for the design of MAP systems containing blueberry at 15 and 25°C; the model was successfully verified by using different types of packaging films with a difference between the experimental and predicted headspace gas composition less than 1%. Mahajan et al. (2008) developed a simple mathematical model for transpiration rate to understand the evolution of mushrooms weight loss as a function of temperature and RH. This model was further modified and used for other products (Caleb, 2013; Sousa-Gallagher et al., 2013). Modified atmosphere packaging (MAP) is an important packaging technology which can help to maintain quality and extend shelf life of produce, by making qualitative or quantitative changes to the atmosphere composition around the product (Torrieri et al., 2008). In order to design an optimum package it must be taken into account also the packaging requirements in terms of water vapour transmission rate to match the high physiological

product requirements (Sousa-Gallagher et al., 2013). In fact, most polymeric films have lower transmission rates to the water vapour compared to the transpiration rates of fresh products; so, a MAP system not properly designed might lead to undesirable results such as moisture condensation, microbial growth and consequently a shorter shelf life.
Iceberg lettuce is packaged in polypropylene and stored under refrigeration temperature. However, most polymeric materials (PP, PE, PVC) used in fresh produce packaging have low water vapour transmission rate relative to the transpiration rates of fresh produce. Therefore, most water molecules evaporated from the produce do not escape through the film and remain within the package, enhancing the water vapour pressure in the package micro environment. Under such conditions, even minor temperature fluctuations result in condensation inside the package causing sliminess, decay, enhancement of microbial growth, and browning of produce surface (Linke et al., 2013). Micro-perforated packaging films are commonly used in fresh produce packaging to enhance O_2 and CO_2 gas permeability and achieve equilibrium MAP. But generally such films do not allow the diffusion of sufficient amounts of water vapour into the environment leading to high humidity levels & condensation of water vapour in the packaged fresh produce. Therefore, the excess condensed water accumulated inside the package is still unresolved problem. The objective of this work was to understand the transpiration behaviour of iceberg lettuce under different storage conditions and quantify the amount of condensation and use it for designing a package to regulate moisture in headspace.

2.2. Materials and methods.

Iceberg lettuce heads (*Lactuca sativa*) of Spanish origin were purchased from a local supermarket, transported to the laboratory and equilibrated at the test temperature. Before the experiment started, the external leaves and the central stern were removed; then the lettuce heads were cut in pieces of 4 x 3 cm wide using a sharp knife, washed in cold tap water for 5 min and centrifuged for 2 minutes to remove the excess of water.

2.2.2. Transpiration rate measurements

The experiment was carried out in three containers, located in walk-in cooling room. RH was controlled by using saturate salt solutions of sodium chloride, potassium chloride, potassium nitrate and water, giving 76, 86, 96 and 100% RH, respectively. Salt solutions were placed on the bottom of the container and covered with a perforated aluminium support on which a plate containing 10 grams of cut iceberg lettuce was placed; the test was performed at 2, 6 and 10°C. Temperature and relative humidity were continuously monitored using air humidity sensor FHA 646R (Ahlborn, Holzkirchen, Germany).

Transpiration rate was calculated from the changes in mass of iceberg lettuce over time :

$$TR=\frac{Mi-M}{t\times(\frac{Mi}{1000})} \quad (2.1)$$

Where TR is the transpiration rate expressed in g Kg^{-1} h^{-1},M_i is the initial weight of the lettuce (g), M is the weight of lettuce (g) at time t (h).
The experiment was carried out in triplicate.

2.2.3. WVTR measurements

The water vapour transmission rate of two films was evaluated. Naturflex(NVS 30 µm) (Innovia Films, Cumbria, UK) is a cellulose-based film characterized by a high moisture barrier. Propafilm (Innovia Films, Cumbria, UK), made by polypropylene, is a common film used for fruit and vegetables
The water vapour transmission rate was measured by a gravimetric method by means of bottle containing deionized water. Films were placed on the top of the bottle and sealed by rubber rings and lids with a hole of 3 cm of diameter; the film area was 7.07 cm^2. The bottles were weighted and then placed in a box containing a saturated magnesium nitrate solution which provided a constant water activity of 57% at 6°C; the bottles were weighted every day. Three replicates for each film sample were performed. The WVTR was expressed as follow:

$$WVTR = \frac{g}{m^2 day} \quad (2.2)$$

where: g are the grams of water, m^2 is the film surface equal to 0.007.

2.2.4. Packaging design and selection of the film

In order to properly design the package for the minimally processed iceberg lettuce it is necessary to evaluate the balance of the water vapour around the package system (figure 1).

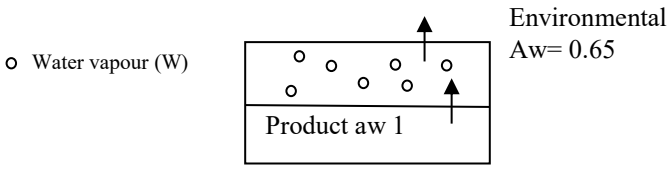

Figure 1. Water vapour balance around the product

The product produce water due to transpiration phenomena. Due to the difference of water activity at film interface, a water transport through the film is activated as function of the film permeability to water. Thus, the water accumulation in the head space is function of the two phenomena: the generation of water due to transpiration rate of the product and negative generation of water due to WVTR through the film:

$$+GEN(p) - GEN\ (f) = ACC \tag{2.3}$$

That is:

$$+TR * M - WVTR * A\ = \frac{dW}{dt} \tag{2.4}$$

In order to avoid moisture condensation, the system must be in equilibrium, thus :

$$TR * M = WVTR * A \tag{2.5}$$

Thus, to properly design a package it is important to choose correctly the film material on the base of the TR of the product, the quantity of product to be packed and the package surface.

Most polymeric materials such as polyethylene and polypropylene, used for fresh product have lower water vapour transmission rate than transpiration rate of fresh produce; thus most water molecules evaporated from the product, do not escape and remain within the package, enhancing the water vapour pressure in the package environment and thus the water condensation. On the other hand, biopolymer based film, due to the hydrophilic nature, could cause an excessive weight loss.

A way to obtain package film with the desired properties is to combine different film in parallel or in series. In this work six packaging films were obtained by combining Propafilm (PP) and Naturflex (NF) in parallel at different ratio as follows:

- 100PP 0NF (PP)
- 98PP 2NF
- 95PP 5NF
- 90PP 10NF
- 85PP 15NF
- 0PP 100NF (NF)

The objective was to obtain a package material with the desired properties in terms of WVTR.

The permeability of the composite structure were calculated considering the theoretical model as shown in equation (2.3):

$$A_{tot}P_{tot} = (A_1P_1) + (A_2P_2) \tag{2.3}$$

Where $A_{tot}P_{tot}$is the total permeability multiply for the total area, given by the sum of the product of the surface (A) and permeability (P) of each components**.**

The criteria used to select the film for the package of the minimally processed iceberg lettuce were that the WVTR had to be in the range of TR of the product. Moreover, as further criteria, was used the maximum weight loss accepted by commercial reason, that is 5%.

2.2.5. Validation

Six packaging film of size 240x180 cm with 150 g of fresh-cut lettuce were used; bags were sealed and stored in a cooling room at 6°C and 57% of relative humidity for 7 days. The package area available for gas exchange was about 864 cm^2. Gas composition inside each package were monitored at the end of the storage time (7 days) by using a gas analyser (Chechmate 3, PBI Dansensor, Ringstead, Denmark). Gas composition was not monitored overtime to avoid alternating environmental or storage temperature around the bags. Temperature and relative humidity inside the package

were continuously monitored using an air humidity sensor FHA 646R (Ahlborn, Holzkirchen, Germany). The water vapour absorption of the bag was gravimetrically determined by measuring increase in weight of the bag at the end of the storage time. The amount of water transmitted over the film was gravimetrically measured by weighing the package at the start and at the end of storage period. The amount of condensation was measured by weighing the bag before and after wiping off the water accumulated on the film.

2.2.6. Data analysis

Transpiration rate as function of temperature and relative humidity were described by using a Transpiration rate model developed by Mahajan et al. (2008) for mushrooms (2.4):

$$TR = Ki \times (a_{w_i} - a_w)(1 - e^{-aT}) \quad (2.4)$$

Where a_w is the water activity of the container (RH/100), a_{wi} is the initial water activity of iceberg lettuce that assume a constant value of 1, T is the temperature (°C) and a is an empiric parameter to explain the effect of temperature.
To describe the TR at 100% of RH, the model has been modified and the following equation (2.5) has been used:

$$TR = b \times e^{cT} \quad (2.5)$$

Where b and c are the constant coefficients estimated using the non linear regression analysis of experimental data (Microsoft Office Excel 2007).
Eq. (2.4) was combined with Eq. (2.5) yielding to a global model that explain the effect of RH in the range 0-100%:

$$TR = (b \times e^{cT}) + Ki \times (a_{w_i} - a_w)(1 - e^{-aT}) \quad (2.6)$$

The mass transfer coefficient Ki and a were estimated by fitting Eq. (2.6) to the experimental data by non linear regression analysis (Microsoft Office Excel 2007).
The effect of temperature and relative humidity on transpiration rate of fresh-cut lettuce were quantified with a Pareto analysis at 95% significant level.

2.3. Results and Discussion:

2.3.1. Transpiration rate:

Transpiration rate of iceberg lettuce is shown in Figure 2.

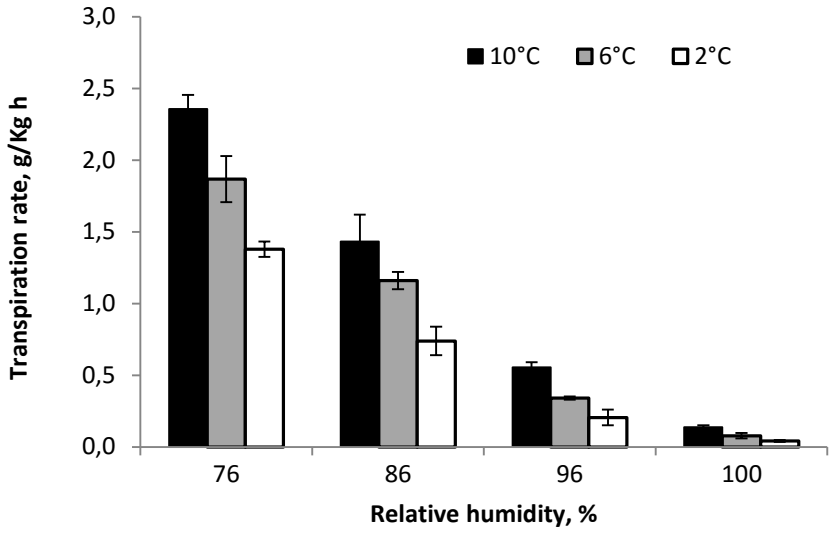

Figure 2. Effect of storage temperature and relative humidity on transpiration rate of fresh-cut iceberg lettuce.

TR ranged from 0.04 g/(Kg h) to 2.36 g/(Kg h) over all the combination of temperature and RH tested. These results are lower than those found by Mahajan et al. (2008) for mushrooms (TR ranged from 0.29 to 5.2 g/(Kg h)), at 4, 10 and 16°C and 76, 86 and 98% RH, but almost the same of strawberry (TR from 0.24 to 1.16 g/(Kg h) (Sousa-Gallagher et al., 2013)) at the same relative humidity and 5, 10 and 15°C. Our values are higher than those reported for cut onion, cut green onion(transpiration rate of 0.447 and 0.369 g/(Kg h), respectively, at 10°C and 82% RH in normal air), apple (TR of 0.0184 at 0°C and 100% RH in normal air) (Kang and Lee 1998), grape tomatoes (TR from 0.018 to 0.107 g/(Kg h)) (Xantopulos et al., 2014), arils and aril sacs (TR from 1.42 to 15.23 and 0.63 to 9.95 g/(Kg day), respectively)) at 5, 10, 15 °C and 76, 86, 96% RH (Aindongo et al., 2014).

