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Doctoral Thesis

BIO-BASED FOOD PACKAGING FILMS: INFLUENCE OF FORMULATION AND PROCESSING ON FUNCTIONAL PROPERTIES

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“We cannot all do great things,

But we can do small things with great love”

Mother Teresa of Calcutta

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Aim of the study

The general aim of this PhD project was the development of a bi-layer food packaging film through the use of materials coming from renewable resources. The use of bio-materials can be explained because the growing reliance on oil-based polymers had raised several environmental and human health issues due to their persistence in the environment. PLA was chosen since it is a thermoplastic material with good processability and can be processed in machines used for conventional plastic. However, it is brittle and has moderate barrier properties to water vapour and oxygen along with low temperature of degradation. For this reasons in this project the main purpose was to improve the properties of PLA firstly through the incorporation of nanoclay and then through the application of a suitable coating in order to produce bi-layer films, entirily produced from renowable resources.

This thesis consists of three main parts where the specific aims are:

- Part I: production of nanostructured PLA-based films. In section A, films were produced by extrusion. The aim was to investigate the combined effects of the addition of four types of OMMT possessing different chemical structures in two PLA matrices consisting of distinct contents of L-isomer, on the physico-chemical properties of the resulting films. In section B, three nanoclays used in the previous section were incorporated in a PLA matrix through sthe use of solvent casting technique. Films obtained in section A are more promising because obtained by extrusion, a technique conventionally used for the processing of plastic. Therefore, they were characterized in a deeper way.
- Part II: the aim of this part was the realization of a suitable coating for PLA in order to improve the barrier properties. High-pressure was applied on film-forming solutions in combination with mild heat treatment and holding time to evaluate if an effect on the barrier to oxygen could be observed.
- Part III: production of a bi-layer by using the films with the best performance as barrier to oxygen from Parts I and II.

Abstract

Bio-based nanocomposite films were produced by adding different types of OMMTs in a PLA polymer matrix and compared with the unfilled materials. For the production of the films, two techniques were used, extrusion and solvent casting.

In particular, with the extrusion process, the nanocomposite films were produced by blending two semicrystalline PLA matrices with four types of OMMTs. In order to study their effect on optical, structural, thermal, mechanical and surface properties, they were added at a fixed nominal concentration of 5 % w/w and compared with pure PLA films. The XRD measurements showed that the incorporation of small amounts of nanoclays resulted in a formation of nanocomposites with the intercalation of PLA chains between the platelets of the nanoclay. An increase in UV barrier properties was observed in nanocomposites, with Closite® C20A showing the best performance. The content of L-isomer in PLA significantly affected the thermal properties of the films, with a decrease in T_m of about 14˚C and increase in T_c of about 20˚C when the matrix, possessing a higher D-isomer content, was used. FTIR results showed an interaction between the nanoclays and the two different PLA matrices, due to the appearance of new peaks in nanocomposites films at around 520 and 627 cm^{-1}. Mechanical properties were differently affected by the addition of nanoclays, thereby seeming to be influenced by the polymer optical purity. In general, the PLA nanocomposites showed a decrease in Young modulus and an increase of elongation at break point. An increase on the water contact angle was observed in PLA nanocomposites in function of the hydrophobicity of the different type of nanofillers.

Nanocomposite films, obtained by solvent casting by addition of the same amount of three different OMMTs in a PLA matrix, showed an intercalated structure, in which the interlayer distance between the clay platelets increased. However, the pristine structure of the nanoclay was preserved. As expected, the barrier properties of the films were inferior compared to the same materials obtained through extrusion.

A Box-Behnken design was developed to determine the optimum combination of three factors, that is pressure (0 – 600 MPa), temperature (20 – 60 ˚C) and time (5 – 30 min), in PSG solutions, in order to evaluate the effects on oxygen transmission rate of the resulting food packaging films. The obtained experimental data were fitted to a second-order polynomial equation, according to the model used in this study. Results showed that the variable response was significantly affected by the three effects. In particular, temperature and time were the main factors that principally affected the OTR. While pressure was not significant, the interaction between pressure and time affected significantly the variable response. The fitted surface plot showed that the treatments applied on the film-forming solutions were able to decrease the oxygen transmission rate of the resulting films. The lowest values were obtained with the high level of pressure (600 MPa) and time (30 min) and low level of temperature (20 ˚C).

Bi-layers with improved barrier properties, obtained using nanocomposite PLA films as base layer and PSG as coating, were subsequently produced and characterized. A significant decrease in the oxygen transmission rate was observed in the bilayer when compared to the base layer, indicating that an appropriate combination of these materials could be used for potential applications in the food packaging sector.

1. General introduction

1.1. Definition and functions of packaging

According to Coles (2003), packaging can be defined as “a means of safely and cost effectively delivering products to the consumer in accordance with the marketing strategy of the organization”. With this statement, the author wanted to highlight the strategic importance of packaging in the exercise of the marketing function.

Packaging can be in general defined as follows:

- A means of ensuring safe delivery to the ultimate consumer in sound condition at optimum cost;
- a coordinated system of preparing goods for transport, distribution, storage, retailing and end-use;
- a techno-commercial function aimed at optimising the costs of delivery while maximising sales.

The basic functions of packaging can be stated as follows:

- *containment*: related to the product’s physical form and nature;
- *protection*: prevention of mechanical damage;
- *preservation*: prevention or inhibition of microbiological spoilage along with chemical and biochemical changes;
- *information about the product*: legal requirements, product ingredients, use, etc.;
- *convenience*: for the pack handlers and users throughout the packaging chain;
- *presentation*: material type, shape, size, colour, merchandising display units, etc.;
- *brand communication*: e.g. use of typography, symbols, illustrations, advertising and colour, thereby creating visual impact;
- *promotion (selling)*:free extra product, new product, money off etc;
- *economy*: efficiency in distribution, production and storage;
- *environmental responsibility*: in manufacture, use, reuse, or recycling and final disposal.

According to Weber et al (2000) food packages fall into three different categories:

1. *Primary packaging*: packaging materials which are in direct contact with foods. Their function is to contain, protect and facilitate distribution and storage of foods. The properties of the primary packaging should be tailored in function of the requirements set by the packaged foods.
2. *Secondary packaging*: it is often used for physical protection of the food product. In particular, it protects the primary packages from mechanical damage during storage and distribution. It can also provide information for distributors and retailers on lot number and production dates.
3. *Tertiary packaging*: it incorporates the secondary packaging and its aim is to facilitate storage and handling. It also protects the packaged product against mechanical damage and weather conditions. Example of tertiary packaging are boxes, pallets and stretch foils.

1.2. Food contact materials legislation

The contamination of foods caused by chemical contamination has been the driving force in the industrialized countries for the regulation of food sector. Migration is the term used to describe undesiderable mass transfer occurring between packaging materials and food products coming into contact with them. The safety of these materials is evaluated by assessing the identity, the toxicological properties and the amount of compounds migrating from the packaging into food during conditions of intended use (Weber et al, 2000). The safety of food packaging materials is therefore generally based on the lack of potential toxic substances and the absence of migration from substances, the latter evaluated through migration testing.

Legislation adopted on materials intended to come into contact with foodstuffs has been adjusted over time in order to fulfill to the need to remove trade barriers through a harmonization of laws in the European Community (EC). However, this process of harmonization has not completed yet. In fact, for food contact materials, at the EU level harmonized Community legislation adopted by EU and nonharmonized national legislations exist in parallel (Schäfer, 2010).

The European Union legislation has five main instruments, such as Regulations, Directives, Decisions, Recommendations and Opinions. Almost all legislation relating to migration has been issued in the form of Directives, which are enacted by the national parliament, pratically unchanged. However, significant changes are often necessary to fit the style of national legislation and procedures.

Within the European Union, food contact materials are regulated by the so-called Framework Regulation (EC) No. 1935/2004 of 27 October 2004, which sets out basic rules for all materials in contact with food, thus removing differences which existed at the time between national legislations. Attached to the framework Regulation, the Good Manufacturing Practice Regulation (EC) No. 2023/2006 of 22 December 2006 set down the requirements/principles of good manufacturing practices for all food contact materials and articles. The Framework Regulation also allows the development of implementing measures for specific materials. Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food is such an implementing measure (so-called "PIM" or Plastics Implementation Measure) addressing plastics specifically. Regulation (EC) No. 10/2011 has been amended and corrected by the Regulation (EU) No. 1282/2011 of 28 November 2011.
According to Rijik and Veraart (2007), food packaging materials have to comply with the following rules:

- shall not endanger human health;
- shall not change the composition of the food in an unacceptable way;.
- shall not change taste, odor, or texture of the food;
- shall be manufactured according to good manufacturing practice (GMP)

Moreover, the consumers, food packer, or converter should be informed on:

- the suitability of the product for food contact through the use of the symbol  or the words "for food contact";
- the person responsible for manufacturing or placing on the market of the product;
- instructions for the safe use of the product;
- means of identification of the product for traceability.

Regulation (EC) No. 450/2009, which is specific to active and intelligent packaging, sets down additional requirements to ensure their safe use and introduces an authorization scheme for substances used for active and intelligent function in food contact materials (Galić et al., 2011). The reference document for the italian legislation is constituted by the Decree Law of 21st March 1973 (D.M. 21/03/1973) and subsequent modifications, containing general and specific regulations (Milana, 2010). However, today is not in force for plastic packages.
Regulation (EC) No. 10/2011 includes definitions and migration limits with the aim to reduce the use of residues associated with specific substances. A migration limit is defined in terms of

weight released per unit area, e.g. 10 mg dm^{-2}, or where this is not feasible, e.g., for caps, gaskets or where the volume of the container is from 0.5 to 10 L, the migration limit into the packaged food is 60 mg kg^{-1}.
This Regulation re-sets rules for migration testing using specific food simulants:

- *simulant A*: 10% v/v ethanol for hydrophylic products with alcoholic content ≤ 10%;
- *simulant B*: 3% w/v acetic acid for acidics foods (pH < 4.5);
- *simulant C*: 20% v/v ethanol for hydrophylic products with alcoholic content ≤ 20%;
- *simulant D1*: 50% v/v ethanol for food products such as milk, yogurt, cream;
- *simulant D2*: rectified olive oil for fatty/oily foods;
- *simulant E*: 2,6 difenil-p-fenilene polyoxide (particle size 60-80 mesh; porous size 200 nm) for dry food products.

1.2.1. Nanomaterials

Nanotechnology involves the manipulation of matter at a very small scale, generally between 1 and 100 nanometers (Cushen at al., 2012). A nanoparticle is defined as a discrete entity that has three dimensions of the order of 100 nm or less (Som et al, 2010). According to the EC Cosmetics Regulation (EC) No. 1223/2009, a nanomaterial is defined as an "insoluble or bio-persistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale form 1 to 100 nanometers". Nanotechnology are set to have a deept impact on the all stages of the food industry production (Silva et al, 2011). In particular, the area of food packaging has been involved in a great innovation through the use of various nanoscale fillers for the improvement of gas barrier properties of packaging materials. Moreover, the incorporation of a nanofiller in a polymer matrix is able also to provide reinforcement to the matrix and improve flexibility, temperature control and moisture stability of food contact materials (FCMs). Nevertheless, it is very important to note that the application of nanomaterials may be represent risks to the consumer due to the migration of particulate nanomaterials form FCMs into foods. The consequences of such kind of exposure have not fully determinated yet, thereby raising questions on the safety of foodstuffs which have been in contact with nanopackaging. Ingestion of this kind of food can be originated by the weakness in packaging performance and subsequent migration of nanoscale particulates from the packaging materials into the food. However, it is not known up-to-date if the consumption of food contaminated with nanomaterials may represent health risks.

Toxicology research and risk assessment in nanotechnologies are pratically non-exixtent especially in the food sector (Tiede et al., 2008) and only few can be considered valuable in terms of their use in assessing toxicity (Card and Magnuson, 2010).

Some nanoparticles have been found to exhibit negative effects on tissues such as inflammation, oxidative stress and signs of early tumour formation (Carlson et al., 2008). For the consideration of the toxicity of nanoscale particles there are few aspects among all that need to be considered and compared to those of the substance in a non-nano form: size, shape, solubility and reactivity. The fact that some materials exhibit toxicity at the nanoscale and not at the macroscale can be confirmed by Cui et al. (2005). These authors showed that single-walled carbon nanotubes inhibited human embryo kidney cell proliferation and negatively impacted on cell growth and cell turnover, thus discouraged from being used in th efood industry. Moreover, since toxicological properties seem to vary among particulate nanomaterials, a risk assessment must be done on a case by case basis (Munro, et al., 2009).

Regarding the applications of nanomaterials in the food packaging area in ralation to the risks that this technology poses, tests on chemical migration from food packaging into food are useful reference since they are reproducible, reliable, and overall well established. They are relatable to the nanomaterial migration test and can be form the basis of a preliminary risk assessment.