The values reported that TR was negatively affected by temperature; in fact, by increasing temperature from 2 to 10°C, TR increased; moreover by increasing RH from 76 to 100% TR decreased.TR was found to be the highest at 76% RH and 10°C and the lowest TR value was obtained at 100% RH and 2°C; at 100% RH iceberg lettuce lose water and the TR is affected by temperature, by decreasing from 10 to 2°C, TR decreased. Similar results were reported by Lareo et al. (2009) for butterhead lettuce leaves; they showed that temperature and storage time had a great influence on weight loss because at low temperature weight loss decreased but increased by increasing the storage time. It is important to underline that also at 100% RH fresh-cut lettuce lose water; this result is in according to Rux et al (2015) that showed that mushrooms at 100% RH continuously lose weight over time; the temperature of the mushroom surface was slightly higher than the surrounding air temperature due to the respiratory heat generation. The driving force of water loss in harvested fruit and vegetables is based on the proportional difference between the water vapour pressure/concentration gradient in the atmosphere surrounding product (Ben-Yehoshua & Rodov, 2003). Moreover the TR is influenced by the resistance of produce surface to water vapour loss. According to Fick's low of diffusion, water vapour will move from the higher to the lower concentration; the transporation process under saturated atmospheric conditions is a complex process which involves different heat components such as the internal heat generated by produce, the evaporative cooling effect on the surface of produce and the convective heat transfer between the product and the surrounding environment. Thus at a saturated atmospheric level there is no potential for transpiration or for evaporative cooling to occur; so, the respiratory heat generation causes an increase in water vapour gradient for the mass transfer between the product and the surrounding conditions. According to our results, Song et al. (2002) proposed a model based on heat and mass transfer balances accounting for the respiratory and transpiratory behaviour of fresh produce, and the transport phenomenon across the package. When the ambient air is below saturation level, the difference in water vapour pressure between the product surface and the ambient air will cause moisture evaporation from the product surface resulting evaporative cooling.

The effect of temperature and humidity on transpiration rate of iceberg lettuce, quantified with a Pareto analysis at 95% significant level is showed in Figure 3:

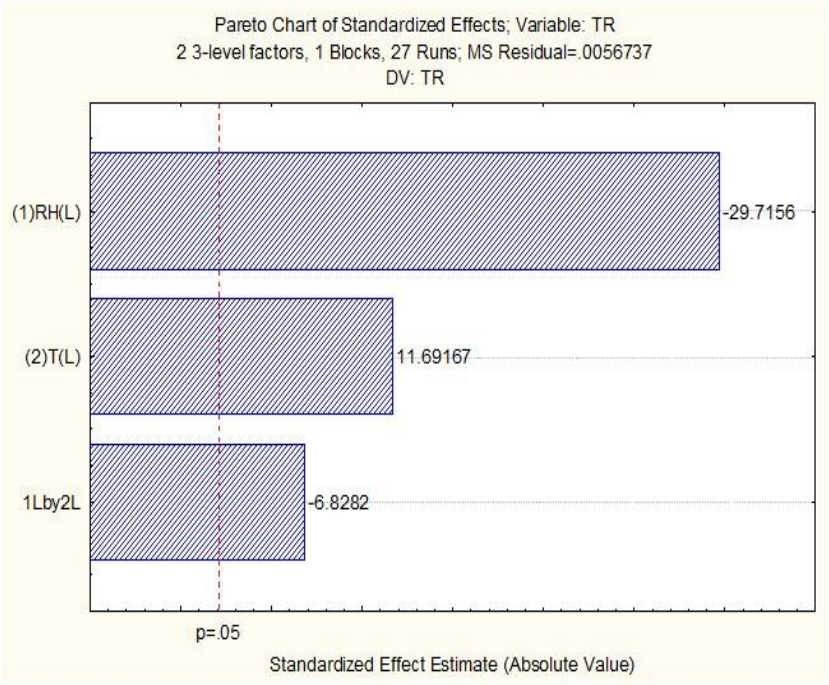

Figure 3. Pareto chart showing the effect of temperature and relative humidity on transpiration rate on iceberg lettuce at 95% significance level (indicated as a vertical dashed line).

This analysis showed that, in the range of conditions studied, both temperature and relative humidity affected significantly the transpiration rate but the effect of humidity was more pronounced than that of temperature; moreover the interactive effects of temperature and humidity were significant, with the effect of humidity on TR that increased with decreasing humidity and the effect of temperature on TR that increased with increasing temperature. This results are in agreement with those obtained by Mahajan (2008) on mushrooms and Sousa-Gallagher (2012) on strawberry.

In table 1 are reported the parameters of the transpiration model obtained by fitting the model to experimental data and the corresponding R^2.

Table 1. Parameters of the mathematical model (Eq. 2.3)

Ki (Kg h)	a ($°C^{-1}$)	b	c	R^2 (%)
8,92	0,42	0,031	0,151	96,6

Figure 4 shows the relationship between experimental and predicted values of weight loss of iceberg lettuce; the values predicted by Eq. (2.4) were in agreement with those obtained experimentally (R>0.97).

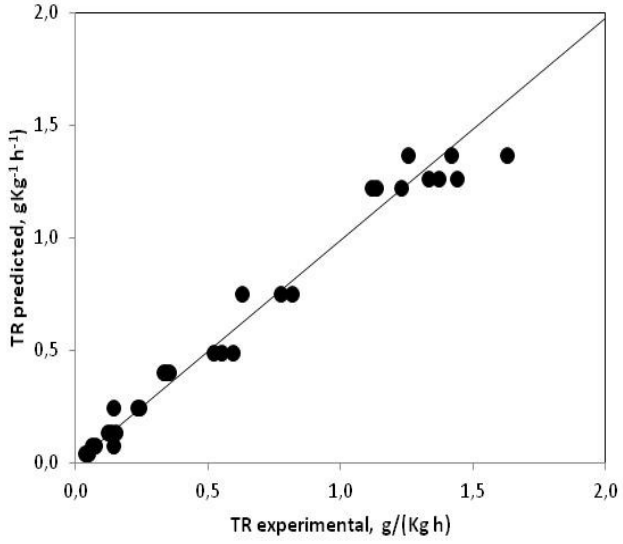

Figure 4. Relationship between experimental and predicted values of TR of fresh cut lettuce

2.3.2. WVTR

The water vapour transmission rate of PP and Nf are showed in Figure 5.

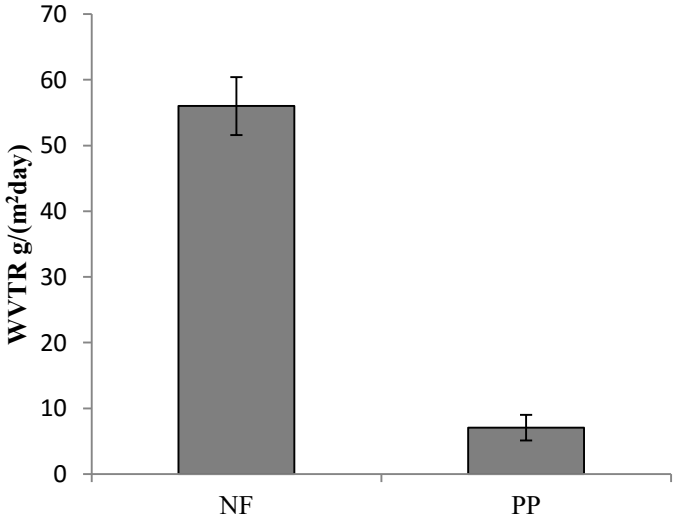

Figure 5.: Water vapour transmission rate of Naturflex (NF) and Propafilm (PP) calculated at 6°C.

Results showed that PP had a very low WVTR, equal to 7,1 g/(m^2 day) compared to NF which had a higher transmission rate equal to 56 g/(m^2 day).

2.3.3. Packaging design

Experimental data based on TR as a function of temperature-humidity conditions was used to calculate the amount of water produced per day in a typical package (size 240x180 cm and area of 864 cm^2) containing 150 g of fresh-cut lettuce stored at a given temperature and a range of relative humidity; the flux of water from 150 g of lettuce at 6°C was found to be 0.036 (g/h) at 98% RH and 0.073 (g/h) at 95% RH. To avoid moisture condensation inside the package, the transmission rate of the packaging materials should be as close as possible to the transpiration rate of lettuce. Considering a typical package both materials didn't satisfy the requirements in terms of transmission rate since polypropylene had a lower flux of water transmitted equal to 0.025 (g/h) whereas naturflex had a higher flux compared to that of the product (0.20 g/h). The transmission rate of composite film, calculated by using eq. (2.3) compared to PP and NF are reported in figure 6:

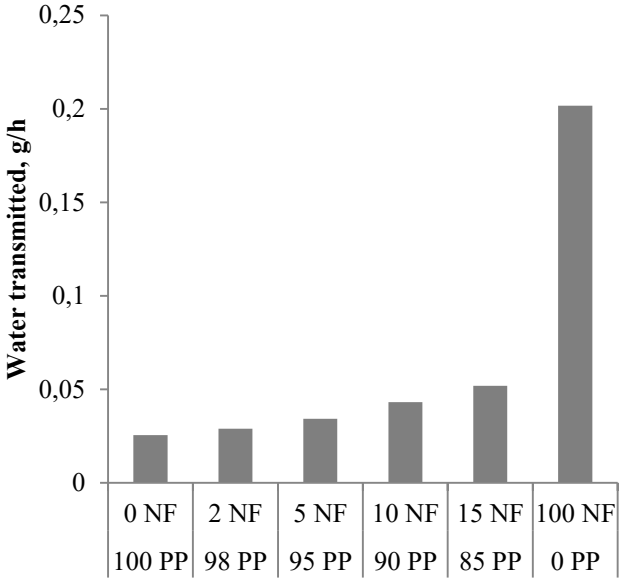

Figure 6. Water transmitted (g/h) of NF, PP and composite film made by combining PP and NF

As showed in the figure 6, by increasing the percentage of NF in the bag from 2% to 15%, the water transmitted per unit time increased. The amount of water transmitted for PP 98-NF 2, PP 95- NF 5, PP 90-NF 10, PP 85-NF 15, was equal to 0.029 (g/h), 0.034 (g/h), 0.043(g/h), 0.052 (g/h) respectively. Since the requirements in terms of water produced from the lettuce is between 0.036 (g/h), and 0.073 (g/h), the films with transmission in these range are 90PP 10NF and 85PP 15NF.

2.3.4 Validation

The package validation was carried out by comparing the amount of water loss by lettuce, water absorbed by the film, water transmitted out from the film and water vapour condensed inside the package. The temperature and relative humidity monitored over time in the lettuce package are reported in figure 7:

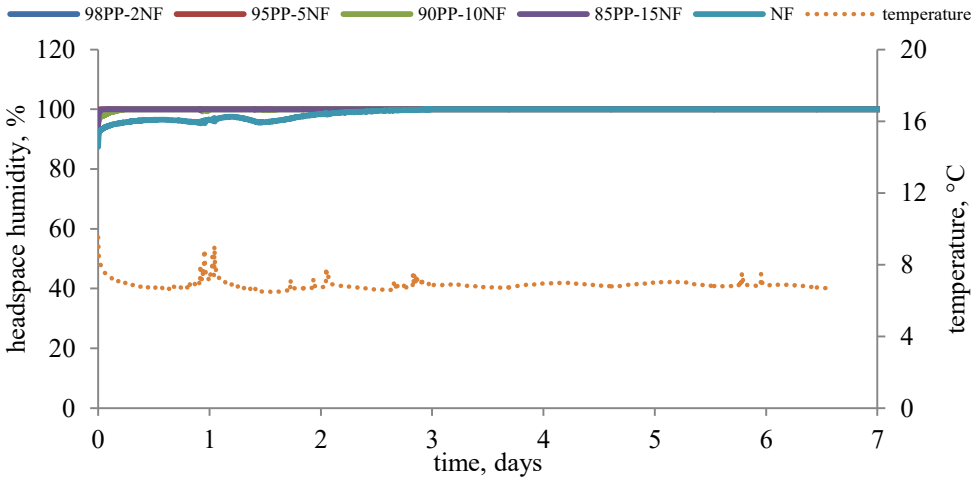

Figure 7. Change in relative humidity inside the package

PP package over all the storage period showed a RH inside the package equal to 100% (data non shown); also for 98PP-2NF, 95PP-5NF, 90PP-10NF and 85PP-15 package the RH was 100%. NF package at the beginning showed a RH of 95%RH but reached 100% in about three days.

The gas composition after 7 days of storage at 6°C is reported in figure 8:

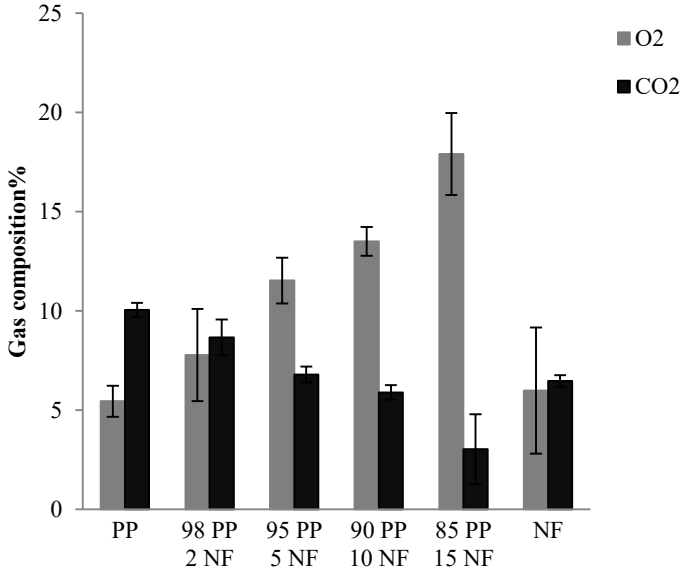

Figure 8. Gas composition inside the packages

After 7 days of storage, for PP and NF the O_2 decreased reaching the value of 5.4%±0.8 and 5%±3 whereas CO_2 increased until 10.1±0.4 and 6.5±0.3, respectively. For the combined packages it was not possible to monitor the gas changes due to the leakages; as shown in figure. 2.7, in fact, by increasing the percentage of NF film the O_2 concentration increased and the CO_2 concentration decreased. However on batch scale, this kind of problem can be easily solved.

In figure 9 is showed the water loss by lettuce**.**

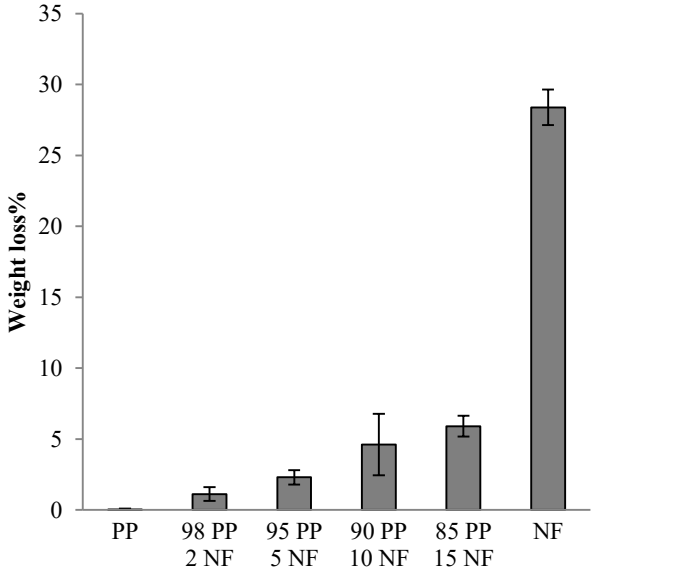

Figure 9.Weigh loss of lettuce

The total water loss of iceberg lettuce ranged from 0.55% in PP to 29.32% in NF; for the combined films the water loss was equal to 1.76%, 2.73% and 6.37% for 98PP 2NF, 90PP 10NF, 85PP 15NF, respectively.

In fig 10 is showed the water transmitted by the film.

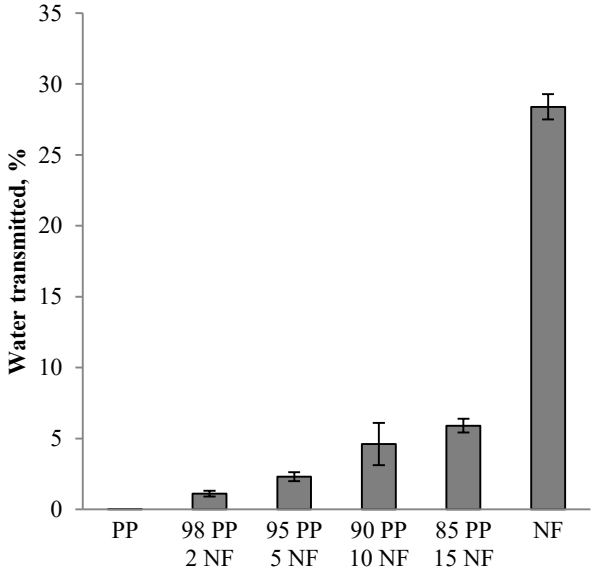

Figure 10. Water transmitted by the film

The water transmitted by the films increased by increasing the percentage of NF in the bag; the water transmitted values were equal to 0.03%, 1.12%, 2.31%, 4.61%, 5.91% and 28.39% for PP, 98PP-2NF, 90PP-10NF, 85PP-15 NF and NF, respectively. As known, bio-based materials have high WVTR compared to the polymeric materials due to the their hydrophilic nature; thus, the higher percentage of NF has the package, the higher water transmitted through the bag will be.

The water condensed values are reported in figure 11.

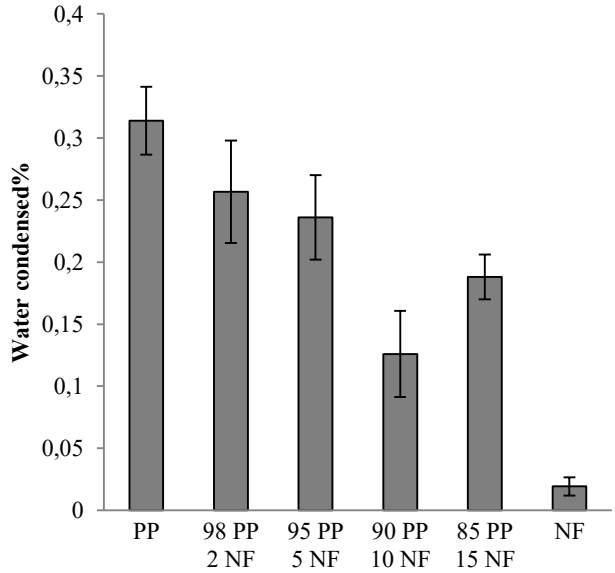

Figure 11. Water condensed inside the package

The PP package showed the highest percentage of condensed water inside the package, equal to 0.31% whereas the package with lowest condensed water inside is NF with 0.02%.The condensation was also observed in PP100 NF0 with water drops.
As general trend, by increasing the NF percentage, the water condensed decreased, except for 90PP-10NF, probably due to a leakage. The higher condensation inside PP is due to the low WVTR of PP that didn't allow to the water molecules to escape and thus they remain inside the package.
In Figure 12 the water absorbed by the package are shown:

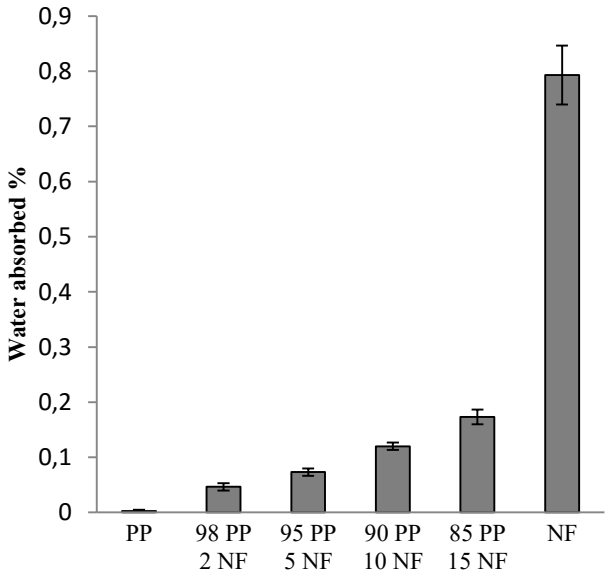

Figure 12. Water absorbed by the package

The water absorbed by the package ranged from 0.003% for 100PP 0NF to 0.79% for 0PP 100NF. As expected, PP100 NF0 did not absorb significant amount of water whereas PP0 NF100 absorbed 0.79% of the water loss by lettuce due to the hydrophilic nature. Thus the water absorbed depended on the percentage of NF: by increasing NF percentage, in fact, the water absorbed increased.

In figure13 are summarized the grams of water loss, water absorbed by the package, water condensed inside the package and water transmitted from the package:

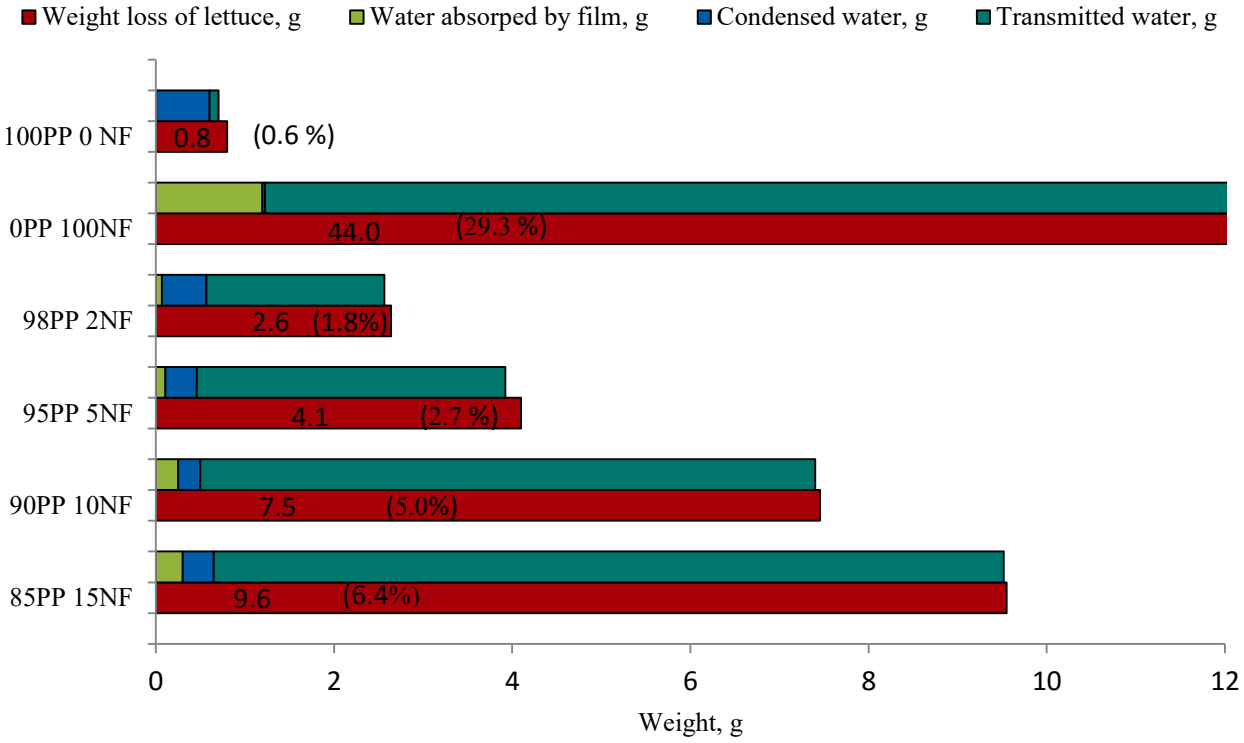

Figure 13. Distribution of water loss in six different package after 7 days of storage at 6°C

For NF, 98PP-2NF, 95PP-5NF, 90PP-10NF and 85PP-15 and NF package, the highest part of water lost by lettuce is transmitted by the package and the amount of water transmitted depends on the NF percentage of the bag. As the NF percentage increased, the condensed water decreased, whereas the water absorbed by the package increased. For PP, the highest amount of water lost by lettuce is the condensed water and just in small part (0.003%) the water is transmitted.

Under the saturate humidity conditions, the water vapour flux from the package headspace to storage environment is dominated by the limiting step of the film resistance to transport. Since the WVTR of the polypropylene is very low most of the water lost by lettuce remained inside the package, whereas the high WVTR of NF allowed the water to migrate outside.
The validation confirmed that the packages able to respond to the transpiration rate requirements of lettuce were 90PP-10NF and 85PP-15NF; however 85PP-15NF led to a weight loss of lettuce higher than 5% which is the maximum value allowed. Thus, the package composed by 90% of PP and 10% of NF was able to reduce the condensed water inside the package and, at the same time, allowed to obtain a product with a weight loss below 5% after 7 days of storage at 6°.

2.4. Conclusions:

Transpiration rate of fresh-cut iceberg lettuce was found to be in the range of 0.04 - 2.36 g/(Kg h) and both temperature and RH had a significant effect on transmission rate. The influence of RH in the range 0-100% and temperature has been well explained by a mathematical model, that showed a good predictability for transpiration rate of fresh-cut lettuce. A packaging validation confirmed that in order to design a proper package, the requirements in terms of transmission rate of the film and transpiration rate must be taken into account; moreover the weight loss of the product do not exceed 5%. The package that better satisfy the requirements of lettuce was found to be the bag made by 90% of polypropylene and 10% of naturflex.