The regulation of nanotechnologies is within the scope of both so-called horizontal and vertical legislation. The horizontal legislation is broad and included characteristics of nanotechnologies even if it does not specifically aim to do so. The most important european horizontal legislation are:

- Directive 2001/95/EC: this is on general product safety. It provides a definition of a safe product and places the responsibility of ensuring that products are safe on the producers. The concept of safety refers to human health but not to the environment.
- REACH (Registration, Evaluation, Authorisation and Restriction of Chemical substances) Regulation (EC) No. 1907/2006: the scope of REACH is to enhance the protection of human health and the environment. This has to be done through a better and earlier identification of the intrinsic properties of chemical substances, ensuring that chemicals are safe on producers. The REACH Regulation places greater responsibility on industry to manage the risks from chemicals and to provide safety information on the substances. Manufacturers and importers must provide to consumers safety information by a new labelling style on the product and safety data

sheets when requested. The information needs to be registered in a central database run by the European Chemicals Agency (ECHA). The Agency purpose is to "act as the central point in the REACH system: it manages the databases necessary to operate the system, co-ordinates the in-depth evaluation of suspicious chemicals and is building up a public database in which consumers and professionals can find hazard information".

- Regulation (EC) No. 1272/2008: it regulates the classification, labelling and packaging substances and mixtures. In particular, a substance produced at the nanoscale could provide a change in the properties and therefore a change in its classification. However, this information has to be reported to the ECHA.

The main european vertical legislations issued in the area of nanomaterials are the following:

- Regulation (EC) No. 10/2011: it regulates the nanoform substances, which can be used if explicity authorized as in the specifications in Annex I of the Regulation.
- Regulation (EC) No. 1333/2008: according to Eisenberger et al (2010), this was the first legislation to explicity mention nanotechnologies, specifically in article 12, stating that in case of change in the starting material used or in the production method of an addictive, it must undergo a new authorization process and safety evaluation. This is the case of changes in particle size of a substance.
- Regulation (EC) No. 1935/2004: it regulates food packaging including new types of materials which actively maintain or improve the condition of the food which is widely viewed as a promising use of nanomaterials.

A recent Scientific Opinion released by the Scientific Commetee has set down the guidelines for the risk evaluation related to the application of nanoscienze and nanotechnology in the food chain (EFSA, 2011). This document states that the risk evaluation is strictly connected to the physico-chemical characterization of nanomaterials and to the knowledge of the interactions with body tissues. The evaluation of the risk has also to begin to outline possible scenarios of exposure, which allow to determine the more appropriate approach to the toxicologic study of nanomaterials on the basis of the six cases defined in the document.

1.2.2. Public perception on namomaterials

The public perception is an important factor in establishing the commercial success of nanotechnology in the food and beverage area, since comsumers are very sensitive to this topic. According to Siegrist et al. (2007), a group of 153 consumers were hesitant to buy nanothechnology-related foods or food packaging improved with nanotechnology. A difference in the perception of product manufactured with the use of nanotechnology between United States and Europe was observed (Cobb and Macoubrie, 2004; Gaskell et al., 2005). While in the United States the perceptions of nanothechology were generally optimistic, in Europe they were more pessimistic.

1.3. Bioplastics

According to European Biolastics (2011), bioplastics are plastic materials that comply with one or both the following requirements:

- to be manufactured from renewable resources, thus reducing the oil consumption and greenhouse gases production;
- to be biodegradable or compostable, providing at the end of the life cycle of the materials an alternative option to the disposal in landfills.

Even whether an agreement on the requirements that allow a clear definition of the bio-derivation of a material does not exist yet, the attribute of compostability can be assigned to plastics employed for food packaging only after the evaluation of compliance to the european standard EN 13432.

1.3.1. Bio-based food materials

On the basis on the definition reported by European Bioplastics, the term bioplastics can refer either to polymers oil-based possessing the properties to be compostable, or to polymers obtained form renewable resources. The latters are in general materials fully biodegradable, with the exception of polymers manufactured through deep chemical modifications (Guilbert and Gontard, 2005; Siracusa et al., 2008).

In food packaging, films obtained from renewable raw materials are considered potentially able to replace those obtained from conventional oil-based, as a consequence of a growing tendency of markets to more eco-friendly materials (Han et al, 2005). According to the definition reported in Weber et al. (2000), biobased polymers used in the production of food packaging are materials derived from renewable resources, suitable for food contact applications. They can be divided into three categories based on their origin and production as shown in Fig. 1 (Petersen et al. 1999; Guilbert and Gontard, 2005; Mensitieri et al., 2011):

1. Category 1: Polymers directly extracted/removed from biomass. Examples are polysaccharides such as starch and cellulose and proteins like casein and gluten.

2. Category 2: Polymers produced by classical chemical synthesis using renewable bio-based monomers. A good example is polylactic acid, a bio-polyester polymerised from lactic acid monomers. The monomers themselves may be produced via fermentation of carbohydrate feedstock.

3. Category 3: Polymers produced by microorganisms or genetically modified bacteria. To date, this group of bio-based polymers consists mainly of the polyhydroxyalkonoates, but developments with bacterial cellulose are in progress.

Materials that belong to one of the above categories are currently being used for food packaging purposes and are considered to possess a great potentiality in this area.

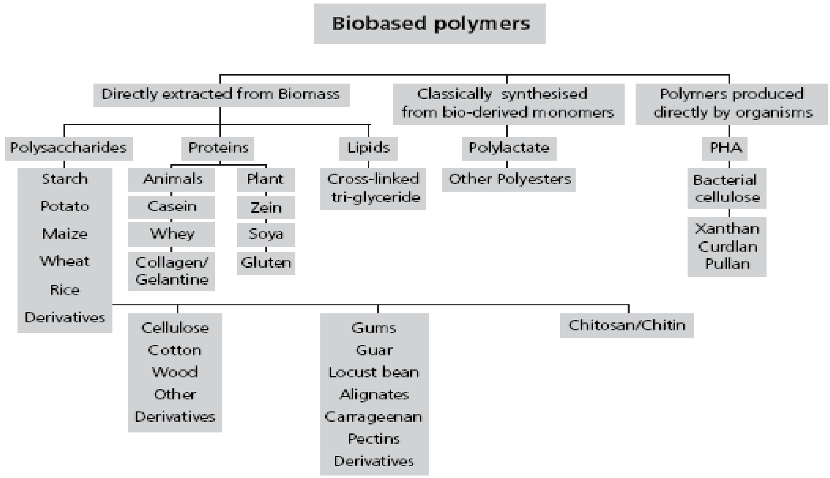


Figure 1. *Schematic presentation of bio-based polymers in function of their origin and method of production (Weber et al., 2000).*

According to Sinha Ray and Bousmina (2005), packaging materials produced from fossil fuels are undegradable wastes. This means that, amounting to 40% of packaging refuse, they are pratically eternal, raising the issue of a global environmental problem. In fact, there are two approaches for keeping the environment free oil-based plastic wastes: the storage of wastes at landfill sites and the utilization processes sucha as incineration an recycling. Nevertheless, the first approach can't be a solution, since the burial of plastic wastes in landfill is a time bomb where today's problems are only postponed to future generations. Regarding the second approach, incineration of the plastic wastes produces a large number of carbon dioxide and sometimes toxic gases, thus being responsible for the creation of global warming and global pollution. Recycling can only be somehow a solution of the problem, but it requires great expenditure of labor and energy because all the steps involved in the process of recycling (removal of plastic wates, separation, washing, drying, grinding and reprocessing) make the final packaging more expensive with a lower quality if compared with the non-recycled materials. As a consequence, green prolymeric materials that would not involve the use of toxic substances and can be biodegradable need to be urgently developed.

Although the global interest to environmental issues, packaging is yet dominated by oil-based polymers such as polyethylene and polystyrene. This means that some drawbacks related to the use of biobased materials still exist (Petersen et al., 1999). An exception is represented by cellulose, which is used in the form of paper and cardboard/carton as an exterior packaging layer. (Petersen et al., 1999; Rhim at al., 2006). The drawbacks derived from the use of bio-polymers deal basically with three main factors: performance, processability and costs. Unlike materials extracted from the biomass (category 1) which are characterized by drawbacks related to performance and production process, bio-polymers obtained from categories 2 and 3 possess good performance and are easily proccessable into films by using conventional plastics techniques (Petersen et al., 1999).

In order to improve the physico-chemical properties of the polymers obtained from renewable resources and to meet specific structural requirements, several chemical compounds and additives such as stabilizers, plasticizers, antioxidants and fillers are added in the formulation of these materials (Siracusa et al., 2008; Mensitieri et al., 2011). Moreover, blendings, composites and laminates obtained with bio-based polymers and petrol-based polymers have been developed to expand the area of application (Nampoothiri et al., 2010; Mensitieri et al., 2011).

One of the challenges that the food packaging industry has to face in the production of primary packaging with the biobased polymers materials is to match the durability of the packaging with product shelf-life. The biobased packaging materials need to remain stable during all along their commercial life (until disposal), maintaining their physico-chemical properties. For this reason, it is necessary to avoid environmental conditions during storage of the food product which are susceptible to lead to the biodegradation of the material (Petersen et al., 1999). In general, bio-based polymers possess inferior barrier properties, in particular to water vapour, when compared to conventional materials. This drawback can be solved in different ways. A more immediate option is to package foods which are compatible with the packaging materials and their properties. An example is given by high-moisture foodstuffs such as fresh vegetables with short shelf-life. An alternative is represented by the possibility to manufacture multilayer packaging through lamination with conventional polymer, or the combination with an exterior packaging layer which is able to overcome the drawbacks of bio-based packaging. A further option is to study and develop bio-based polymers chemically modified, co-polymers and composite materials, possessing new properties to comply with the requirements for the packaged food (Petersen et al., 1999). Barrier properties of films obtained with bio-based polymers can be improved through the addition of wax, fatty acids and lipids (Han et al., 2005). The business of bio-based plastic materials is facing a period of strong growing due to some driving factors such as an increasing cost of oil, favourable legislation and the image linked to the environmental sustainability. PLA is in a unique position in the field of bio-based product, due to its qualities related to processability, appearance, and properties such as mechanical strength and barrier (Södergård and Stolt, 2010).

PART I

PRODUCTION OF BIO-BASED NANOCOMPOSITE FOOD PACKAGING FILMS

I.1. Introduction

PLA is a compostable polymer derived from renewable sources, in particular from starch and sugar. PLA is an aliphatic polyester produced by condensation polymerization directly from lactic acid, or through conversion of lactide, the cyclic dimer of lactic acid (Garlotta, 2001; Groot et al., 2010).

It is a thermoplastic polymer with high strength and high modulus, possessing rigidity and clarity similar to PET (Gupta and Kumar, 2007; Groot et al., 2010). During the last decade, it has been used primarily for medical applications such as: implant devices, tissue scaffolds and internal sutures, due to its high cost, low availability and limited molecular weight (Datta and Henry, 2006). Recently, production costs have been lowered through the introduction of new technologies, such as: the ROP process and large-scale production. Thus, the use of PLA has been extended to other areas, such as: packaging, textiles and composite materials (Drumright et al., 2000; Garlotta, 2001; Groot et al., 2010). PLA is classified as GRAS (Generally Recognized As Safe) in the USA by the FDA (Food and Drug Administration) and approved for use in all the applications related to food and beverage packaging (Conn et al., 1995, FDA, 2002, Datta and Henry, 2006). While the main advantages of PLA consist of being a thermoplastic material with good processability and processed in machines used for conventional plastic, its drawbacks such as brittleness, moderate barrier properties to water vapour and oxygen and low temperature degradation (200°C) limit its use to films, thermoformed and blow molded containers, food service ware and short shelf-life bottles (Gross and Kalra, 2002). It is therefore suitable for use with fresh products and foodstuffs not requiring a packaging with low oxygen permeability performance (Jamshidian et al., 2010).

Lactic acid (2-hydroxy propionic acid) is the simplest hydroxyl acid with an asymmetric carbon atom and exists in two optically active configurations, the L(+) and D(−) enantiomers (Garlotta, 2001; Gupta and Kumar, 2007). The two enantiomers are shown in Fig. I.1.

Lactic acid can be produced by chemical synthesis or fermentation. The latter route is undertaken by all the major manufacturers of lactic acid (Datta and Henry, 2006; Nampoothiri et al., 2010). The interest in the fermentative production of lactic acid is due to the prospects of environmental friendliness and of using renewable resourses instead of petrochemical.

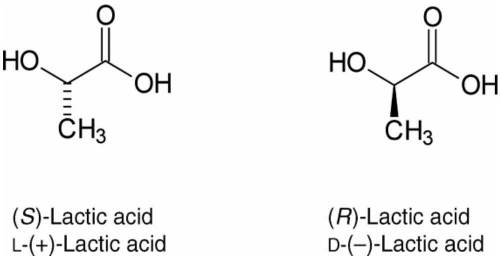


Figure I.1. *The two enantiomeric forms of lactic acid: L(+) and D (−) 2-hydroxypropionic acid.*

High product specificity, low cost of substrates, low production temperatures and low energy consumption are yet another advantages in pursuing lactic acid fermentation (John et al., 2007). According to Vink et al. (2003), fermentation-derived lactic acid typically consists of 99.5% of the L-isomer and 0.5% of the D-isomer. The fermentative process is carried out through the use of homolactic organisms such as various optimized or modified strains of the genus *Lactobacilli*, which exclusively produce lactic acid (Hatmann, 1998). Among the microorganisms used, *L. amylophilus*, *L. bavaricus*, *L.casei, L. maltaromicus* and *L. salivarius* predominantly yield the L(+)- isomer. Strains such as *L. delbrueckii*, *L. jensenii* and *L. acidophilus* yeld the D-isomer or mixtures of both (Hartmann, 1998; Nampoothiri et al., 2010). All these strains can yield high carbon conversions from feed stocks at standard fermentation conditions (Kharas et al., 1994).