2.5. References

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III STUDY CASE

Application of edible biopolymers coating on minimally processed fruit

ABSTRACT

Recently, there has been an increasing market demand for minimally processed fruits and vegetables due to their fresh-like character, convenience, and human health benefits. Minimal processing including washing, sorting, peeling, slicing and chopping, cause a quality deterioration with an increase in water loss, softening, microbial contamination, respiration rate, ethylene production, and cut-surface browning; thus minimally processed products become more perishable. The use of edible coatings can prolong product shelf-life, providing a semi-permeable barrier against oxygen, carbon dioxide (CO_2), moisture, and solute movement. The effect of coatings (1% chitosan, chitosan 2%, caseinate/chitosan ratio=2) in combination with anti-browning agent (1% citric acid, 1% l-ascorbic acid agents, 1% CaCl2) on minimally processed apple slices were studied during storage . The different coating types were compared against uncoated controls dipped in anti-browning solution, by monitoring parameters such as respiration rate, weight loss, and colour at 5, 10 and 20°C. Results showed that coating treatments were able to reduce the respiration rate of minimally processed apples. Only the caseinate/chitosan blend was efficient to retard the browning and was not significantly different ($p<0.05$) from the control sample. All formulations tested did not performed very well as water vapour barriers.

3.1. Introduction

Recently, minimally processed fruits and vegetables are gaining importance in the market due to their convenience and freshlike properties conformed to consumer demands. During minimal processing, peeling, slicing, shredding, chopping, and cutting cause injury to the tissues of fruits and vegetables and provide opportunity for microbial contamination, an increase in respiration rate, transpiration rate and a reduction in tissue firmness. Thus, compared to whole fruits and vegetables, minimally processed fruits and vegetables generally have a shorter shelf life due to being more perishable (Olivas et al., 2007). Respiration rate is the process by which plants take in oxygen and give out carbon dioxide; during the process, carbohydrates are metabolized in carbon dioxide and water, with produce of energy in order to maintain life of the produce. This process decreases with falling concentration of oxygen in the environment and increases as the concentration of reaction product increases; generally controlled atmosphere (CA) and modified atmosphere (MAP) are widely used for fruit and vegetables to make low O_2 and high CO_2 in the environment to reduce the respiration rate and extend the storage life (Bai and Plotto, 2012).Edible coating can help to reduce the respiration rate because they provide a semi permeable barrier against oxygen and carbon dioxide; however the atmosphere modification could induce some undesirable effects such as fermentation and production of off odours and flavours, especially at O2 levels that cannot sustain aerobic respiration. The use of edible coatings and films in food application is conditioned by the achievement of diverse characteristics such as cost, functional attributes, mechanical and optical properties, barrier effects against gas and water and sensory acceptability; these characteristics are influenced by the composition, molecular weight distribution of the material implemented, external parameters such as pH, type of solvent, components concentration or ratio, the use of plasticizer, antioxidant and other additives (Falguera et al., 2011).Edible films can be derived from proteins, polysaccharides, lipids, and resins. By and large, proteins have received attention in edible film research because of their abundance as agricultural by-products and food processing residuals. Most of the work in the literature consists of coating of whey protein in order to improve their barrier and mechanical properties through optimization of protein denaturation conditions (e.g., temperature, pH, time, and protein content), addition of cross-linking agents and additives (Reinoso et al., 2008; Lee et al., 2003; Perez-Gago et al., 2006).Whey proteins produce transparent and flexible water-based edible coatings with excellent oxygen, aroma, and oil barrier properties at low relative humidity. However, the hydrophilic nature of whey protein coatings causes them to be less effective as moisture barriers. Casein based edible coatings are attractive for food applications due to their high nutritional quality, excellent sensory properties, and good potential for providing food products with adequate protection against their surrounding environment (Vargas et al., 2008).Research conducted showed that casein-lipid coatings provide protection for fruits and vegetables from moisture loss and oxidative browning (Baldwin *et al*. 1995; Certel *et al*. 2004).Tien *et al*. (2001) found that edible coatings based on casein, whey protein and carboxymethyl cellulose show important antioxidant capacity; while Krochta *et al*. (1993)found that carrots coated with sodium caseinate and stearic acid had lower whitish index and could help moisturize the carrot surface. Polysaccharides are the most widely used components found in edible coatings for fruits; they show effective gas barrier properties although they are highly hydrophilic and show high water vapour permeability in comparison with commercial plastic films. The main polysaccharides that can be included in edible coating formulations are starch and starch derivates (Pan et al., 2013; Chiumarelli et al., 2012), cellulose derivates (Perez-Gago et al., 2005; Baldwin et al., 1999), carrageenan (Ribeiro et al., 2007; Lee et al., 2003), chitosan (Chien et al., 2007; Qi et al., 2011; Leceta et al., 2015) pectin (Maftoonazad et al., 2007). Polysaccharide -protein complexes could exhibit better functional properties than that of the proteins and polysaccharides alone (Pogaku and others 2007).Chitosan (CH) with positive charged groups, can interact and form 3-dimensionalnetworks with molecules containing opposite charges, such as sodium caseinate (SC).It was demonstrated that films prepared from sodium caseinate and chitosan exhibited better mechanical properties (tensile and impact strengths)and lower equilibrium moisture content than either chitosan and sodium caseinate alone due to the electrostatic interactions developed during the polyelectrolytic complexation of the biopolymers (Pereda et al. 2008, 2009).Moreover the antimicrobial effectiveness as food coatings/wrappers of sodium caseinate-chitosan on three different native microfloras (cheese, salami, and carrots) was evaluated; in *vivo* assays were also performed on sodium caseinate, chitosan and sodium caseinate/chitosan blend applied as film-forming solutions and wrapping on the different food substrate (Moreira et a., 2011).The objectives of this study were to (1) develop and optimize the procedure for the use of edible coatings in combination with anti-browning agent. The choice of the optimal formulation was carried out by analysis of the surface of apples using a scanning electron microscope. The choice of the best formulations has been done evaluating the homogeneity of the coating on the sample surface. (2) To evaluate the effects of CH, SC/CH coating on respiration, moisture and colour loss on minimally processed apples; since the temperature is a critic parameters for the preservation of fresh-cut apple, the experiment was carried out at different temperatures.

3.2. Materials and method

3.2.2. Materials

Marlene Royal apples (Trentino Alto Adige, Italy) were bought from a local farmer in Portici (Naples, Italy) and stored into a climatic chamber at each temperature of experiment for approximately 1 h to equilibrium the temperature before the test started. The fruits were selected on the basis of absence of defects. Chitosan medium molecular weight (MMW, deacetilation degree 75-85%), sodium caseinate, glycerol, acetic acid, sodium hydroxide, Phosphate buffered saline , citric acid, L-ascorbic acid, calcium chloride were purchased from Sigma-Aldrich (Milano, Italia).

3.2.3. Preparation of film forming solution

Chitosan solutions with a concentration of 1% and 2% (w/v) were prepared by dissolving the powder in:

(1) acetic acid (1% v/v, pH=2.8-3)
(2) citric acid (pH=1,6 e 2,6)
(3) anti-browning solution (pH=1.5-1,2)

All the solutions were stirred for about 16 hours at room temperature to obtain complete dispersion of chitosan. An exact amount of glycerol was added to achieve GLY/CH weight ratio of 0.1. In order to eliminate solid impurities, the dispersion was centrifuged at 3500 rpm for 10 min. CH dissolved directly in anti-browning solution showed a phase separation after 16 hours, maybe due to the interaction between CH and $CaCl_2$that didn't allow a complete solubilisation. CH dissolved in citric acid was not completely solubilized after 16 hours; since CH showed best solubility in a 1% (v/v) acetic acid solution (pH = 4.4)after 16 hours, the CH solution was prepared by using this solvent.
Sodium caseinate (SC) in a concentration of 4% was dissolved in a
(1)Phosphate-buffered saline (PBS) (pH= 6,8)
(2)Citrate buffer (pH= 7; pH=6,2)
(3)TRIS buffer (pH=8)

All the solutions were stirred for 4 hours at room temperature. An exact amount of glycerol was added to achieve GLY/SC weight ratio of 0.1.
TRIS buffer is not food grade but was chosen as reference because allows the complete solubilisation of SC; both citrate buffer and PBS were suitable for dissolving the SC powder after 4 hours.
Preparation of the polyelectrolyte complex between SC and CH required a careful control of the solution pH. SC is remarkably heat-stable at pH = 6.5 and highly insoluble at the isoelectric point, pH between 3.8 and 4.0. but remains soluble at pH lower than 6.4 (Moreira et al., 2011). In this work, the pH of the CH dispersion was adjusted to 5.0 using NaOH 1M. Film forming solution were obtained by mixing CH2% and all the solutions of SC in a proportion of 1:1 under stirring for 1h. Then the film forming solutions were degassed under vacuum for 15 min to remove air bubbles.
The SC solution (3) added to the CH, gave rise to an homogeneous, white and stable solution, with a final pH of 5,3 e 5,4. The SC solution (2), added drop to drop to CH, gave rise to a instable solution, with flocs and evident phase separation, maybe due to the interactions that can occur between CH and citrate ions which compete with SC for the interaction (Honary et al., 2010). The SC solution (1) showed a behaviour similar to the solution (3) with a final film forming solution homogeneous, opaques and milk white colour with a final pH between 5,2 e 5,4.

The film forming solution used for apple slices fruit coating are showed in Table 1.

Table 1.:Biopolymer concentration (g/100ml) of the film forming solution used to coat the apple slices (CH=chitosan, SC=sodium caseinate, Gly=glicerol), D4=SC/CH blend).

	g/100 ml			
	CH	**SC**	**Gly**	**SC/CH**
CH 1%	1	-	0.1	-
CH 2%	2	-	0.2	-
D4	1	2	0.3	2

3.2.4. Preparation of apple slices

Apples were selected for uniform size and appearance, without damages and gently washed with tap water for 3 minutes. Then apples were peeled and cut in 16 slices with a sharp stainless steel knife. The apple pieces were first dipped into the anti-browning solution for 2 minutes and drained for 1 minute. Anti-browning solution were prepared by dissolving 1g of acid citric, 1 g of L-ascorbic acid and 1 g of calcium chloride in 100 ml of deionized water in order to obtain a final solution with a pH between 1.5 and 2. The apples treated only in the anti-browning solution has been used as control. Then, samples were coated as reported in the next paragraph.

3.2.5. Coating application

The apple slices were dipped for 2 minutes in CH and SC/CH blend in order to obtain three samples as follows: (1) chitosan 1% (w/v), (2) chitosan 2% (w/v), (3) D4. The residues of slices solutions were allowed to drain for 1 min. Coated and uncoated apple slices were put on a grid and allowed to dry into a tunnel (Armfield tray drier)at 20° and 30°C. The proper drying time was experimentally determined by weighing the apple samples at regular intervals (2minutes) until the achievement of the initial weight before the coasting application.

3.2.6. Respiration rate measurements

O_2 consumption (RRO_2) and CO_2 production ($RRCO_2$) rates of minimally processed apples were measured using a modified closed system (Torrieri et al., 2009). The system (Figure) is composed of a cylinder (a), a humidification chamber (b), a splitter (c), manual on⁄off valves (d), micrometric valves (e), sample jars (4 L) (f) and a circulatorDC30 (ThermoHaake) (g). The jars are provided with a jacket where the thermostated water, coming from the circulator, keeps the internal jar space at a controlled temperature. The gas passes through the humidification chamber and then enters the jars where the product is located. The gas flow rate is controlled by the valves (e). The inlet section is located at the bottom of the jar and the outlet at the top to ensure uniform flushing of the gas mixture through the product, which is placed on top of a perforated plate located at a given distance from the bottom of the jar (f).The jar was provided with a septum for gas sampling. The gas tightness of the jars was tested by inserting a gas mixture of known composition into the empty sealed jar and subsequently monitoring possible changes in gas composition over time. The product (0.5 kg) was placed in a steel jars(0.004 m3), conditioned for 1 hour at temperature test and hermetically closed. The temperature and relative humidity inside the jar were monitored by means of a data logger (Escort Data Login Systems Ltd, Naples, Italy).For all the formulations, analysis were performed at 5,10 and 20°C in ambient air.

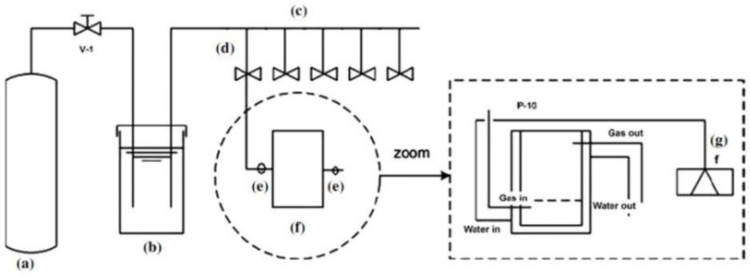


Figure1. Schematic diagram of experimental modified closed system for measuring fresh respiration rate: (a) cylinder, (b) humidification chamber,(c) splitter, (d) on⁄off valves, (e) micrometric valves, (f) sample jars, (g) a circulator DC30. (fromTorrieri et al., 2009)

At regular time intervals (Δt), 3 mL of gas mixture was drawn from the jar headspace and analysed using a gas analyser for O_2 and CO_2% (PBI; Dansensor, Ringsted, Denmark). The rate of consumption of O_2, RRO_2 , and production of CO_2, $RRCO_2$, expressed as ml/(kg h), were determined as :

$$\boldsymbol{RRO_2} = \frac{\boldsymbol{Vf}}{\boldsymbol{W}\,\boldsymbol{100}} \times \frac{\boldsymbol{dO_2}}{\boldsymbol{dt}} \tag{3.1}$$

$$\boldsymbol{RRCO_2} = \frac{\boldsymbol{Vf}}{\boldsymbol{W}\,\boldsymbol{100}} \times \frac{\boldsymbol{dCO_2}}{\boldsymbol{dt}} \tag{3.2}$$

where W is the mass of the sample (kg) and Vf (mL) is the head space inside the jar given by:

$$\boldsymbol{Vf} = \boldsymbol{V} - \frac{\boldsymbol{W}}{\boldsymbol{\rho}} \tag{3.3}$$

where V is the volume of the jar, ρ the apparent density of the apples (0.810 g/mL) and W the weight of the apples (kg).

Respiration rate expressed as mol/(kg h) were determined as follows:

$$\boldsymbol{RRO_2} = \frac{\boldsymbol{Vf}}{\boldsymbol{W100}} \times \frac{\boldsymbol{dyO_2}}{\boldsymbol{dt}} \times \frac{\boldsymbol{P_{tot}}}{\boldsymbol{RT}} \tag{3.2}$$

$$\boldsymbol{RRCO_2} = \frac{\boldsymbol{Vf}}{\boldsymbol{W100}} \times \frac{\boldsymbol{dyCO_2}}{\boldsymbol{dt}} \times \frac{\boldsymbol{P_{tot}}}{\boldsymbol{RT}} \tag{3.5}$$

where dyO_2 and $dyCO_2$ are the volumetric concentration of O_2 and CO_2, respectively, at time t (% v/v) Ptot is thetotal pressure (Pa), R is the universal gas constant(Pa m^3/mol/K) and T is the temperature (K).

The respiration quotient, RQ, was calculated as follows:

$$\boldsymbol{RQ} = \frac{\boldsymbol{RRCO_2}}{\boldsymbol{RRO_2}} \tag{3.3}$$

An Arrhenius-type equation was applied to the experimental respiration rate data to quantify the influence of temperature on respiration rate of minimally processed apples:

$$\boldsymbol{RRO_2 = RRO_{2_0}.\exp\left[\left(-\frac{Ea1}{R}\right)\times\left(\frac{1}{T}-\frac{1}{T_0}\right)\right]} \quad (3.7)$$

$$\boldsymbol{RRCO_2 = RRCO_{2_0}.\exp\left[\left(-\frac{Ea2}{R}\right)\times\left(\frac{1}{T}-\frac{1}{T_0}\right)\right]} \quad (3.8)$$

Where RRO_{20} (ml/(kg h)) is the value that respiration rate assumes at a given reference temperature (T0). Ea1 and Ea2 are the activation energy (kJ/mol) constant for O_2 and CO_2,respectively, and R is the universal gas constant(kJ /(mol K)).

Arrhenius parameters were estimated by linear regression using Excel 2007 software (Microsoft Office 2007).

3.2.7. Water vapour resistance

The determination of water vapour resistance (WVR) of coatings was carried out according to Chiumarelli et al.(2012). Apple cylinder samples of a diameter of 16 mm and 20 mm of height were coated and uncoated . The exposed area, taken as the upper surface and lateral area of the cylinders, was 12.06 cm^2. The samples were equilibrated for 24 h at 20±1°C in desiccators maintained at 0.97 with a K_2SO_4 solution. After 24 h, the samples were placed in small test cups, weighed in an analytic balance and placed in two different conditioned system: (1) at 20°C into a desiccators equilibrated at aw=0.6; (2) at 5, 10 and 20°C into a desiccators containing silica gel with a aw=±0.06. Weight was taken periodically during a 24 h period and the water vapour flux was determined as follow:

$$\boldsymbol{WF = \left(\frac{dP}{dt}\right)\times\left(\frac{1}{A}\right)} \quad (3.4)$$

Where WF is the water vapour transferred per unit of area exposed (g s^{-1} cm $^{-2}$);dP / dt is the water vapour transferred (gs^{-1}); A is the area exposed.

The water vapour resistance (WVR) which is the resistance of the coating to water diffusion (s cm^{-1}) was calculated as:

$$\boldsymbol{WVR = \left[\left(\frac{a_w-\frac{RH}{100}}{RT}\right)\times P_{WV}\right]\times\left(\frac{1}{WF}\right)} \quad (3.5)$$

Where: a_w = water activity of the sample; RH =relative humidity of the environment; P_{WV} = saturated water vapour pressure (mmHG);R=gas constant (3464,629 mmHg cm3 K^{-1} g^{-1});T =temperature (K). Each measurement was conducted in triplicate. Water activity of the apples were measured with an Aqualab (Water activitymeter 4TE, USA) at 20°C.

3.2.8. Colour measurements

The colour of apple slices was measured with a colorimeter (Minolta Chroma MeterMod. CR 300, Osaka, Japan) after 4 days in different storage conditions. The degree of browning was expressed as the change in the L * value, a *, b *, □E *. The results were expressed as a mean value of three replicates of 10 samples measured. The Hunter parameters L* (from 0=black to 100=white), a* (- a*= greenness to + a*= redness), and b* (-b*=blueness to +b*= yellowness) were measured and averaged from three randomly position for each sample the total colour difference (ΔE) was calculated according to the following equation (Ghorpade, Li, Gennadios, and Hanna, 1995):

$$\boldsymbol{\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}} \quad (3.6)$$

Where ΔL*, Δa*,Δb*are the differences between a sample colour parameter and the colour parameter of a standard taken as the apple treated with anti-browning agent at time 0 (L = 84, a= -5.5, b=+26.3) used as background.

3.2.9. Microstructure

Before being observed with a scanning electron microscopy (SEM) (LEO EVO 40 SEM, Zeiss, Germany), the samples (~ 3 mm of thickness and 6 mm of diameter) were frozen (-18 ° C for 1 h) using a chiller (Sirman), lyophilized (-50 ° C for 24 h) using a lyophilizer (Alphal-2LD plus) Subsequently they were metallized with a metallizer (AGAR B7340, Agar Scientific Ltd, Stansted, UK). The microstructure of sections of lyophilisates apples were examined with a scanning electron microscope with a 20kV accelerating voltage to a variable magnification from 200 to 1500 X.

3.2.10. Data analysis

Data were submitted to analysis of variance by means of SPSS v20.0 for Windows (SPSS, Milan, Italy). Duncan's test was carried out to find the source of the significant differences within the samples examined; significance of difference was defined at $p<0.05$.

3.3. Results and discussion

3.3.1. Coating application

The drying time for the coatings depends on the concentration of biopolymers; thus, in the first part, preliminary tests were carried out in order to standardize the proper drying time for each formulation. Apple slices were weighed at time 0, than dipped in the film forming solution and weighed afterward. The samples were placed in an air stream at 20 and 30°C, and their weight was monitored over time; as the final weight reached the initial one, the sample was considered dried. In Figure 2 is showed the weight loss of the control sample at both temperature.

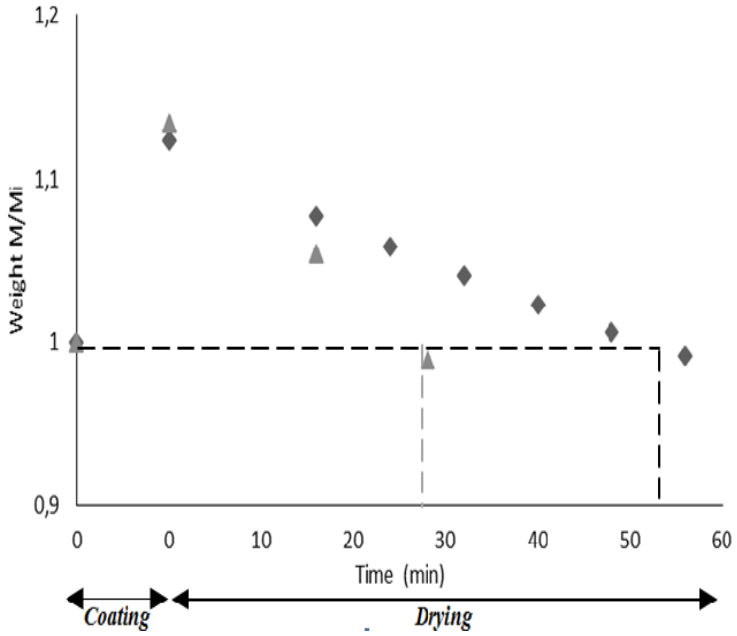
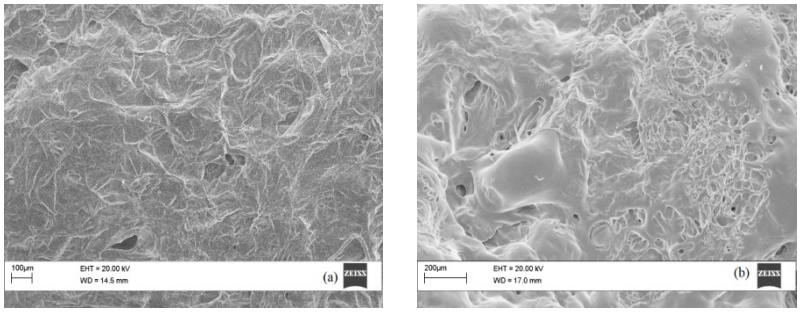


Figure 2.: Drying curve at 20°C (♦) and 30°C (▲) of the control sample

At 20°C the sample took about 50 minute to reach the initial weight and to be dried on the surface; at 30°C the sample took about 25 minutes. Since at the end of drying process, both samples didn't show differences in terms of colour and texture, it was chosen to use 30°C to dry apples slices for all the formulation tested.
At 30°C CH 2% took about 40 minutes to be dried; the apples coated with CH1% took 35 minutes and the samples coated with the blend D4 took 30 minutes.

3.3.2. Microstructure

Characteristic SEM images of surface sections of the apple samples coated at different formulation are showed in Figure 3.



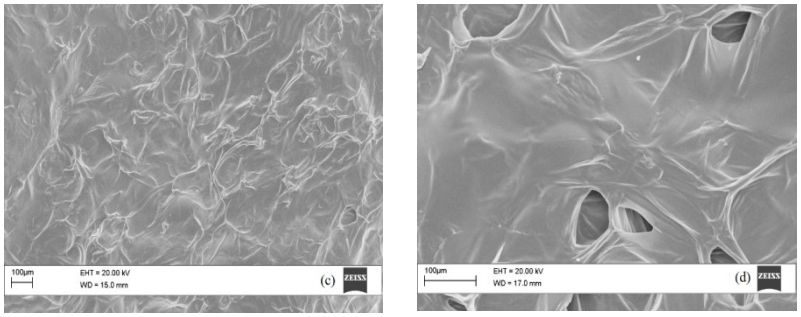


Figure 3. SEM micrographs of apple uncoated without anti-browning (a), CH2% without anti-browning (b), CH2% with citric acid(c); CH2% with anti-browning(d). Magnification 200x.

Scanning electron microscopic examination was performed to understand better the structure of the different apples coated. Sample coated with CH2% without a preliminary treatment in anti-browning solution compared to samples CH 2% treated before in an acid solution showed a non-homogeneous coating; this phenomena could be due to the positive charge of chitosan that didn't establish interaction with pectin on the surface of apples, due to the low negative charges.
In figure 3 the sample (c) has been immersed previously in citric acid and then in CH2%; this treatment has involved a better coating distribution, maybe because citric acid can charge negatively the pectin; however the use of citric acid has led to a more rapid browning.
In figure 4 scanning electronic microscopy of blend D4 (SC/CH=2) with different solvent for SC and pretreatment are reported.