The different types of carbohydrates that can be utilized in the fermentation depend on the particular strain of *Lactobacillus*. In particular, substrates are generally constituted by simple sugars such as glucose, maltose and dextrose (obtained from corn or potato starch), sucrose from cane or beet sugar, lactose from cheese whey (Garlotta, 2001). The selection of a specific source is related to the cost, availability and purity (Datta and Henry, 2006) However, along with the carbohydrates, microorganisms need proteins, amino acids, complex nutrients (B-vitamins) and nucleotides, which can be supplied by corn steep liquor, yeast extract, cottonseed flour, soy flour (Garlotta, 2001). Several agro-food industry products and residues are more economic alternative substrates compared to refined sugars for lactic acid production. Inert agro residual substrates like sugarcane bagasse are also reported (John et al., 2007). Among agricultural sources, starch, lignocellulose/hemicellulose hydrolyzates, cotton seed hulls, Jerusalem artichokes, corn cob, corn stalks, beet molasses, wheat bran, rye flour, sweet sorghum, sugarcane press mud, cassava, barley starch, cellulose, carrot processing waste, molasses spent wash, corn fiber hydrolyzates are all byproducts that can be used as potential substrates (Nampoothiri et al., 2010).

The purification process of lactic acid obtained through fermentation is very important for the production of PLA. Crude lactic acid is characterized by the presence of impurities which decrease the quality of the polymer obtained (Gupta and Kumar, 2007). Acids, alcohols, esters are examples of such impurities (Groot et al., 2010).

The PLA properties such as crystallization, processability and thermal degradation are in function of the polymeric chain structure and composition. In particular, it depends on D- and L- lactic acid enantiomers ratio. PLA stereochemical structure can be modified through copolymerization of mixtures of L-lactide, *meso*-lactide and D-lactide (Fig. I.2), resulting in a high-molecular weight semi-crystalline or amorphous polymer, characterized by a melting point ranging from 130 to 185˚C (Hartmann, 1998; Garlotta, 2001).

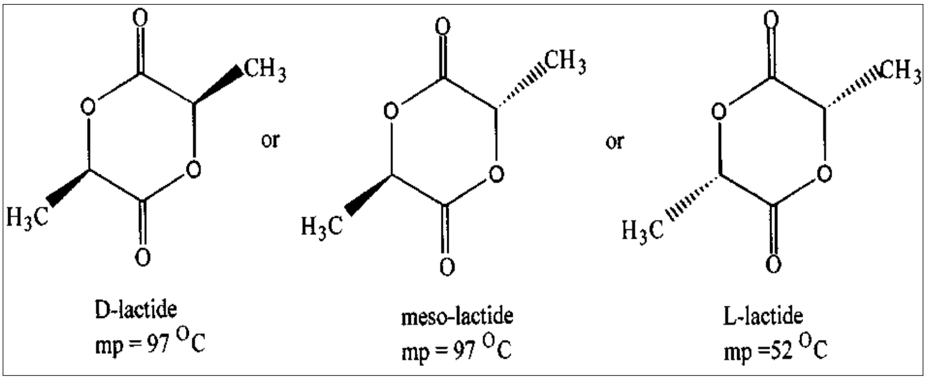


Figure I.2. *The three diastereomeric structures of lactide (3,6-dimethyl- 1,4-dioxane -2,5-dione). Adapted from Garlotta 2001.*

Unlike isotactic PLLA homopolymer (constituted by L-lactide only), which is a semicrystalline material with the highest melting point, PLA copolymers possessing higher D-isomer content exhibit lower melting points and much slower crystallization behaviour. With a D-content higher than 12-15%, PLA becomes amorphous (Grijpma and Pennings, 1994; Kolstad, 1996; Sinclair and Lipinsky, 1996). PLA undergoes thermal degradation at temperatures above 200˚C, due to hydrolysis, lactide reformation, oxidative main chain scission, and inter-or intramolecular transesterification reactions. For this reasons, PLA homopolymers have a very narrow processing windows. The most largely used method for the enhancement of PLA processability is based on melting point depression through a random incorporation of small amounts of lactide enantiomers of opposite configuration into the polymer. Nevertheless, the melting point depression leads to a decrease in crystallinity and crystallization rates (Spinu et al, 1996).

According to Nampoothiri et al. (2010), Nature Works LLC (Minnetonka, Minnesota, USA) is currently the leader in lactic polymer technology and markets. Over the last ten years, this company has done a remarkable work in developing lactic acid based products such as polylactide-based resins, used in the packaging field, and polylactide-based fibers, used in textiles and fiber applications.

I.1.1. Synthesis of PLA

The synthesis of lactic acid into high-molecular-weight PLA can follow different routes of polymerization (Fig. I.3). The techniques used include:

1. Direct condensation polymerization;
2. azeotropic dehydrative condensation;
3. polymerization through lactide formation.

Generally, lactide ring-opening polymerization is the most utilized production process.

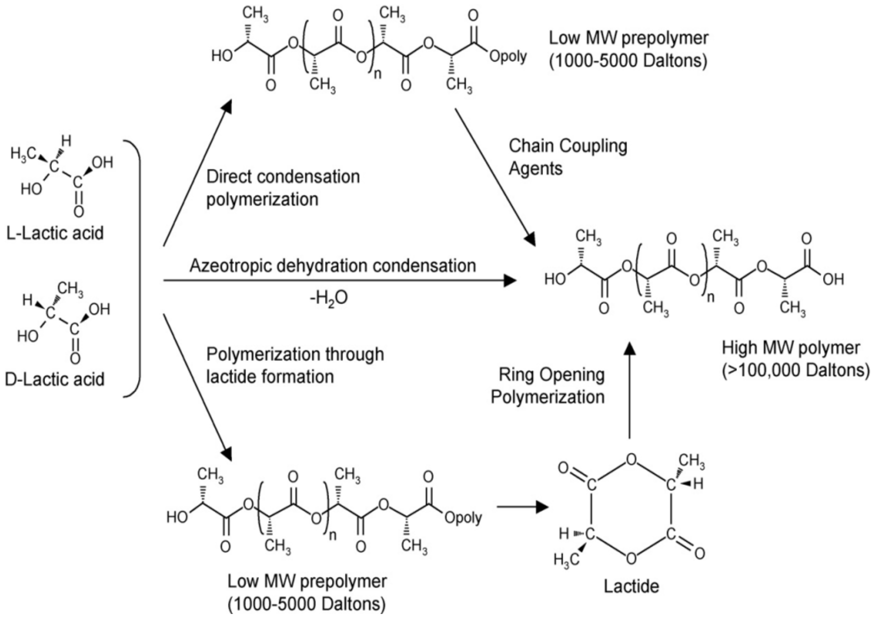


***Figure I.3**. Synthesis of PLA from D- And L- lactic acids. Adapted from Auras et al. (2004).*

I.1.1.1. Direct condensation polymerization

PLA can be formed by lactic acid through a reaction of the hydroxyl and carboxylic acid groups of lactic acid. The removal of water produced during this condensation process allows the reaction to proceed towards the product (Södegärd and Stolt, 2010).
The removal of water becomes more difficult with the advancement of the reaction. This can be explained by the increase in viscosity due to the higher molecular weight. However, the water removal can be improved by applying vacuum (Gupta and Kumar, 2007).
The synthesis of PLA from lactic acid by direct condensation polymerization can be divided into three principal stages:

a) Removal of the free water content with a system possessing good heat transfer. This process can be carried out in commonly available evaporators, such as falling film evaporators. Flash evaporation is another technique used for the removal of water.
b) Olygomer polycondensation: production of low molecular weight PLA or olygo(lactic acid) by polycondensation through the use of catalysts such as strong acids and organometallic compounds.
c) Melt polycondensation and production of PLA possessing high molecular weight. An efficient method to improve the polycondensation reactions is through the use of an apparatus with a good renewal of phase boundary layers that provides a homogenization of the reaction mixture and carries out the reaction under vacuum conditions in an inert atmosphere.

Along with the removal of water, other factors that prevent the production from reaching high-molecular-weight PLA include either the presence of impurities in the reaction mixture, which behave as chain terminators like carboxyilic acids and hydroxylic alcohols, or intramolecular transesterification side-reactions (*back-biting*), which lead to cyclic structures such as lactide (Garlotta, 2001; Södegärd and Stolt, 2010). The polymer obtained through direct polycondensation is characterized by a low molecular weight and poor mechanical properties that make it unsuitable for many applications (Garlotta, 2001; Gupta e Kumar, 2007; Södegärd and Stolt, 2010).

I.1.1.2. Azeotropic dehydrative condensation

The azeotropic dehydrative condensation is a method to obtain high-molecular-weight polymers without the use of chain extenders or adjuvents can be obtained. This method is basically based on the removal of the majority of the condesation water through a reduced pressure distillation of lactic acid for 2 to 3 hours at 130°C, a subsequent addition of catalyst and diphenyl ether, and an isolation of the polymer formed as it is or dissolved and precipitated for further purification (Garlotta, 2001; Ajioka et al., 1995a; Ajioka et al., 1995b). The main drawbacks of this method are represented by the use of solvents and residual catalysts. In particular, the low boiling point of the solvent sets a limit on the polycondensation temperature that can be used (Södegärd and Stolt, 2010). Moreover, residual catalysts can determine many problems during further processing, among which unwanted degradation and uncontrolled or unreprodudible hydrolysis rates (Garlotta 2001).

I.1.1.3. ROP

The most widespread route to obtain high-molecular-weight PLA is through the ROP of lactide. The intermediate lactide is formed in the first step when the condensation product water is removed by evaporation during oligomerization (Nampoothiri et al., 2010). This method allows to obtain polymers possessing the required properties by means of a high control of the product stereopurity (Gupta and Kumar, 2007). According to Södegärd and Stolt (2010), the process consists of three steps:

a) polycondensation;
b) lactide manufacturing;
c) ROP

Polycondensation is targeted to obtain low-molecular-weight polymers which constitute the substrate for the lactide production. This can occur either in presence or in absence of a catalyst. In the second step, olygomers are depolymerized under reduced pressure at high temperature (130-230°C) through a "back-biting" reaction in presence of specific catalysts, leading to a formation of three stereoforms of lactide: L-lactide, D-lactide and *meso*-lactide (Nampoothiri, 2010). However, the crude lactide in most cases contains different impurities that make the monomer mixture unsuitable for direct ROP. Consequently, the lactide needs to be purified. There are three main purification approaches known:

- *Solvent-assisted purification*: the most used method for the purification of the lactide is by crystallization from ethyl acetate or from toluene and subsequent drying of the lactide under vacuum (Stolt and Södergård, 1999; Leenslag and Pennings, 1987).

- *Melt crystallization*: the separation of the different lactides from each other and the impurities is carried out considering the differences in the melting points of D- or L-lactide and *meso*-lactide.
- *Separation in the gas phase*: it is based on the different boiling point of the different lactic acid species. Water and lactic acid are the more volatile compounds, the lactide is the less volatile and lactic acid olygomers are often in the liquid phase. A final step consists in the production of pure lactide by purifying *meso*-lactide from L-lactide and/or D-lactide by distillation.

In the third step (ROP) high-molecular weight PLA are obtained by catalytic ring-opening polymerization. The most commonly catalyst used is stannous octoate. However, for laboratory demonstrations, tin (II) chloride is often employed. The ROP involving L-lactide is generally the preferred route to obtain high-molecular-weight PLA due to the possibility to control the reaction. The most simple and reproducible method used for the polymerization of the lactide is melt polymerization in a rection vessel provided with an agitator.

At the end of the ROP process, PLA undergoes post-polymerization treatments to improve the processing and processability of the polymer. Södegärd and Stolt (2010) have reported different techniques that can be used for this purpose. In particular, catalyst deactivation with phosphorous containing compounds, antioxidants, acrylic acid derivatives, and organic peroxides is one important feature that has been applied to PLA. Moreover, PLA has to be dried before processing to minimize the thermo-htdrolysis and molecular weight reduction during the melt processing due to water residues. Suggested drying conditions are 60˚C under vacuum and the use of hot dry air (Södegärd and Stolt, 2010).

I.1.2. Physical and chemistry properties of PLA

I.1.2.1. Structure

Most of the physical properties of PLA are affected by the amount and distribution of the lactic acids sterocenters in the polymer chain, which reflect its history including the stereochemistry of the feed composition (D-lactide, L-lactide, *meso*-lactide, racemic DL-lactide) and the kinetic of polymerization (Gonçalves et al., 2010). The chiral properties of lactic acid produce different forms of PLA, such as PLLA (poly-L-lactide), PDLA (poly-D-lactide), PDLLA (poly-D/L-lactide) (Gupta and Kumar, 2007). Both stereoisomers of lactic acid are present in nature, with the L- isomer being the main fraction constituting the PLA obtained from biological sources. In particular, the fact that the lactic acid is produced in the human body mostly under L-enantiomeric form, along with the strong interest of the biomedical applications for this polymer, have led both research and production to focus on L- and DL-lactide polymers (Lehninger, 1977; Lunt, 1988; Maharana et al., 2009). The D-isomer is only used in particular medicinal chemicals applications. Oil-based production provides today a 50/50 mixture of the L- and D- forms.

PLLA and PDLA are crystalline polymers because of the enantiomeric purity of the pristine monomers and the stereoregularity of the polymer chain. On the contrary, PDLLA is completely amorphous due to its irregular structure made of equimolar random copolymer of L- and D-lactic acid (Fambri and Migliaresi, 2010).

The stereochemical configuration of PLLA (or PDLA) and PDLLA are isotactic and atactic respectively. Syndiotactic PLA can be considered as an alternating DL-lactic acid copolymer and can be polymerized from a streroselective polymerization of *meso*-lactide.

I.1.2.2. Optical properties

The absorption and transmission of UV-Vis radiation by polymers is very important in the food packaging industry where the packaged goods are sensitive to light.

Lipids, aromas, vitamins and pigments are components which may undergo degradation reactions when exposed to light irradiation. Factors that deeply influence the food quality are the spectrum and the intensity of the light source, the conditions of light exposure and the degree of light transmittance of the packaging materials (Gonçalves et al, 2010). The transmission of visible light (400-700 nm) and of ultraviolet radiation (100-400 nm) are key parameters in designing the appropriate packaging in order to preserve and protect food until they reach the consumers. The photochemical degradation of plastic materials is generally due to the UV-B radiation (315-280 nm), because its energy content is able to break certain chemical bonds (Auras et al., 2004b). From the comparison with the oil-based conventional polymers, PLA is characterized by a significant increase of transmittance at 225 nm, reaching 95% at 300 nm. However, no transmission of UV radiation has been observed in the wavelength region between 190 -220 nm. Polymers such as PET, polystyrene and cellophane transmit less radiation in the UV range where most food are sensitive, with PET and LDPE (low density polyethylene) showing the best and worst performance in terms of barrier to UV light respectively (Fig. I.4).

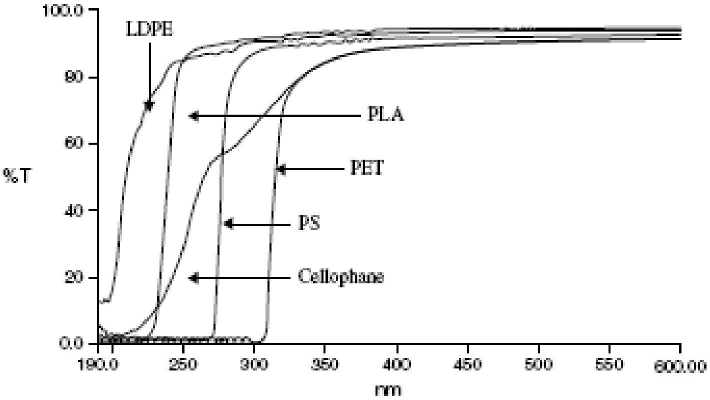


Figure I.4. *Transmission versus wavelength for PLA possessing 98% L-lactide, PS, LDPE, PET and cellophane films. Adapted from Auras et al., 2004b.*

Holm et al (2006) compared the transmittance of two different thermoformed materials (PLA and PET/PE with a thickness of 500 and 102 μm respectively) for cheese packaging. They found that the transmittance for both testing materials was around 90% in the range of visible light. Nevertheless, while the control PET/PE showed a cut-off in the UV region at 320 nm, PLA was characterized by a lower protection (cut off at 250 – 300 nm). In their study, Rhim et al (2006) compared PLLA films manufactured through thermocompression and solvent casting (the latter obtained using chloroform as a solvent) and found percentages of transmittance at 660 nm greater that 90%. In a further study, Rhim et al. (2009) reported values of transmittance at 660 nm slightly higher than 80% in PLLA films obtained by solvent casting and subsequently desolvation under vacuum conditions at 60˚C.

The specific optical rotation [α] of PLLA and PDLA was measured in chloroform at a concentration of 1 g/dL at 25˚C through a polarimeter and wavelength of 589 nm. The values of α ranged from +150˚ to +156˚ for the D-isomer and from -150˚ to -160˚ for the L-isomer form. (Perego et al., 1996; Tsuji e Ikada, 1996; Garlotta, 2001).

I.1.2.3. Crystallization and thermal properties

Crystallization, crystallinity degree, and thermal properties of PLLA depend on a number of factors such as polymer molecular weight, polymerization conditions, thermal history and purity (Fambri e Migliaresi, 2010).

Unlike PDLLA, three main thermal events can be observed in a typical thermogram of PLLA obtained by Differential Scanning Calorimetry (DSC) analysis from the heating scans (Fig. I.5):

glass transition (T_g), cold crystallization (T_{cc}) and melting (T_m). PLA exhibits values of T_g from 50 to 70°C depending on the optical purity of the monomer, the production process and the polymer thermal history (Perego et al., 1996; Auras et al., 2004; Kubies et al., 2006; Pluta, 2006; Tsuji et al., 2006; Krishnamachari et al., 2009; Papageorgiou et al., 2010; Picard et al., 2011).

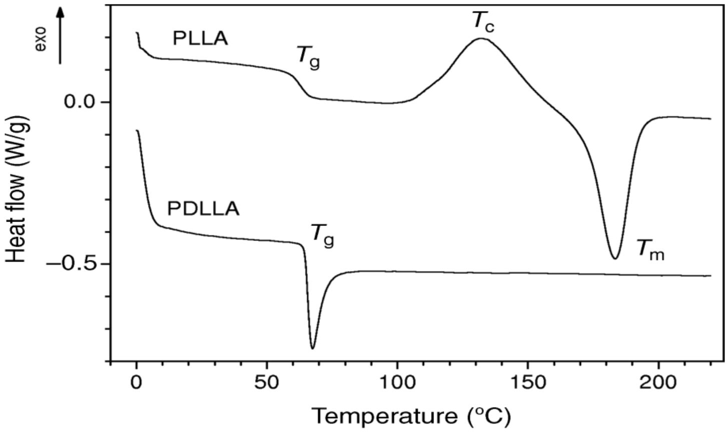


Figure I.5. *DSC thermograms of amorphous PLLA and PDLA (heating rate 10°C/min).*

Higher temperatures are generally reported in literature for the purest polymers. A variation of specific heat (ΔC_p) of 0.50 J/(g K) was also reported as T_g was achieved (Fambri and Migliaresi, 2010). Moreover, Papageorgiou et al., (2010) observed a correlation between the heating rate of DSC scans and the measured values of T_g, which tended to increase with the increase of the scan rate.

The crystallization behaviour of PLA has been studied by many researchers over the last years (Garlotta, 2001; Sarasua et al., 2005; Kister et al., 1995; Kawashima et al., 2002; Hartmann, 1999; Kister et al., 1998; Kishore et al., 1988; Kishore et al, 1984). They showed that crystallization was strictly related to stereochemistry and thermal history of PLA, which were important factors for the production of either amorphous or semicrystalline PLA. The temperature of crystallization was found to range from 100 to 140°C (Sinha Ray et al., 2003b; Sarasua et al., 2005; Pluta, 2006; Rhim et al., 2006; Tsuji et al., 2006; Picard et al., 2011). However, crystallization of PLA can be induced by thermal treatments such as *annealing* at temperatures between 75°C and T_m through a cold-crystallization process, with kinetics depending on comonomers ratio and the presence of nucleation agents (Bigg, 1996). This process leads to the formation of semi-crystalline organized structures called spherulites (Fig. I.6).

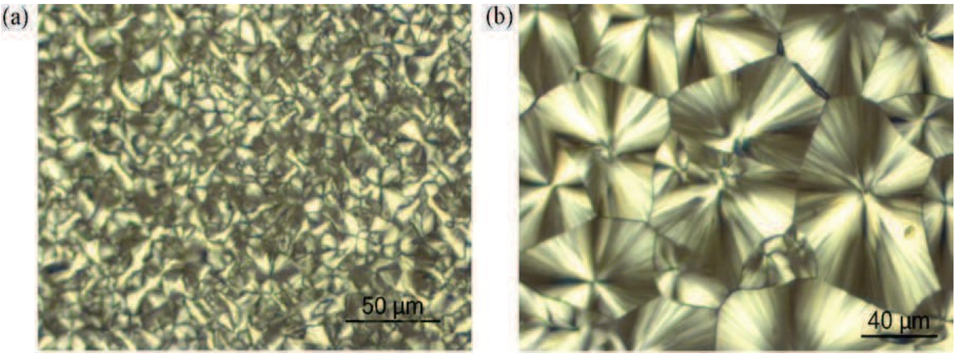


Figure I.6. *PLA spherulites obtained by melt-quenching at 100°C (a) and 120°C (b). Adapted from Papageorgiou et al. (2010).*

The increase of the polymer crystallinity can be also obtained through either a slow cooling of the polymer from the melt (melt-crystallization) or mechanical treatments of orientation (Lim et al., 2008). Like PET, PLA is a material that crystallizes slowly (Garlotta, 2001). The quick cooling (quenching) of the polymer from the melt carried out at temperatures below T_m produces an amorphous structure (Lim et al., 2008).

The T_m of PLA generally ranges between 160 and 190°C depending on the optical purity of the monomer (Perego et al., 1996; Sinha Ray et al., 2003a,b; Sarasua et al., 2005; Kubies et al., 2006; Tsuji et al., 2006; Zaidi et al., 2010; Picard et al., 2011). The presence of *meso*-lactide in the PLA structure can depress the T_m by as much as 50°C, depending on the amount of D-lactide incorporated to the polymer (Lim et al., 2008).

The polymeric chain of PLA possesses a helical shape, with a orthorhombic unit cell. PLLA crystals have three different structural conformations that can be originated through different process conditions. In particular, melt-crystallization and cold-crystallization phenomenons can induce a production of α-form crystals, while the orientation and lamination treatments at high temperature lead to a formation of β-form crystallites (Cartier et al., 2000; Fambri e Migliaresi, 2010). Values of T_m related to α- and β- forms are 185 and 175°C respectively (Jiang et al., 2010). Cartier et al. (2000) found a third crystalline form (γ) by means of epitaxial crystallization techniques.

Crystallinity affects the properties of polymers. For instance, a decrease in elongation at break and an increase in resistance to traction and modulus of elasticity were observed when PLLA was annealed (Perego et al., 1996). The formation of crystallinity may or may not be favorable depending on the end-use requirements of the PLA articles. In particular, high crystallinity is desiderable for injection molded articles which require good thermal stability, but it is not for injection molded performs intended for further blow molding, since rapid crystallization of the polymer would not provide a good stretching of the perform and optical clarity of the resulting bottle (Lim et al., 2008).

An increase in the crystallinity of PLA can be obtained through the incorporation of nucleating agent in the polymer during the extrusion process. This is due to a decrease in the surface energy barrier for nucleation that favors crystallization at higher temperatures upon cooling. According to Kolstad (1996), the addition of talc (6%) to PLLA reduced the crystallization half-time from 3 min at 110˚C to approximately 25 s. Moreover, Li and Huneault (2007) found that by adding 1% (w/w) of talc in 4.5% D-PLA the crystallization half-time was reduced from few hours to 8 min. However, the incorporation of montmorillonite (MMT) in the polymer was less effective as a nucleating agent.

I.1.2.4. Solubility

The solubility of PLA polymers depends on molecular weight, degree of crystallinity and purity of comonomers (Gupta and Kumar, 2007). In general, polylactides are soluble in chlorinated solvents such as chloroform, dichloromethane, 1,1,2-trichloroethane and dichloroacetic acid. Ethyl benzene, toluene, acetone and tetrahydrofuran only partly dissolve polylactides when cold. However, they can be solved in these solvents when heated to boling temperatures (Nampoothiri et al., 2010). PLA cannot be solved in water, alcohols (ethanol, methanol, propylene glycol) and non-substututed hydrocarbons (hexane and heptane) (Gupta and Kumar, 2007).

I.1.2.5. Barrier properties

Permeability is the transfer of small molecules called *permeants* or *penetrants* through a membrane Vieth and Eilenberg (1972) Permeation processes take place through a membrane that separates two fluid phases (gas, vapor or liquid) containing low molecular weight species with differing chemical potential. The molecules at the side of the higher chemical potential tend to diffuse through the membrane and equilibrate the chemical potential on both sides. The permeation process in continuous polymeric materials is accomplished by molecular diffusion within the polymer matrix (Fig. I.6).