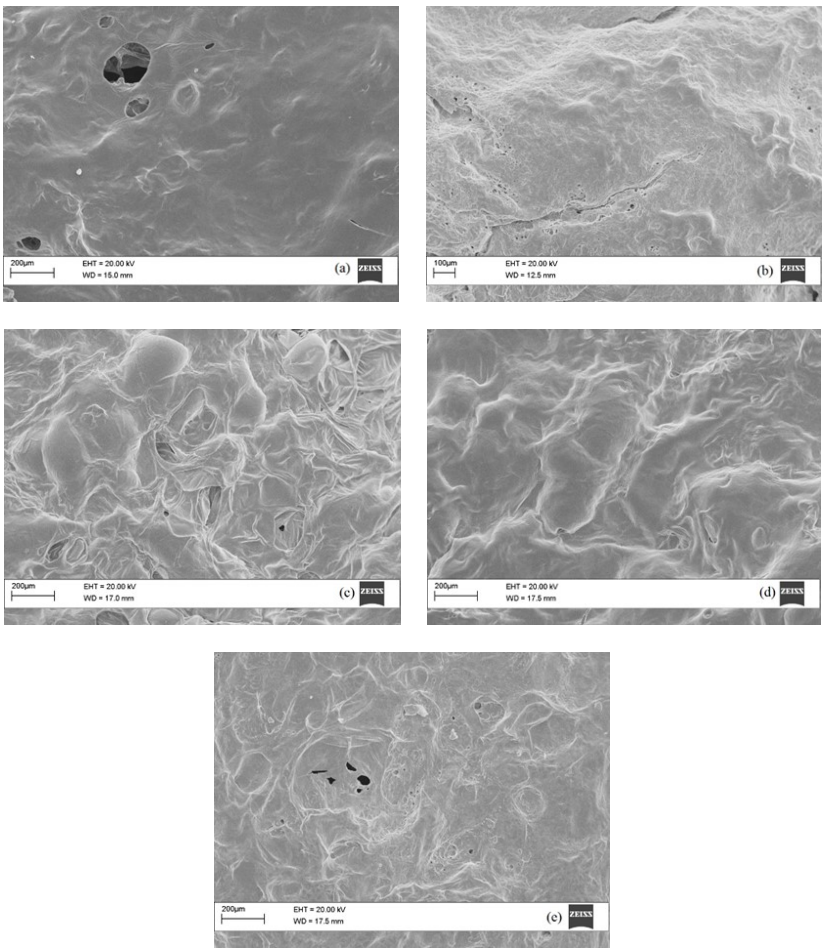


Figure 4. SEM micrographs of sample D4 with SC solubilized in TRIS buffer (a), sample D4 with SC solubilized in TRIS buffer and previously dipped in citric acid (b), sample D4 with SC solubilized in TRIS buffer and previously dipped in anti-browning solution (c), sample D4 with SC sample D4 solubilized in PBS buffer (d), sample D4 with SC solubilized in PBS buffer and previously dipped in anti-browning solution (e). Magnification 200x

The samples immersed in the blend D4 without any pretreatment (figure 4 (a) and (d)) in both solvent (TRIS and PBS buffer) showed an homogeneous surface; however the samples underwent to browning quickly. The sample (b), treated previously with citric acid, didn't allow an homogeneous surface. The samples (c) and (e) even were not as homogeneous as samples (a) and (d), were found to be the best since they have helped to limit browning.

3.3.3. Respiration rate measurements

The wounding and damage caused by cutting, peeling, slicing and shredding could raise the respiration rate and ethylene production of minimally processed fruit; thus it is important to control or reduce the respiration rate in order to extend shelf life. At higher temperature the respiration rate is higher, so it is important to understand how the coating affects the respiration rate at different temperature. The following figures show the variation of O_2 and CO_2 for all the formulation tested at different temperature.

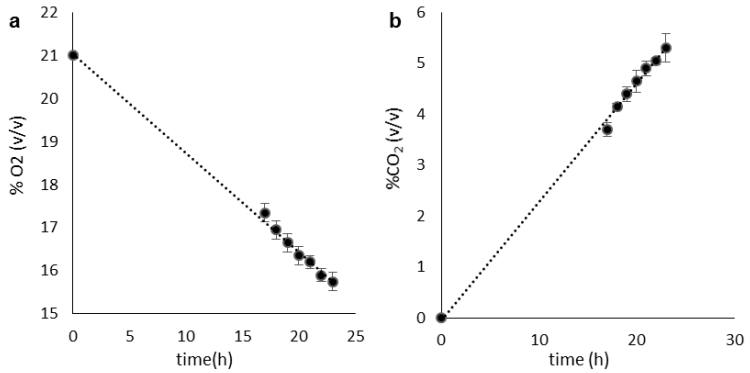


Figure 5. Headspace gas analysis for control during 24 hours of storage in air at 20°C, **a** O_2 concentration; **b** CO_2

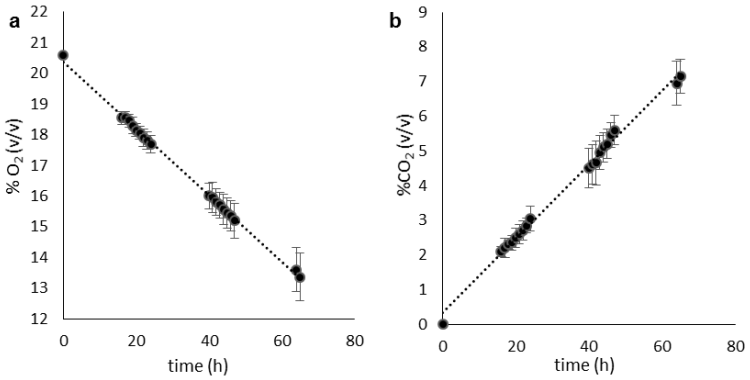


Figure 6. Headspace gas analysis for control during 65 hours of storage in air at 10°C, **a** O_2 concentration; **b** CO_2

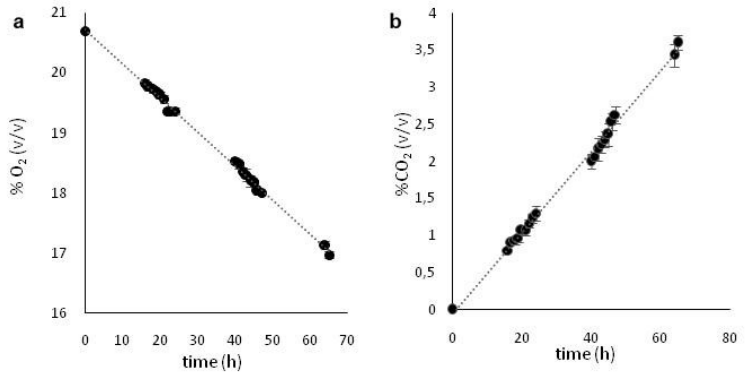


Figure 7. Headspace gas analysis for control during 70 hours of storage in air at 5°C, **a** O_2 concentration; **b** CO_2

At 20 °C (Figure 5) the O_2 concentration of the control sample reached1 5.75% after 23 hours. 10°C (Figure 6), O_2 concentration decreased from 20.7% to 13.35% after 65 hours, and CO_2 increased from 0% to 7.15%. At 5°C (Figure 7) O_2concentration ranged from 20.7% to 17% during 65 hours and the CO_2 reached 3.6%.

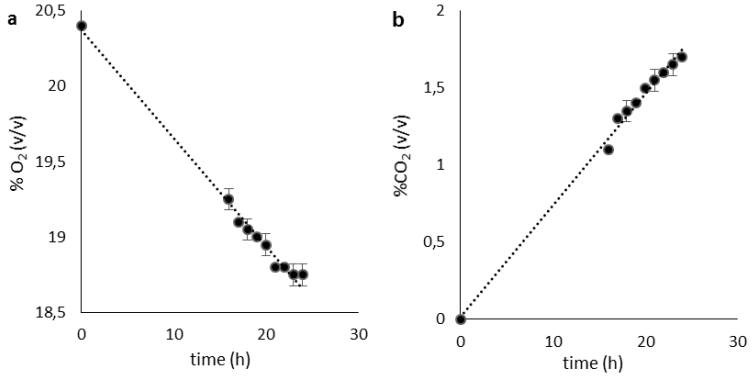


Figure 8. Headspace gas analysis for CH2% coated apple during 24 hours of storage in air at 20°C, **a** O_2 concentration; **b** CO_2

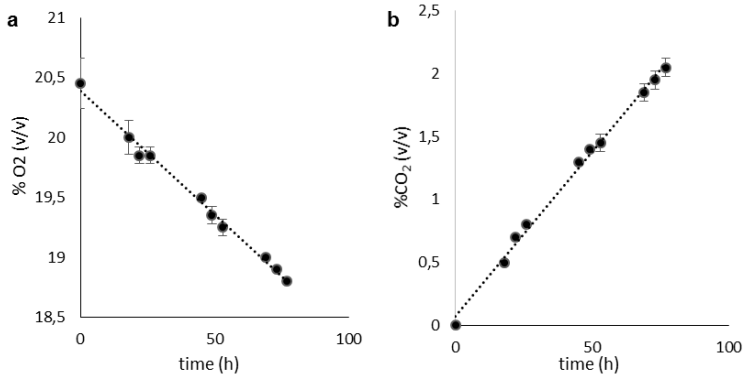


Figure 9. Headspace gas analysis for CH2% during 80 hours of storage in air at 10°C, **a** O_2 concentration; **b** CO_2

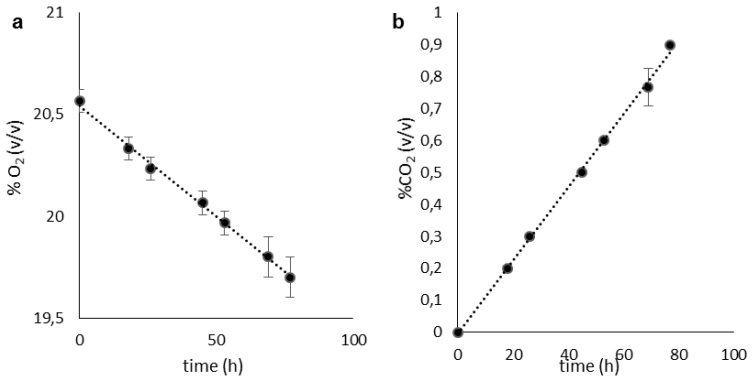


Figure 10. Headspace gas analysis for CH2%coated apple during 80 hours of storage in air at 5°C, **a** O_2 concentration; **b** CO_2

At 20 °C (Figure 8) the O_2 concentration of the CH2% sample ranged from20.4% to 18.7% after 24 hours, whereas CO_2 reached 1.7%. At 10°C (Figure 9), O_2 concentration decreased from 20.4% to 19.7%, and CO_2 increased from 0% to 2%. At 5°C (Figure 10) O_2 concentration ranged from 20.6% to 19.7% whereas the CO_2 reached 0.9%.

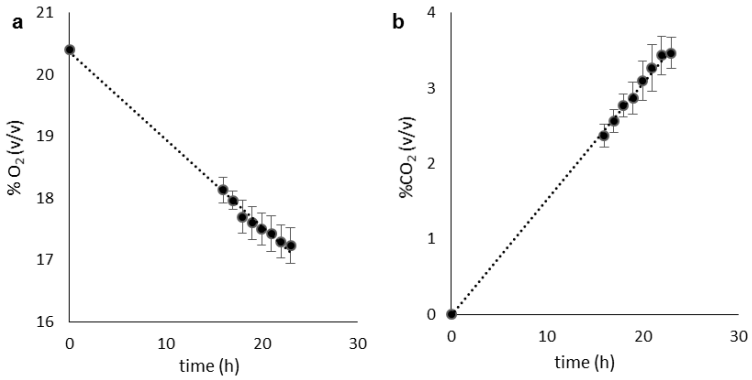


Figure 11. Headspace gas analysis for D4 coated apple during 24 hours of storage in air at 20°C, **a** O_2 concentration; **b** CO_2

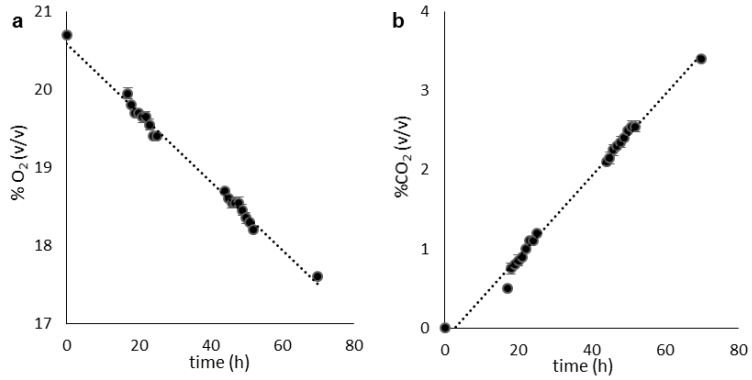


Figure 12. Headspace gas analysis for D4 coated apple during 70 hours of storage in air at 10°C, **a** O_2 concentration; **b** CO_2

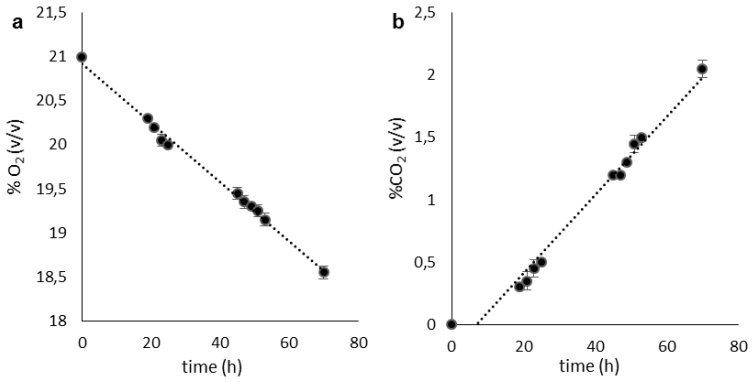


Figure 13. Headspace gas analysis for D4 coated apple during 70 hours of storage in air at 5°C, **a** O_2 concentration; **b** CO_2

At 20 °C (Figure 11) the O_2 concentration of the D4 sample ranged from 20.4% to 17.2% after 24 hours, whereas CO_2 reached 3.5%. At 10°C (Figure 12) O_2 concentration decreased from 20.9% to 17.6%, and CO_2 increased from 0% to 3.4%. At 5°C (Figure 13) O_2concentration ranged from 21% to 18.5% whereas the CO_2 reached 2%.