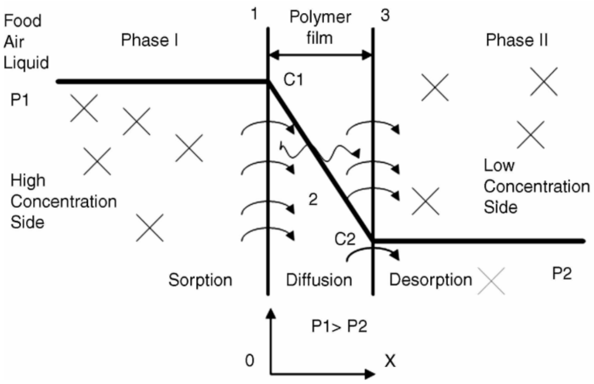


Figure I.6. *Permeation mechanism of permeants from higher (C_1) to lower (C_2) through the continuous polymeric material of thickness l. Steps: (1) sorption, (2) diffusion, and (3) desorption (Almenar and Auras,2010).*

Diffusion is controlled by temperature and it can be modeled by the Arrhenius equation. According to Almenar and Auras (2010), above the T_g, of a polymer, the molecules (phase I) are first sorbed into the polymer and then diffuse across the polymer through random movements originated by the Brownian motion of the polymer chains. Below T_g, the permeants dwells in a cavity of the polymer before performing a quick jump into an adjacent cavity. The molecules primarily diffuse through the free volume of polymer amorphous regions. Free volume is an intrinsic property of the polymer matrix, originated by the gaps between entangled polymer chains. Generally, sorption increases as the amount of free volumes increase. In membranes, the molecules of permeant are desorbed from the membrane to the low chemical potential side (phase II). The diffusion of permeants in non-continuous polymeric materials occurs through pores. Consequently the solubility–diffusion phenomenon is not the main mechanism regulating the transport of molecules.

The diffusion process for the steady state is described by the general Fick's first law (Almenar and Auras, 2010):

$$F = D\nabla\mu \qquad \text{(Eq. I.1)}$$

where F is the flux of permeant, D is the diffusion coefficient, and $\nabla\mu$ is the gradient operator of the chemical potential on the two sides of the membrane. The permeability coefficient (P) can be obtained from the general Fick's first law:

$$P = \frac{F_{\infty} l}{A \Delta p} \qquad \text{(Eq. I.2)}$$

Where F_{∞} is the value of permeant flow at steady state, Δp is the partial pressure gradient across the polymer film, and A is the area of the film. High values of permeability are attributed to materials possessing poor barrier properties.

PLA exhibits values of oxygen permeability (P_O) ranging from 7 to 24 mL mm m^{-2} d^{-1} atm^{-1}, depending on the method used for its determination (Siró et al., 2010). These values are comparable to those of conventional oil-based food packaging materials. For instance, PLA has lower P_O than polystyrene and low-density polyethylene, thus providing better barrier properties, but it shows a worse performance than PET (Auras et al., 2005; Mensitieri et al., 2011).

PLA possesses poor barrier properties to water vapor in comparison with the most widespread conventional petrochemical materials (PET, LDPE, HDPE and PP), with values of permeability (P_{WV}) ranging between 1-2 g m m^{-2} s^{-1} Pa^{-1} (Mensitieri et al., 2011).

Barrier performance of PLA to water vapor and oxygen in comparison to other bio-based and conventional oil-based packaging materials are shown in Fig. I.7.

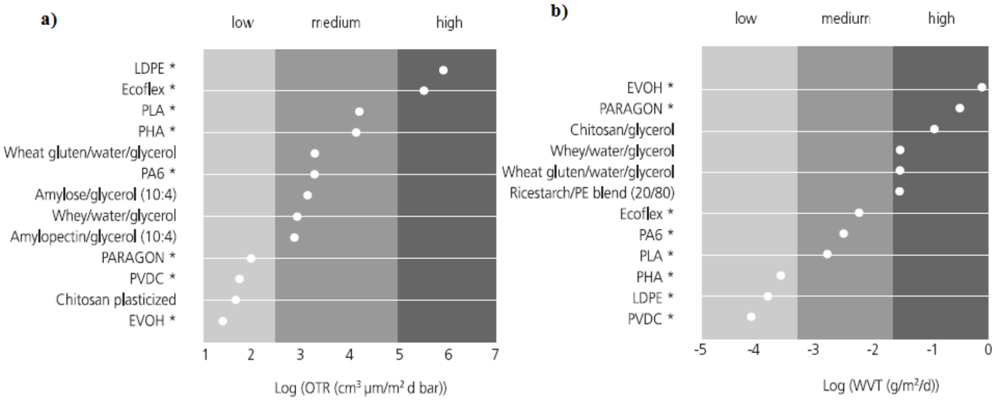


Figure. 1.7. *Oxygen (a) and water vapour (b) transmittance of biobased materials compared to oil-based packaging materials. Materials marked with * were measured at 23° and 50% RH. (Weber et al., 2000).*

The permeability of PLA is affected by several factors such as stereochemical purity, presence of plasticizers, crystallinity, free volume, temperature and water content (Fambri and Migliaresi, 2010). Tsuji et al. (2006) studied the correlation between P_{WV} and degree of crystallinity (X_c) in annealed PLA. They obserbed a reduction in P_{WV} by 50% when crystallinity increased from 0 to 35%. The increase in the water vapor barrier was explained by the formation of spherulites, which are characterized by a greater structural order, thus constituting an obstacle to the diffusion of the molecules of permeants. Conversely, plasticizers increased the permeability of PLA due to the higher mobility of the polymer chains (Rhim et al., 2006; Ahmed et al., 2010).

I.1.2.6. Mechanical properties

PLA is generally characterized by good mechanical properties that can make it a suitable material in replacing conventional polymers in many applications. It has an elastic modulus of 3000-4000 MPa and tensile strength of 50-70 MPa (Perego and Cella, 2010).

PLLA possessing semi-crystalline structure is generally preferred for applications requiring improved mechanical and thermo-mechanical properties. The major limiting factor is represented by the poor elongation at break (2-10%). Due to its T_g (60- 70°C), PLA is brittle at room temperature, fracturing through a crazing mechanism. Amorphous PDLLA is typically used for biomedical devices (slow drug release) and its mechanical properties are quite different from those of PLLA. This different behavior is principally due to the stereoregularity of the polymer chains, which are characterized, in the case of PLLA, by the presence of only one type of chiral centers. Mechanical properties are also affected by the molecular weight. In particular, the increase of molecular weight from 107,000 to 550,000 g/mol results in a 20% increase in tensile strength (Engelberg and Kohn, 1991). In general, the variation of mechanical properties is less pronounced for high molecular weights.

Mechanical properties can be improved through different techniques. One of the most used is mechanical orientation. This process converts a brittle polymer into a versatile and useful material for practical tools such as packaging by undergoing the polymer to tensile strength at controlled temperature (Piergiovanni and Limbo, 2010). The best way to obtain an effective PLA film with a reduced structural anisotropy and improved mechanical properties in both the longitudinal and lateral directions is through a biaxial orientation. The stretching is generally carried out at a temperature to be selected in function of the stretching method between T_g and T_m of the material (Kumar et al., 2005). Typical values of the most important mechanical parameters related to biaxially oriented films of semi-crystalline PLLA are: 100-200 MPa (tensile strength), 50-150% (elongation at break), 3300 MPa (elastic modulus) (Yu et al., 2008; Kumar et al., 2005).

I.1.2.7. Surface properties

Surface tension characterization is a critical parameter in the field of food packaging materials. It allows to understand mechanisms involving solid-liquid interactions such as wetting and spreading. The comprehension of the wetting properties of a material is an important tool to improve and develop new surfaces and interfaces by means of artificially changing compatibility of a material with other phases (Karbowiak et al., 2006). This is particularly useful for the optimization of many processes involving an interaction with the surface of the packaging material, such as coextrusion and lamination, printing, coating and surface tratments and the understanding of phenomena such as permeation and adhesion of microorganisms to the packaging (Cava et al., 2007).

One of the potential ways for the characterization of the surface of solid materials consists in the measurement of the contact angle (θ), formed by a liquid drop deposited on a solid surface of the testing material (Fig. I.8). The drop can be considered in equilibrium with the three forces involved: the solid-liquid interfacial tension γ_{SL}, the solid-vapor interfacial tension γ_{SV}, and the liquid-vapor interfacial tension γ_{LV}. The value of the contact angle θ obtained between the solid surface and the tangent drawn on the drop surface and originating from the triple-point of atmosphere-liquid-solid, indicates how hydrophobic or hydrophilic the surface of the material is (Karbowiak et al., 2006). The relationship involving the interfacial tensions of the three phases constituting the system is described by the Young's equation:

$$\gamma_{LV} \cdot \cos\theta = \gamma_{SV} - \gamma_{SL} \qquad \text{(Eq I.3)}$$

where θ , γ_{LV}, γ_{SV}, γ_{SL} have been previously described. This equation is fulfilled in the ideal conditions of a smooth and rigid solid phase.

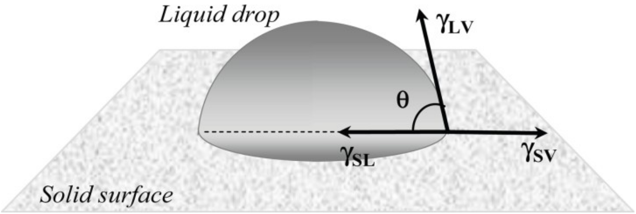


***Figure I.8**. Contact angle of a liquid drop on a soli surface and representation of surface tensions at the triple point (Karbowiak et al., 2006).*

Few authors have investigated the surface properties of PLA. They reported values of contact angles θ ranging between 69° and 96° (Cai et al., 2002; Yang et al., 2002; Zhu et al., 2002; Paragkumar et al., 2006). Such values were obtained by using water as the liquid drop.

I.1.2.8. Migration

Food packaging materials may represent a source of contamination due to the potential migration of substances from the packaging into the foodstuffs contained in them. In section 1.2 (General introduction), the European Union and national legislation adopted on materials intended to come into contact with foodstuffs has been discussed. In Europe, the Europe Union legislation regulates the materials currently in production and/or placed on the market intended to come in contact with food and, like conventional oil-base polymers, bio-based materials such as PLA must comply with it. In general, legislation on migration regulates the food packaging materials with the purpose to decrease the consumer's exposure to chemicals by assuring the safety of these materials when in contact with food.

The regulation of substances may include lists of polymers, monomers, and additives permitted to be used in packaging materials, global and specific migration limits, composition limits, and procedures to calculate the estimated daily intakes (EDI) for certain migrants. PLA migrants include lactic acid, its linear dimer (lactoil lactic acid) and other oligomers containing up to 13 carbon atoms (Soto-Valdez, 2010). Lactic acid is considered the main migrant from PLA, since the other species are expected to hydrolyze to lactic acid in both acqueous and acidic media generally found in food systems or in the human digestive tract (Conn et al, 1995).

Lactic acid is largely used in food and is considered safe when either used both as an intentional food ingredient or when naturally found in food (Datta and Henry, 2006).

The two L- and D-isomers are metabolized in different manners in mammals. For instance, L-lactic acid is a common compound of intermediary metabolism in mammals that is formed by the utilization of glycogen for energy in muscle. L-lactic acid is then oxidized to pyruvic acid in the liver by L-lactic acid dehydrogenase. The other stereoisomer, D-lactic acid, is also absorbed from the intestinal tract, but it is poorly metabolized by mammalians due to the lack in the enzyme D-lactic acid dehydrogenase in their body. This isomer is not utilized as a source of energy, and it can accumulate in cells to produce an organic acidosis. It can penetrate tissues such as brain and interfere with its normal metabolic process (Conn et al, 1995).

PLA is relatively stable in aqueous media at a temperature of 40°C for 6 months. Above T_g, the polymer undergoes degradation and the amount of lactic acid increases, especially for polymers possessing a high content of D-isomer. Lactic acid is characterized by low levels of EDI (0.054 mg d^{-1}) if compared to the average intake of lactic acid from foodstuffs (924 mg/day). For this reason, the PLA is considered safe when it is used at temperatures below the T_g (Soto-Valdez, 2010). New PLA-based materials containing additives, comonomers and fillers must comply with current legislation in order to be placed on the market.

I.1.3. Processability and applications

During the last decade, it has been used primarily for medical applications such as: implant devices, tissue scaffolds and internal sutures, due to its high cost, low availability and limited molecular weight (Datta and Henry, 2006). Recently, production costs have been lowered through the introduction of new technologies, such as: the ring-opening polymerization (ROP) process and large-scale production. Thus, the use of PLA has been extended to other areas, such as: packaging, textiles and composite materials (Drumright et al., 2000; Garlotta, 2001; Groot et al., 2010). Although PLA can be processed on standard converting equipment with minimal modifications, its unique material properties must be taken into consideration in order to optimize the conversion of PLA to molded parts, films, foams, and fibers. Today, the main conversion methods for PLA are based on melt processing. This approach involves heating the polymer above its melting point, shaping it to the desired forms, and cooling to stabilize its dimensions (Lim et al., 2008).

Some of the examples of melt processed PLA are injection molded disposable cutlery, thermoformed containers and cups, injection stretch blown bottles, extruded cast and oriented films, and meltspun fibers for nonwovens, textiles and carpets (Lim et al., 2008). PLA also finds applications in other less conventional applications, such as for the housing for laptop computers electronics (Lim et al, 2008). With relation to the food packaging field, PLA can be used for products that are not to be heated, such as single use disposable drinking cups. PLA packages have already been commercialized for eggs, vegetables, fruits, bakery, and blister pack products (Obuchi and Ogawa, 2010) as shown in Fig. I.9. Further applications are food beverages such as flat water beverages and pasteurized milk (Lim et al., 2008). Packaging bags made of PLA tend to produce a cracking noise when handled. Moreover, it can’t be used for microwave containers due to the heat deflection temperature, which is 10-15°C lower than that of amorphous PET.

Figure I.9. *Examples of applications of PLA thermoformed containers (Obuchi and Ogawa, 2010).*

I.1.4. Nanofillers in PLA

Over the years, many studies have been carried out as a means of improving the properties of PLA films (Pluta, 2006). For example, orientation has been used to improve the mechanical properties of PLA (Auras et al., 2005). Similarly, new formulations and plasticizers, blending with other polymers, co-polymerisation, co-extrusion and lamination with more flexible and higher barrier polymers, coating, and the formation of new structures with the addition of fillers ranging from non-biodegradable to biodegradable materials have all been used to improve the properties of PLA-based packaging materials.

The progress which has developed recently in the field of nanoscience and nanotechnology has given an unique opportunity to develop revolutionary materials which overcome the typical performance compromises of the conventional materials by benefiting through a sinergism occuring between components below certain dimensions (Sinha Ray, 2010). For instance, nanoparticles have been shown to improve the properties of polymeric materials through their low level addition, from 2 to 8% w/w (Lim et al., 2008), thereby allowing them to function as nanofillers. The incorporation in a material of particles that have at least one dimension or contain components with at least one dimension in a length of approximately 1-100 nm is able to produce a nacomposite possessing physical and chemical properties significantly different from those reported by a microcomposite made out of the same substance (Duncan, 2011).

Nanostructured materials or nanocomposites based on polymers have recently received great attentions from both industrial and academic research (Sinha Ray, 2010). Nanocomposites are materials where interface interactions between two phases are maximized. They are characterized by a homogeneous dispersion of the nanofiller in the polymer matrix as separate particles. According to Sinha Ray (2010), there are wide variety of nanoparticles and a way to classify them is based on the number of nanoscale dimensions they possess. Their shape include:

- One-dimensional needle- or tube-like structures (inorganic nanotubes, carbon nanotubes, sepiolite);
- Two-dimensional platelet structures (layered silicates);
- Sphere-like three dimensional structures (silica, zinc oxide).

To date, various types of reinforcements have been used for the manufacturing of PLA nanocomposites (Sinha Ray, 2010). Examples are constituted nanoclay, cellulose nanowhiskers, ultrafine layered titanate, nanoalumina and carbon nanotubes. Among all the nanomaterials considered, layered silicate have been the most studied. Their popularity could be probably explained by the easy availability of the starting clay materials, the low cost and effectiveness ratio, the high stability and benignity (Gorrasi et al., 2002; Duncan, 2011). Moreover, layered silicates have shown to dramatically improve material properties of the nanocomposite structures as compared with the pure PLA, such as machanical and flexural properties, barrier properties and biodegradetion (Lim et al., 2008).

I.1.4.1. Montmorillonite

I.1.4.1.1. Structure and properties

Montmorillonite (MMT) is a phyllosilicate characterized by a crystalline structure constituted of stacks of clay platelets (Fig. I.10). Each platelet has an average thickness of about 1 nm and contains two external layers of silicon oxide tetrahedra with a central sheet of aluminium or magnesium oxide octahedra. The lateral dimensions range from hundreds to thousands nanometers. A net negative charge is located on both faces of platelets, so that electrically positive cations (Ca^{2+} , Na^{+}, etc.) are attracted in the gallery area between them (Duncan, 2011; Pavlidou and Papaspyrides, 2008). This structure is able to improve several properties of the material in which montmorillonite is incorporated, such as gas barrier, mechanical and thermal properties (Burgentzelé et al., 2004). Moreover, these benefits can be obtained with only a small percentage of clay load. In fact, a performant concentration of the MMT in a polymer matrix has been demonstrated to be around 4-5 % w/w (Lim et al., 2008; Pluta, 2006).

MMT in its pristine state is characterized by a hydrophilic nature as a consequence of the presence of inorganic cations located in the interlayer gallery. However, alkali counter-ions are exchanged with cationic-organic surfactants in order to make MMT more compatible with hydrophobic polymers. This allows polymers, within which MMTs are dispersed, to be improved in terms of their properties, such as: barrier to permeants and mechanical strengths (Pilla, 2011).

I.1.4.1.2. Cloisite®

Cloisite® (Southern Clay Products Inc., Gonzales, Texas, USA) is a commercially available additive for plastics to improve various physical properties of a matrix polymer. This denomination corresponds to different types of MMT, which are obtained by modification of natural MMT with different ammonium salts (OMMT). These silicates are suitable for the realization of nanocomposite plastic materials by using the main thermoplastic polymers. Polymers loaded with 3-5% (w/w) of Cloisite® exhibit enhancements in several properties, such as heat distortion temperature, mechanical, barrier, recyclability and stability at high temperatures. The different types of Cloisite® are characterized by different organic modifiers in order to make them suitable to be incorporated in various polymers (Southern Clay, 2011).

I.1.4.2. Characteristics of the nanocomposites

In the preparation of nanocomposite materials, two factors have to been considered. Firstly, phyllosilicates can be dispersed in single layers when incorporated in a polymer matrix. Secondly, surface properties of phyllosilicates can be changed through ionic exchange reactions with organic and inorganic cations. These two properties are interrelated because the degree of dispersion of layered silicate in a polymer matrix is in function of the interlayer cation (Sinha Ray and Okamoto, 2003).

Nanoclays constituted by layered silicates contain electrically positive cations (hydrated Na^+ and K^+) in their pristine structure that make them miscible with hydrophilic polymers. In order to be suitable for the realization of nanostructures with PLA, the hydrophilic surfaces of silicates must be converted into more hydrophobic organophilic, which are suitable for the intercalation of PLA chains between the layers of nanoclays (Sinha Ray, 2010). The nanocomposite materials polymer-phyllosilicate exhibit several advantages. For instance, they are characterized by an improved mechanical behaviour, enhanced barrier and thermal properties (Burgentzelé et al., 2004).

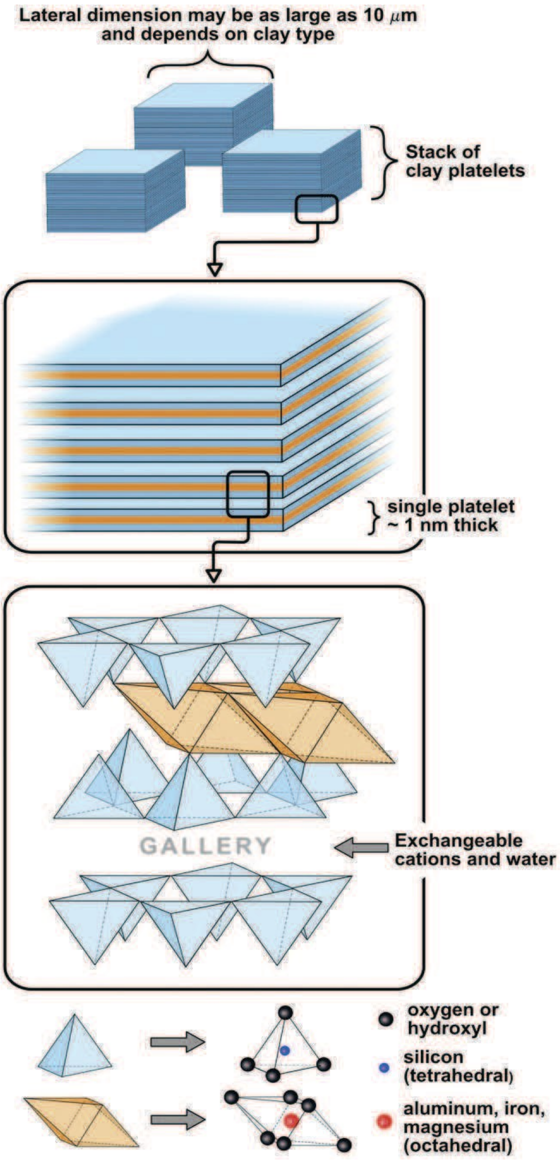


Figure I.10. *Structure of montmorillonite (Duncan, 2011).*

The addition of nanoclays in a polymer matrix does not always result in a nanocomposite material. In several cases, a phase separation is observed due to the immiscibility between the two components, thus generating an agglomeration of the filler with a degrading of the mechanical properties of the material. The unseparated MMT layers surrounded by the polymer are often referred as tactoids (Lim et al., 2008). In this case, the resulting materials tend to exhibit similar properties as conventional microcomposites. However, the insertion of the polymer chains into the galleries of phyllosilicates produces nanocomposite polymers possessing two different structures: (Pavlidou and Papaspyrides, 2008):

- *Intercalated structure*: it is obtained when the polymer chains are inserted between the clay platelets, creating an ordered structure in which polymer and inorganic layers are alternated. The distance between layers is around 20-30 Å.
- *Exfoliated structure*: it is obtained when the polymer chains are individually dispersed in the polymer matrix with distances between each other greater than 80-100 Å.

The different structures obtained with the incorporation of layered silicate in a polymer matrix are shown in Fig. I.11.

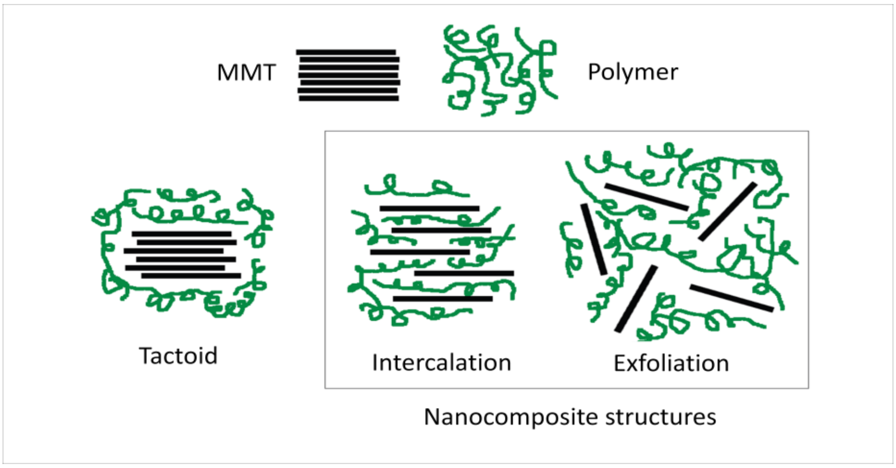


Figure I.11. *Tactoid, intercalated and exfoliated polymer-clay nanocomposite morphologies*.

The exfoliated structure is of particular interest because it minimizes the interactions between polymer and clay, leading to the most significant changes in mechanical and physical properties (Beyer, 2002). However, it is not easy to achieve complete exfoliation of clays, since the majority of the polymer nanocomposites studied were found to possess intercalated or mixed intercalated-exfoliated nanostructures (Chin et al., 2001). The achievement of an exfoliated structure is largely dependent on the chemical compatibility of the clay and the polymer matrix, as well as the process conditions used to disperse the silicate layers of the clay (Lim et al., 2008). To date the techniques mainly used for the preparation of polymer-layered silicate nanocomposites are the following (Alexandre and Dubois, 2000; Fornes at al., 2001; Beyer, 2002; Kim et al., 2001a; Denis et al., 2001; Kim et al., 2001b).

- *in situ template synthesis*: it consists in the synthesis of clay minerals within the polymer through the use of an aqueous solution (or gel) containing the polymer and the silicate building blocks;
- *intercalation of polymer or prepolymer from solution*: the polymer and the layered silicate are mixed together using a solvent in which the polymer is soluble. Firstly, the layered silicate is swollen in the solvent, then the polymer is added to the solution and intercalates between the clay layers. Finally, the solvent is removed by evaporation under vacuum or by precipitation.
- *In situ intercalative polymerization*: in the first step, the layered silicate is swollen by a liquid monomer or a monomer solution. The monomer is intercalated between the galleries of the clay. In the second step, the polymerization reaction can occur between the intercalated layers.
- *Melt intercalation*: this technique consists in blending the layered silicate with the polymer matrix in the molten state.

The intercalative polymerization and melt intercalation are considered the most interesting methods from the industrial point of view. Nevertheless, the former is environmentally unfriendly and economically prohibitive due to the use of solvents (Sinha Ray and Bousima, 2005).

The effect of the incorporation of layered silicates on the physico-chemical properties of PLA has been investigated by a number of studies over the last years (Jiang et al., 2007; Fukushima et al., 2009a; Pluta, 2006; Thellen et al., 2005).

In general, the properties of nanostructured materials seem to be affected by two factors: the physical presence of the nanoclay and the effects of the nanoclay on the interfacial regions of the polymer. The latter is the property that mostly differentiates the effect of nanoparticles from the conventional micro-sized filler, and it’s linked to the high specific surface of the nanoclays. However, results obtained from the nanoincorporation are often contradictory and the performance of the material depends on the amount of filler added and its characteristics, the typology of the resulting structure and the polymer in which nanoclays are incorporated (Pavlidou and Papaspyrides, 2008).