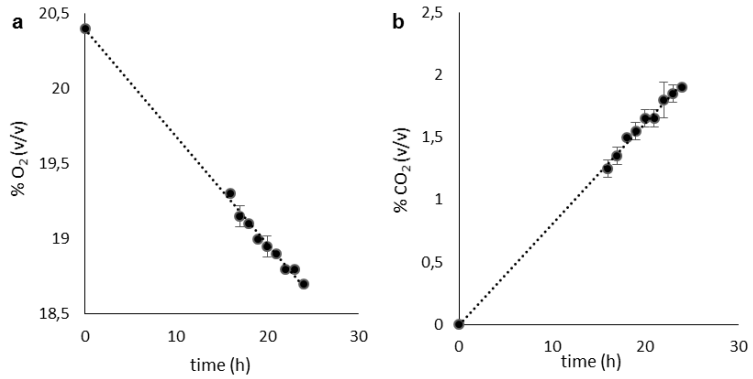


Figure 14. Headspace gas analysis for CH1% coated apple during 24 hours of storage in air at 20°C, **a** O_2 concentration; **b** CO_2

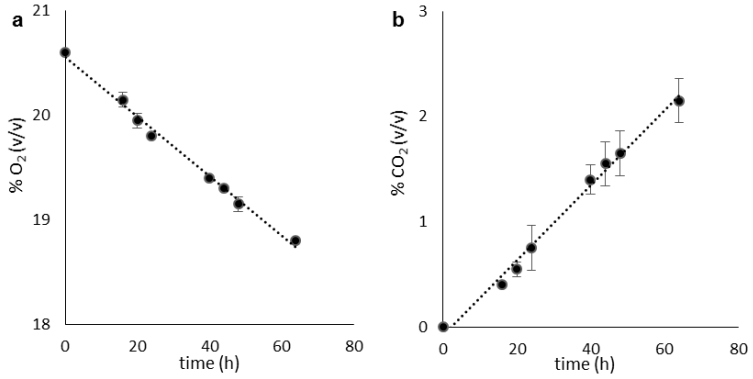


Figure 15. Headspace gas analysis for CH1% coated apple during 65 hours of storage in air at 10°C, **a** O_2 concentration; **b** CO_2

At 20 °C (Figure 14) the O_2 concentration of the CH1% sample ranged from 20.4% to 18.7% after 24 hours, whereas CO_2 reached 1.9%. At 10°C (Figure 15) O_2 concentration decreased from 20.4% to18.8% after, and CO_2 increased from 0% to 2.15%. At 5°C the values was calculated experimentally by using Arrhenius equation.

Table 2 shows the respiration rate expressed in ml/(Kg h) of O_2 and CO_2, the respiration quotient of all the formulation for the three temperatures tested.

Table 2. Respiration rate of O_2, and CO_2 and respiration quotient (RQ) of the samples coated and uncoated at 5, 10 e 20°C

Sample	Temperature	RRO_2	$RRCO_2$	RQ
-	°C	ml/(kg h)	ml/(kg h)	-
	5	3.86±0.07	3.7±0.1	0.95
Control	10	7.3±0.6	7.2±0.6	0.98
	20	15.6±0.6	15.7±0.5	1.01
	5	0.74±0.14	0.8±0.0	1.05
CH2%	10	1.4±0.2	1.8±0.1	1.27
	20	4.8±0.2	4.9±0.1	1.01
	5*	**1.2**	**1.56**	**1.29**
CH1%	10	1.9±0.7	2.4±0.3	1.25
	20	4.8±1.6	5.4±0.2	1.13
	5	2.3±0.8	2.4±0.0	1.05
D4	10	3.0±1	3.5±0.0	1.17
	20	9.3±2.2	10.3±0.8	1.10

theoretically estimated by Arrhenius equation

For all the formulation tested *RQ* remains within the range considered normal for a type of aerobic respiration and never falls below 0.95. The maximum value is 1.29 at 5°C for the sample CH1%.
With regard to the effect of temperature on respiration rate of minimally processed apples, as expected, O_2consumption and CO_2 production increased with temperature for all the coated and uncoated samples. The coated apple pieces generally showed lower initial respiration rate than that for sample uncoated.
In the following figures the temperature and coating effect expressed as ml/(kg h) of O_2 is reported.

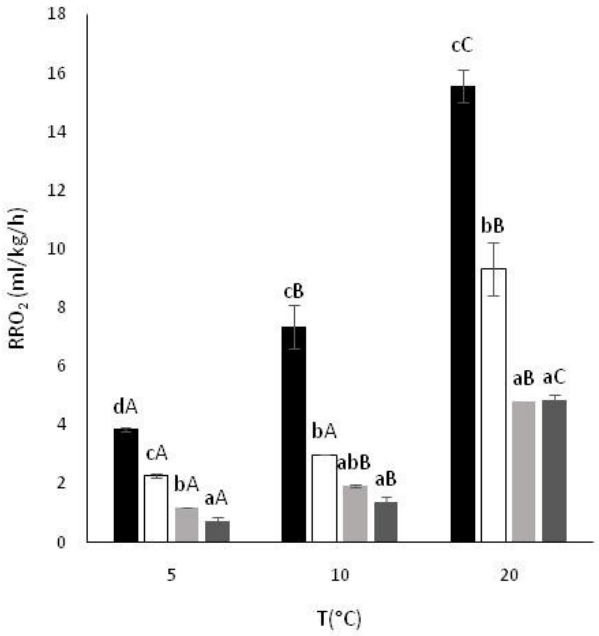


Figure 16. Respiration rate of the control (■), D4 (□), CH1% (■), CH2%(■). Different letters indicate significant differences ($p<0.05$); lowercase indicate coating effect; uppercase indicate temperature effect.

At 5°C all the sample are significantly different; the control showed the highest respiration rate equal to 3.86±0.07 ml/(kg h) whereas CH2% had the lowest value equal to 0.74±0.14 ml/(kg h). At 10°C CH1% and CH2% were not significantly different from each other, with D4 not different from CH1%; even at 10°C control showed the highest respiration rate equal to 7.3±0.6 ml/(kg h). At 20 °C CH1% and CH2% are equal to each other and different from D4 and control. As showed in Figure 16 there is a temperature effect for CH2% and CH1% and control; by increasing the temperature from 5 to 20°C the respiration rate of these samples changed significantly; instead D4 didn't show difference between 5 and 10°C, whereas is significantly at 20°C.

Our results confirm the positive role of the coating on respiration rate reduction according to Chiumarelli et al.(2012); the coating could form a thin film on the surface of processed fruit to retard gas exchange, aging as barrier. In this way there is less oxygen available for the tissues respiration and there is less release of carbon dioxide by the product. The positive role of edible coating on Fuji apples is confirmed also by Qi et al. (2011); they studied the effects of coatings in combination with anti-browning agents (1% Chitosan; 1%L-ascorbic acid+0.5% $CaCl_2$ and 2%ascorbic acid+ 0.5% $CaCl_2$ +1% Chitosan) and they found that chitosan-coating applied to cut apples were able to control initial respiration rate. Our results are in disaccording to Leceta et al.(2015), that used a chitosan-based coating (1% w/v) on baby carrots applied by spraying and dipping; they found that independently of the coating technology used, a the application of chitosan coating increased the respiration activity of carrots.

The effect of temperature on respiration rate of minimally processed apples was significant and its effect was well described by an Arrhenius-type relationship, as shown in Figure 17.

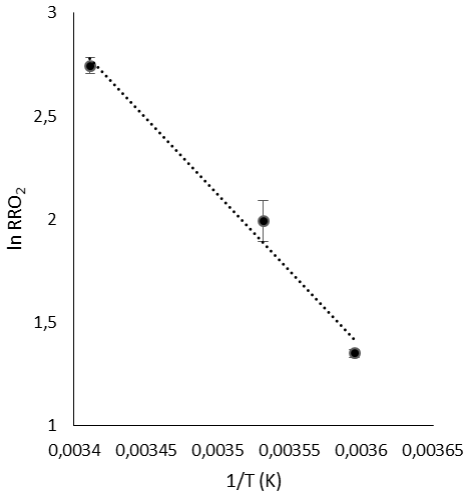


Figure 17. Effect of temperature on respiration rate of minimally processed apples. Symbol represents the experimental data with the standard deviation; the line represents the fit of Arrhenius equation on experimental data.

In the following table activation energy and respiration rate values at reference temperature for all the formulation tasted are reported.

Table 3. Activation energy (Ea) and RRO_{20} at T_0 (reference temperature equal to 284,6 K) of apple coated with different formulation and uncoated.

Sample	RRO_2	Ea
	ml/(kg h)	KJ/mol
Control	7.3	61.6
CH2%	1.7	85.6
CH1%	2.2	63.5
D4	4.2	65.7

3.3.4. Water resistance

The WVR of apple cylinders coated and uncoated at 20°C with a partial pressure difference equal to 0.3 are reported in Figure 18:

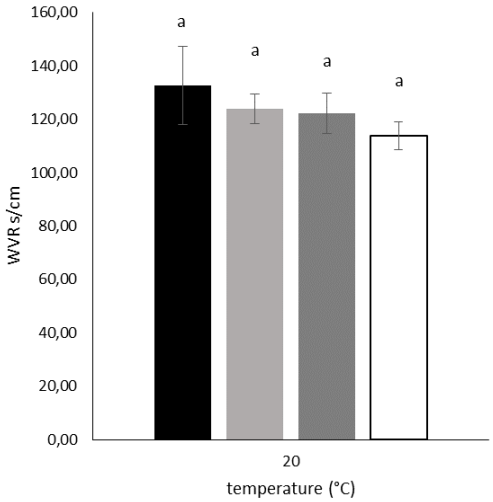


Figure 18. WVR of sample control (■), D4 (□), CH1% (■), CH2% (■) at 20°C with a partial pressure difference of 0.3

The WVR of the control was 133±14 (s/cm), CH1% 124±6(s/cm), n CH2% 122±6 (s/cm) and D4 114±5 (s/cm). These results indicate that there were not significant difference between coated and uncoated samples on weight loss; this can be justified considering that polysaccharides and protein have hydrophilic nature and higher permeability to water vapour. Similar results were reported by Qui et al. (2011); Leceta et al. (2015) showed that the weight loss for chitosan dipped samples was higher compared to the sprayed one and the control. Natural and synthetic waxes and lipids can help to obtain more efficient barrier to water vapour because they have a hydrophilic nature and these molecules cannot easily interact with water. This is confirmed by Garcia et al. (2000), Han et al. (2006) and Chiumarelli et al. (2012).
In order to verify if the partial pressure difference had an influence on WVR, the test was performed by using a partial pressure difference equal to 0.89 (0.06 environment-0.95 sample) at 5, 10 and 20°C. In the

Figure the WVR values of all the formulation tested at different temperature are shown.