PART I

SECTION A

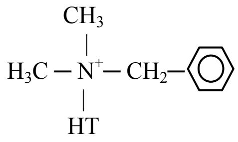
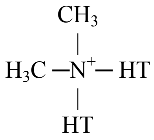
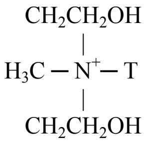
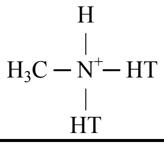
EFFECT OF NANOCLAY-TYPE AND PLA OPTICAL PURITY ON THE CHARACTERISTICS OF PLA-BASED NANOCOMPOSITE FILMS OBTAINED USING EXTRUSION

I.A.1. Materials and methods

I.A.1.1. Materials

PLA 4032D and 4042D with 98.5 and 96% L-isomer lactide content, respectively, were used as the polyester matrix (NatureWorks® LLC, Blair, NE, USA). Four types of nano-sized Cloisite®, C10A, C20A, C30B and C93A (Southern Clay Products Inc., Gonzales, TX, USA), were purchased and used as fillers. These organoclays are obtained by modification of natural montmorillonite with different ammonium salts of organic modifiers. In particular, C10A, C20A, C30B are modified with a quaternary ammonium salt, while C93A with a tertiary ammonium salt. The main characteristics of organoclays used are displayed in Table I.A.1 in accordance to the data provided by the supplier.

Table I.A.1. *Modifier concentration, chemical structure of organic modifier and anion of nanoclays.*

Clay Type	Modifier concentration (meq/100g clay)	Chemical structure of organic modifier	Anion
C10A	125		Chloride
C20A	95	 $\mathrm{H_3C-\overset{CH_3}{\underset{HT}{N^+}}-HT}$	Chloride
C30B	90	 $\mathrm{H_3C-\overset{CH_2CH_2OH}{\underset{CH_2CH_2OH}{N^+}}-T}$	Chloride
C93A	95	 $\mathrm{H_3C-\overset{H}{\underset{HT}{N^+}}-HT}$	Bisulfate

HT is is Hydrogenated Tallow (~65% C18; ~30% C16; ~5% C14).

I.A.1.2. Preparation of PLA/OMMT nanocomposites

Films were manufactured using the facilities of the Department of Chemical and Food Engineering, University of Salerno, (Fisciano, SA, Italy). PLA and PLA-based nanocomposite films were prepared in two steps. Firstly, PLA was blended with a nanoclay (nominal content 5% w/w) using a co-rotating intermeshing twin-screw extruder (ZK25, Dr Collin, Ebersberg, Germany) with a *L/D*=42 and D=25 mm. Due to their high hygroscopic nature and to prevent excessive hydrolysis, PLA and layered silicates were dried at 80°C for 16 hours under vacuum before processing. The screw speed was set at 150 rpm. While dried PLA pellets were fed to the extruder by the primary feeder in area 1 (T1), nanoclays were added through an inlet located in the third (T3). PLA and free-flowing nanoclay powder were fed to the extruder under a nitrogen flux to limit moisture absorption, in order to have a better dispersion of OMMTs in the polymer, since PLA was already in a melt status at this stage. The profile of temperatures used in the different areas of the twin-screw extruder is shown in Fig. I.A.1.

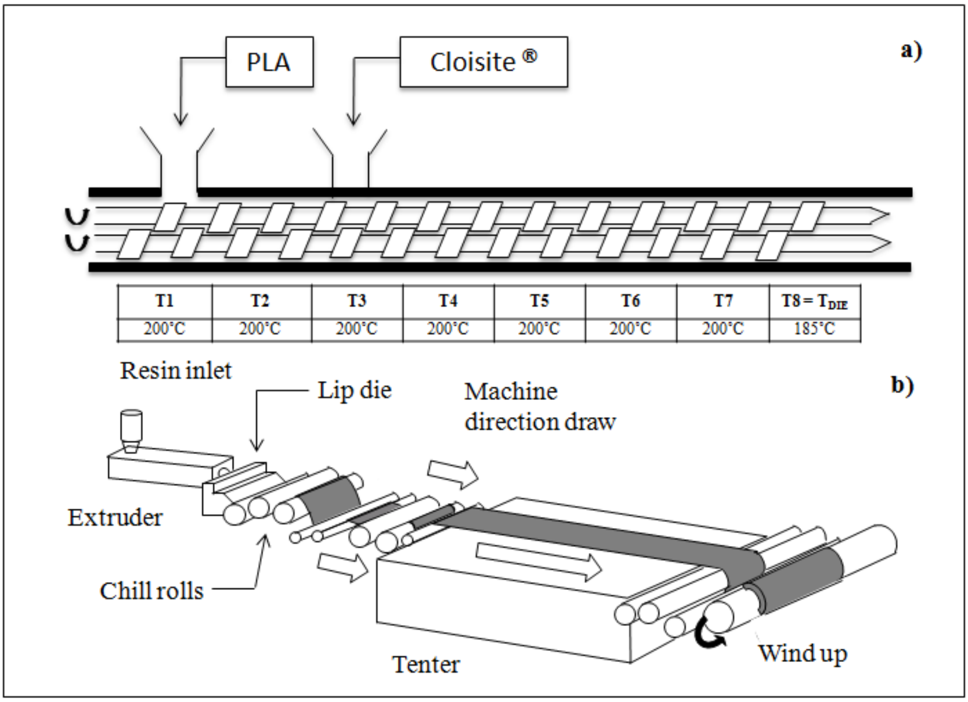


Figure I.A.1. *Scheme of the two steps of a) resin pellets production and b) film production related to the process extrusion for both unfilled and nanoccomposite materials.*

Successively, resin PLA pellets containing OMMT were casted into uniaxially oriented films by a single–screw extruder cast film machine (Gimac, Varese, Italy) with a L/D=24, rectangular die 0.25x200 mm^2. The thermal profile of the latter was 200, 195, 190 and 185˚C at T1, T2, T3 and T_{die}, respectively. The temperature of chill rolls was set at 10˚C. The speeds of screw and wind-up rolls were 50 rpm and 2.5 m/min, respectively. The scheme of both first step (with the profile of temperatures used in the different areas of the twin-screw extruder) and the second step is shown in Fig. I.A.1.

Controls (PLA 4032D and 4042D) and nanocomposites were both manufactured using the same conditions so that there would be no differences in the thermal history between manufactured films. Films produced for this work are specified in Table I.A.2.

Table I.A.2. *Codes of manufactured films.*

Film	Code
PLA 4032D	U
PLA4032D/Cloisite® 10A	U/C10A
PLA4032D/Cloisite® 20A	U/C20A
PLA4032D/Cloisite® 30B	U/C30B
PLA4032D/Cloisite® 93A	U/C93A
PLA4042D	Z
PLA4042D/Cloisite® 10A	Z/C10A
PLA4042D/Cloisite® 20A	Z/C20A
PLA4042D/Cloisite® 30B	Z/C30B
PLA4042D/Cloisite® 93A	Z/C93A

I.A.1.3. Characterization

I.A.1.3.1. Thermal stability

Thermal stability of the films was assessed using a TGA Q500 (TA Instruments, New Castle, Delaware, USA). Samples were analysed from 30-600°C under nitrogen flow with a heating rate of 10°C/min and held in isotherm at this temperature for 1 hour. The temperature range of interest reported in this work is 300-400°C since no changes were observed beyond this interval. The initial mass of the samples tested is in the range of 0.0065 g - 0.014 g. The organoglay load was calculated by subtracting the residue corresponding to the unfilled polymer. Onset and peak temperatures were calculated on the first derivative. Results are based on a mean of three replicates.

I.A.1.3.2. Thermal analysis

Thermal analysis was carried out on a DSC 200 F3 (Netzsch Group, Selb, Germany). Sample (~5 mg) were weighed into an aluminium pan and hermetically sealed. Samples were scanned at 10°C/min in the temperature range of 0 to 200°C. An empty pan was used as reference. In order to cancel the effect of the thermal history, the samples were re-scanned after cooling and T_g, T_{cc} and T_m were evaluated from the DSC thermogram obtained from the second scanning. X_c of samples was calculated based on the following equation (Fukushima et al, 2009b):

$$X_c\ (\%) = 100 \text{ x } (\Delta H_m/(146.0 \text{ x } (1- (\%\mathrm{wt}_{clay}/100)))) \qquad \text{(Eq. I.A.1)}$$

where ΔH_m is the heat of fusion, and 146.0 J/g is the heat of fusion for 100% crystalline PLA homopolymer (Tsuji, 2005; Tsuji et al, 1992). Results are based on a mean of three replicates.

I.A.1.3.3. Structural analysis

Investigation on the structure of nanoclays and nanocomposite films were carried out in a XRD instrument (X'Pert Pro, PANalytical, Almelo, Netherlands) using a Cu Kα radiation at a wavelength of 0.1541 nm and operating at 40 kV and 35 mA. Scans were performed with a step size of 0.0084° from $2\theta = 1.0$ to 10.0°.

The basal spacing of of the silicate layer was calculated using the following Bragg's equation:

$$n\lambda = 2d\ \mathrm{sen}\theta \qquad \text{(Eq. I.A.2)}$$

where n is a integer, λ wavelength of the incident beam, d the spacing between layers and θ is the measured diffraction angle.

FTIR analysis of the films was performed on a Varian 660 FTIR spectrometer using ATR Golden Gate (Specac). Spectra were taken with 32 scans at 4 cm^{-1} resolution in a wavenumber range from 2000 to 500 cm^{-1}.

The cross-section of films were imaged using a FEI Quanta 650 FEG High Resolution SEM. All images of films were taken under similar resolutions.

Films were prepared for imaging using the Cryo-Rupturing Image Sample Preparation (CRISP) method (Flynn et al., 2012), in which a section of the film was submerged in liquid nitrogen and then broken to preserve the structural integrity of the fractured edge (which would be damaged by cutting the film). All film samples required gold-coating (~30 nm) to provide a conductive surface to allow imaging.

I.A.1.3.4. Dynamic frequency sweep test

Frequency sweep test was performed in the linear viscoelastic range from 0.1 rad/s to 100 rad/s with a ARES rheometer (Rheometric Scientific), using a parallel plate geometry with the diameter of 25 mm with a gap of 1 mm at a temperature of 190 ˚C. The complex viscosity was reported against the frequency.

I.A.1.3.5. Transmittance and transparency

Barrier to UV and visible light of films were carried out using a UV-2501PC UV-Vis Recording Spectrofotometer (Shimadzu, Kyoto, Japan) at wavelengths ranging from 200 to 750 nm. The absorbance values at 280, 350, 400, 500, 600 and 700 nm were recorded. The software used was UVProbe v2.31 (Shimadzu, Kyoto, Japan).

Transparency of the films was calculated using the following equation (Han & Floros, 1997):

$$\text{Transparency} = A_{600}/ X \qquad \text{(Eq. I.A.3)}$$

where A_{600} and X are the absorbance at 600 nm and the film thickness (mm) respectively.

Results are based on a mean of three replicates.

I.A.1.3.6. Colour

Surface colour of the films were measured using a Chromameter CR-300 (Minolta Camera Co., Osaka, Japan). The chroma meter was calibrated on the CIE L^*, a^*, and b^* scale before each measurement using a white ceramic ($L^* = 97.15$, $a^* = -5.28$, $b^* = +7.82$). L^* values represent lightness and a^* and b^* values represent redness and yellowness, respectively. The total colour difference (ΔE^*) between control PLA films and PLA/OMMT nanocomposite films was calculated as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{(Eq. I.A.4)}$$

The classification scale for total colour difference reported by Cruz-Romero et al. (2007) was used to classify the films according to the total colour difference. Results are based on a mean of ten replicates.

I.A.1.3.7. WVTR

WVTR of films was calculated according to the ASTM E-96 standard method as outlined by Wang et al. (2009). Distilled water (6 ml) was pippeted in each Perspex^{TM} circular test cup and a PLA film sample was mounted across the cup opening using vacuum silicone grease. The film was secured in place and sealed into test cups with the four screws symmetrically located around the cup circumference. A constant air velocity of 152 m/min was maintained over the cups to ensure uniform movement of air across the test cells. The weight loss of the test cells were monitored over a 24 h period with weight loss recorded every 2 h intervals. WVTR (g/d^{-1} m^{-2}) was determined from the slope obtained by the regression analysis of the data of weight loss as a function of time, once the steady state was reached. Results are based on three replicates.

I.A.1.3.8. OTR

The O_2 content inside a known test cell volume chamber, flushed previously with nitrogen, was monitored non-invasively over a 24 h period with O_2 concentration recorded at 2 h intervals using an optical measuring system Optech^{TM} Platinum O_2 sensor device and disposable O_2 sensor stickers from Mocon (Minneapolis, USA). The optical O_2 sensor works on the principle of a phosphorescent dye that is incorporated in a polystyrene polymer membrane as adherent agent and for environmental protection. The Optech device uses LED technology to take measurement in ~10 s. For O_2 measurement, the instrument need to be brought in an optical contact with the sensor (5-10 mm distance) to produce an O_2 reading (% of O_2, compensated for temperature and pressure variation). Therefore, the sensors were sticked on the top of a hand-made support placed in the center of a lower part of a Perspex^{TM} cup at a distance of 3 mm from the testing film as shown in Fig. I.A.2.