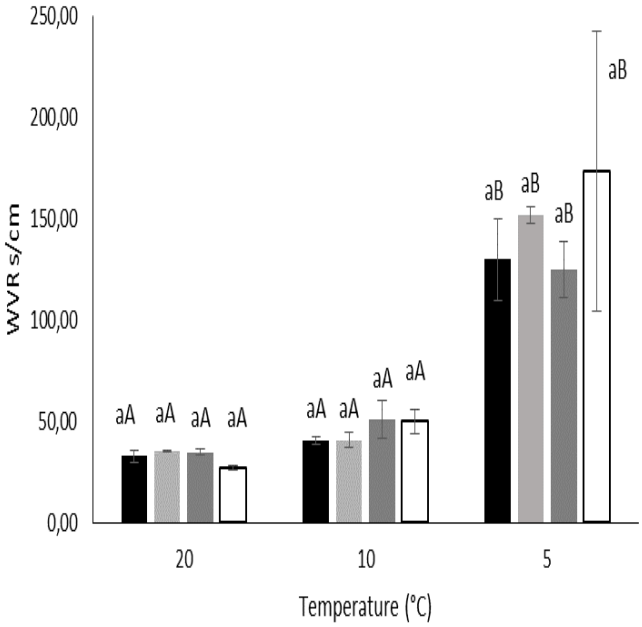


Figure 19. WVR at 5, 10 and 20°C; control (■), D4 (□),CH1% (■), CH2% (■). At different letters correspond significant differences ($p<0,05$); lowercase indicate coating effect, uppercase indicate temperature effect.

At 20°C WVR for the control was equal to 33±3 (s/cm), 35,5±0,6 (s/cm),for CH1%, 35±1 (s/cm) for CH2% and 27±1 (s/cm) for D4.
At 10°C WVR for the control was equal to 41±2 (s/cm), 41±4 (s/cm) for CH1%, 51±9 (s/cm) for CH2% and (s/cm) 50±6 for D4.
At 5°C WVR for the control was equal to 130±20 (s/cm), 152±4 (s/cm) for CH1%, 125±14 (s/cm) for CH2% and (s/cm) 174±69 for D4.
Statistical analysis showed that CH1%, CH2% and D4 didn't affect the WVR. Moreover there is a significant effect on temperature; in particular there were not differences between 10 and 20°C compared to 5°C.

3.3.5. Colour measurements

In Table 4, L*, a*, b* and ΔE at 5, 10, 20 °C for the coated and uncoated apple slices are reported:

Table 4. Colour parameters (L*, a*, b*, ΔE) of apple slices at 5, 10, 20 °C

Sample	T(°C)	L*	a*	b*	ΔE
Control	5	71 ± 2^{aA}	$(-1)\pm1^{aA}$	25 ± 3^{bA}	16 ± 3^{aA}
	10	75 ± 3^{bA}	$(2,8)\pm0,5^{aA}$	25 ± 3^{aA}	13 ± 4^{aA}
	20	74 ± 2^{bA}	$(-3)\pm1^{aA}$	26 ± 2^{aA}	15 ± 3^{aA}
D4	5	74 ± 4^{aA}	$(3,4)\pm0,8^{aA}$	19 ± 5^{aA}	12 ± 3^{aA}
	10	75 ± 4^{bA}	$(-3)\pm1^{aA}$	22 ± 6^{aA}	12 ± 4^{aA}
	20	75 ± 3^{bA}	$(-2)\pm1^{aA}$	24 ± 2^{aA}	13 ± 3^{aA}
CH1%	5	74 ± 4^{aA}	$(-2)\pm2^{aA}$	26 ± 3^{bA}	14 ± 5^{aA}
	10	70 ± 5^{bB}	2 ± 2^{bB}	30 ± 3^{bB}	21 ± 5^{bB}
	20	64 ± 2^{aB}	$3,7\pm0,8^{bB}$	34 ± 1^{bC}	29 ± 2^{bC}
CH2%	5	74 ± 3^{aB}	$(3)\pm1^{aA}$	25 ± 2^{bA}	14 ± 4^{aA}
	10	63 ± 4^{aA}	3 ± 1^{bB}	$31,8\pm0,9^{bB}$	27 ± 3^{bB}
	20	64 ± 4^{aA}	$2,2\pm0,5^{bB}$	32 ± 4^{bB}	27 ± 5^{bB}

- At different letters correspond significant differences ($p<0,05$); lowercase indicate temperature effect, uppercase indicate coating effect.

The effect of temperature and coating for each parameter are shown in the following figures:

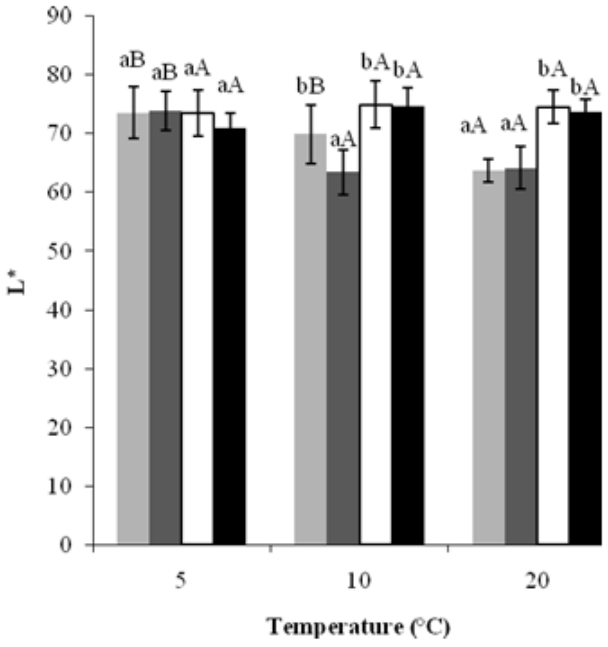


Figure 20. Temperature and coating effect for L* parameter (control (■), D4 (□), CH1% (■), CH2% (■)). At different letters correspond significant differences ($p<0,05$); lowercase indicate temperature effect, uppercase indicate coating effect

Temperature was not significant for L* for control and D4; for CH1% L* was significant different at 20°C compared to 5 and 10°C. CH2% showed differences ($p<0.05$) at 5°C. Statistical analysis showed that there were no differences at 5°C between all the samples; at 20 °C only CH2% was significant different from the others, whereas at 20°C CH1% and CH2% differed significantly from control and D4.

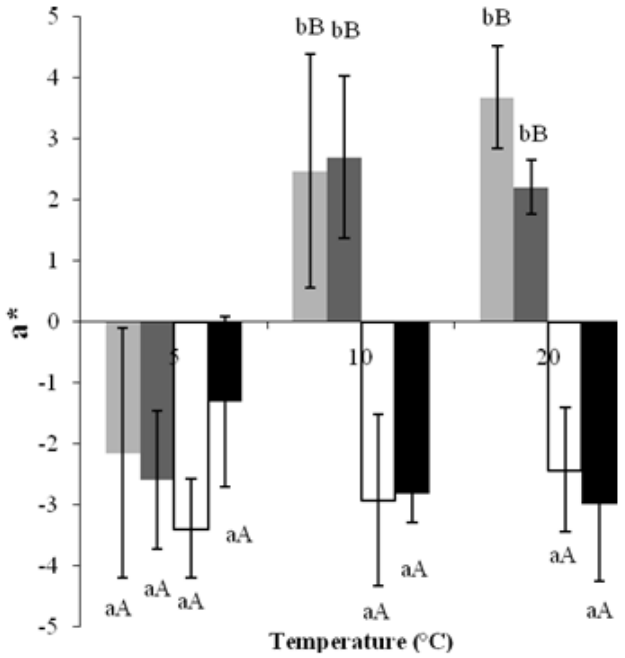


Figure 21. Temperature and coating effect for a* parameter (control (■), D4 (□), CH1% (■), CH2% (■)). At different letters correspond significant differences ($p<0,05$); lowercase indicate temperature effect, uppercase indicate coating effect

Temperature was not significant for a* for control and D4; for CH1% a* was significantly different at 20 and 10°C compared to 5 °C. CH2% showed differences ($p<0.05$) at 5°C. Regarding the effect of coating, at 5°C the samples were not significantly different; at 10 and 20°C D4 and control sample differed from CH1% and CH2%.

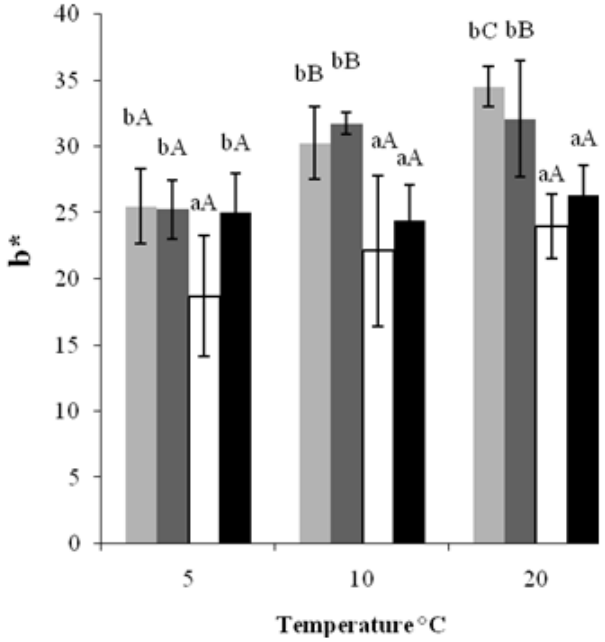


Figure 22. Temperature and coating effect for b* parameter (control (■), D4 (□), CH1% (■), CH2% (■)). At different letters correspond significant differences ($p<0,05$); lowercase indicate temperature effect, uppercase indicate coating effect

Temperature was not significant for b* for control and D4 at 5, 10 and 20°C; for the sample CH2%there were not differences at 10 and 20 °C compared to 5°C whereas CH1% showed difference at 5, 10 and 20°C. Regarding the effect of coating, at 5°C the samples D4 were significantly different from the other samples; at 10 and 20°C D4 and control sample differed from CH1% and CH2%.

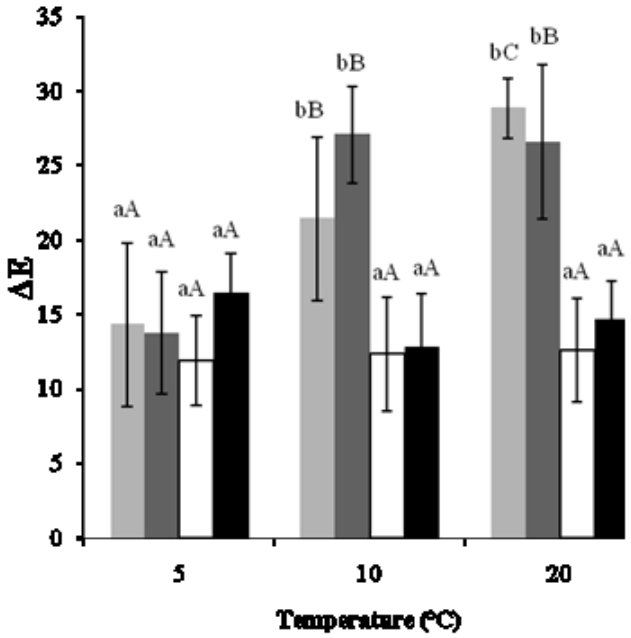


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Figure 23. Temperature and coating effect for Δe parameter (control (■), D4 (□), CH1% (■), CH2% (■)). At different letters correspond significant differences (p<0,05); lowercase indicate temperature effect, uppercase indicate coating effect

Temperature was not significant for ΔE for control and D4 at 5, 10 and 20°C; for the sample CH1% there were differences at 10 and 20 °C compared to 5°C whereas CH2% showed difference at 10 and 20°C compared to 5°C. Regarding the effect of coating, at 5°C all the sample were not significantly different from each other; at 10 and 20°C D4 and control sample differed from CH1% and CH2%.

All the samples tested, after 4 days, showed a browning. These results suggest that on batch scale the equipment employed were not able to ensure a correct preparation sample; on lab scale, in fact, apples didn't show browning (data not shown). However low temperature retarded the changes in browning as confirmed by Qi et al. (2011) and al the samples were not significantly different. ΔE increased ($p<0.05$) by increasing temperature for CH1% and CH2%, whereas remained constant for control and D4. In literature is reported that chitosan coating delay browning on mango fruit (Chien et al., 2007), preserve the colour of baby carrots (Leceta et al., 2015), inhibited the browning in Fuji apples (Qui et al., 2011). In this work we can therefore say that a coating of SC/CH blend is more efficient to preserve the colour.

3.4. Conclusion

Our results showed that coating treatments were able to reduce the respiration rate of minimally processed apples. In particular, apples coated with CH1%, CH2% showed at 20°C a reduction of about 70% compared to the control and D4 (SC/CS blend, ratio=2) of 40%. At 10°C apples slices showed a reduction for CH1%, CH2% and D4 of 75%, %81 and 60%, respectively. At 5°C the respiration rate was reduced of 81% for CH2%, 70% for CH1% and 41% for D4 compared to the control. Regarding the colour, only the sample D4 didn't show difference from the control sample, which was treated with a commercial anti-browning solution. All the coating treatments did not performed very well as water vapour barriers in apple slices.

3.5. References

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