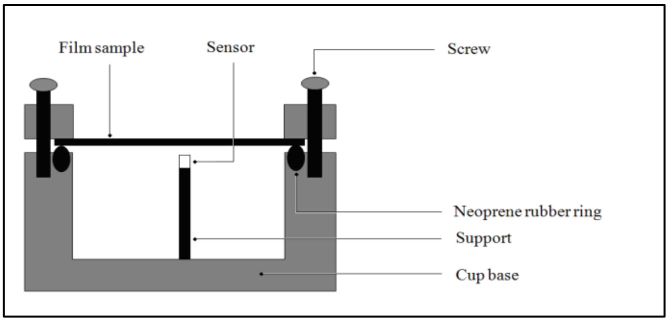


Figure I.A.2. The modified Perspex^{TM} cup used for OTR testings.

Nitrogen was blown into the chamber, the films were mounted between the upper lid and rubber ring with silicon lubricant and fixed to the lower cup by screws. The O_2 concentration in the cup can be measured as many times as necessary as no need of gas sampling/extraction is required. A Windows®-based software was used for the recording of measured parameters (oxygen concentration) and data storage. The cups were stored under controlled temperature and humidity as described in section 2.3.1 and a constant air velocity of 152 m/min maintained over the cups to ensure uniform air movement across cells. OTR was successively calculated considering the oxygen concentration, the volume of the test cup chamber and the area of the film exposed for gas permeation using the following equation (Siró et al., 2010):

$$OTR = V * (slope/100) * 24 * (10000/A) \qquad \text{(Eq. I.A.5)}$$

where *OTR* is expressed as ml m^{-2} day $^{-1}$, *V* is the volume of the receiving chamber, and *A* is the area of the film available for gas permeation. The *slope* is obtained by the regression analysis of the data of the increase of oxygen concentration in the receiving chamber as a function of time. Results are based on three replicates.

I.A.1.3.9. Mechanical properties

TS, E, YM and PS of films were evaluated according to an ASTM Method D 882-02 on the casting machine direction using an Imperial 2500 (Mecmesin, Slinforld, UK). Results are based on ten replicates.

I.A.1.3.10. Contact angle

The hydrophobicity of the surface films was determined by static sessile drop method using a contact angle goniometer (Krüss Gmbh, Germany) provided with an image analysis software (Drop Shape Analysis, Krüss Gmbh, Germany). A 10 μL droplet of distilled water was deposited with a precision syringe (needle diameter = 0.5 mm) on the surface of films. The The contact angle (θ), monitored at t = 0 s, was estimated from the first stable automatic image of the droplet (TRACKER). Values were successively calculated from the intersection beetween the baseline of the drop and the tangent at the drop boundary, the last determined through the use of sessile drop fitting (L-Y) method. The definition of the surface properties of films (hydrophobic or hydrophilic) was made considering $\theta = 65^\circ$ as the discriminating value (Karbowiak et al., 2007). All films were pre-conditioned in a chamber under the same environmental conditions to avoid interferences due to competing moisture exchange at the surface around the droplet (Marcuzzo et al. 2010). Results are based on thirty replicates.

I.A.1.3.11. Antimicrobial properties

Antimicrobial activity of PLA and PLA/clay nanocomposite films against *Escherichia coli* (NCIMB 11943), *Staphylococcus aureus* (NCIMB 13062) and *Pseudomonas Fluorescens* (NCIMB 9046) was carried out using the disk diffusion method. All films were cut into square form of 10 x 10mm and sterilised under UV light for 15 mins before use. Sterilised film samples were placed on Mueller-Hinton agar (MHA) (Oxoid Ltd., Basingstoke, England) plates that had been swabbed on the entire surface of MHA with each bacterial strain containing concentrations approximately 10^6 CFU/ml . The inoculated plates were incubated for 24 hr at 30°C (*Ps. Fluorescens*) or 37°C (*E. coli, S. aureus*). Sterile films without the presence of cloisite were used as a negative control, whereas discs (6 mm) containing 10 µg streptomycin (Oxoid) was placed on the seeded plate as a positive control. After incubation, the area of the inhibition zone developed around the PLA film squares were measured using an absolute electronic calliper (Moore& Wright, UK). The results reported here are the averages of 6 measurements.

I.A.1.3.12. Statistical analysis

Full factorial ANOVA was used to assess the effects of L-isomer content and OMMTs as well as their interaction. Homogeneity of variance was evaluated using the Levene test, and logarithm transformation used when required. Tukey's HSD test was used to compare treatment means when significant differences were found with the ANOVA. The significance level was always set to 0.05. All the analyses were carried out using STATISTICA 8.0 for Windows.

I.A.2. Results and Discussion

I.A.2.1. TGA

The effective organoclay load in the PLA 4032D and 4042D series as assessed by TGA is shown in Table I.A.3.

Table I.A.3. *Nanofiller load, onset and peak temperatures of decomposition on films obtained by TGA*.*

Films	**Nanoclay load (%w/w)**	**Onset (°C)**	**Peak (°C)**
U	-	311.1±3.0^{a}	361.1±3.2^{a}
U/C10A	3.7±0.3	309.1±0.3^{ab}	358.4±1.0^{a}
U/C20A	3.7±0.5	307.7±3.1^{ab}	356.5±6.6^{a}
U/C30B	3.7±0.3	307.5±0.2^{ab}	359.5±1.5^{a}
U/C93A	3.7±0.5	310.5±0.3^{ab}	358.7±0.8^{a}
Z	-	306.6±0.9^{b}	360.5±1.2^{a}
Z/C10A	3.2±0.3	311.2±1.3^{a}	359.6±0.7^{a}
Z/C20A	3.7±0.2	310.5±0.2a^{b}	359.8±0.6^{a}
Z/C30B	3.1±0.2	308.7±0.6a^{b}	360.2±0.2^{a}
Z/C93A	3.7±0.2	311.8±0.6^{a}	360.1±0.3^{a}

*Values are mean ± standard deviation.

Unlike unfilled control samples, a black residue was observed on the platinum sample holders following the decomposition of nanocomposite materials and may be attributable to the presence of clay in the film. The residue measured was higher than the standard deviations measured for each sample and this may be due to the presence of inorganic contaminant traces or as a consequence of incomplete decomposition.

According to the TGA results, the effective content of nanoclays in PLA was 3.7% w/w. Only Z/C10A and Z/C30B had a slightly lower filler load. This may be related to differences in blended interaction between nanoclays and PLA possessing different L-isomer contents.

The TGA curves, along with first derivatives, are shown in Fig. I.A.3.

Results showed that the incorporation of OMMTs had a weak effect on the decomposition process of both PLA grades under non-oxidative conditions. Generally, small difference in values observed in most nanocomposites of the two series compared to the respective controls.

In general, a decrease shift of 3-4˚C was reported in the U series onset, while in the Z series it was noted that there was a delay of about 2-5˚C (Table I.A.3).

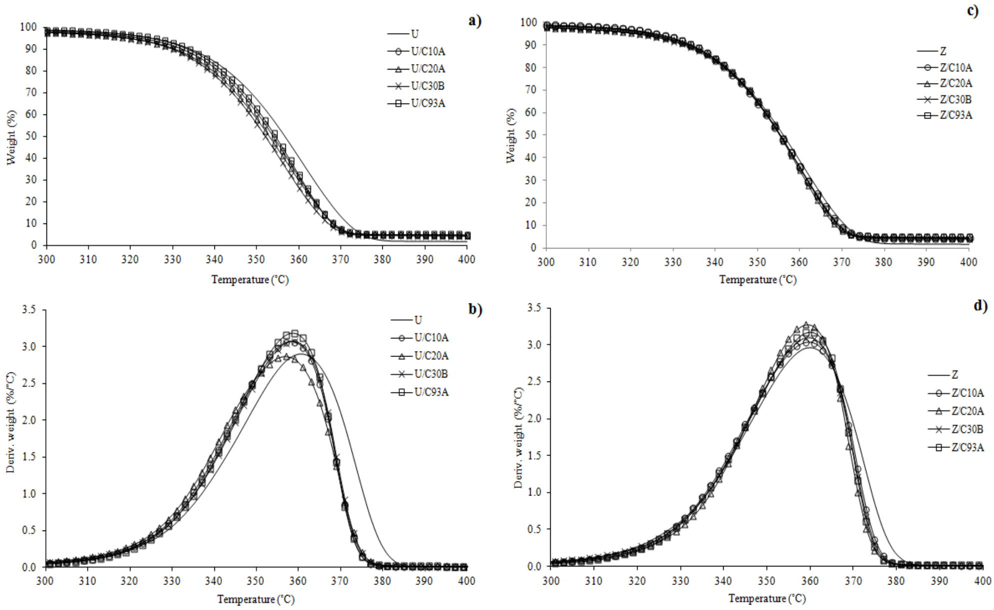


Figure I.A.3. *TGA curves of (a) PLA 4032D series and (b) first derivative, (c) PLA 4042D series and (d) first derivative.*

This may suggest a different interaction between the nanoclays in relation to the two grades of PLA used in this study as indicated by a leveling-off effect at the onset temperatures of degradation. The shift towards lower onset temperatures of the nanocomposites in the U series indicated a lowering of their thermal stabilities upon intercalation at higher temperatures (Chang et al, 2003). In both series of PLA-based films, those manufactured with C93A had the highest onset decomposition temperature among all of the nanocomposites studied. According to Cervantes-Uc et al. (2007), this could be due to the presence of the bisulphate anion in the nanoclay. In the Z series, the incorporation of OMMTs probably act as a heat barrier during the beginning of the decomposition process, thus increasing the thermal stability by slowing down the heat flow eminating from an external source (Zhou and Xanthos, 2009). For both PLA series, once peak temperatures were reached (Table I.A.3), the heat stored in the proximity or between the platelets in the intercalated structure slightly accelerated the decomposition of the nanocomposites. In this instance, the effects delayed oxygen permeation, due to the increased barrier properties, originating by the longer diffusion of the permeant through a “tortuous pathway” (Dubois and Alexandre, 2000; Gilman, 1999), may not be considered since the analysis was carried out under a nitrogen flow. The percentage of crystallinity (Table I.A.4) also seemed to play a role on the thermal stability of the two neat PLA films (Solarski et al., 2008). In fact, the lower cristallinity of Z resulted in a lower temperature onset of 5.5˚C, probably due to a initial increase in polymer hydrolysation.

I.A.2.2. DSC

The thermograms for control and nanocomposite film samples are reported in Fig. I.A.4.

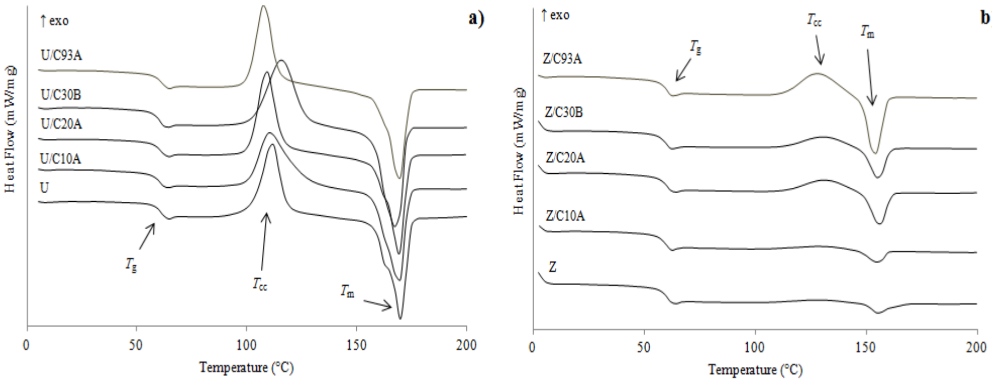


Figure I.A.4. *DSC thermograms of (a) PLA 4032D and (b) PLA 4042D series nanocomposites and unfilled PLA.*

Three thermal events were observed during analysis: T_g, T_{cc} and T_m (values reported in Table I.A.4). T_g is associated with changes in the amorphous region of PLA and is dependant upon the mobility of its chains, T_m is determined by transition of the crystalline regions and T_{cc} is the transition process from amorphous to crystalline states, where the molecules may obtain at a certain stage, sufficient freedom of motion in order to arrange themselves into a crystalline form in a spontaneous manner. The thermograms revealed the presence of a shoulder in the melting peak of PLA 4032D. This behaviour was reported previously in the scientific literature and was proposed to explain what was observed in two different ways: the first as a polymorphism or different crystal structures existing in the PLA, the second as a melt-recrystallization mechanism where small and imperfect crystals change successively into more stable crystals (Yasuniwa et al., 2004).

In the first case, the structures are represented by the α-form and β-form, where the former is the most common polymorph, characterized by a pseudo-othorhombic, pseudo-exagonal or orthorhombic that melts at higher temperatures (Yasuniwa et al., 2006; Yasuniwa et al. 2008). The latter is considered an imperfect crystal constituted by an othorhombic or trigonal structure, melting at a lower temperature in correspondence with the endothermic region (Fukushima et al., 2009b). In agreement with these authors, values of ΔH_m and ΔH_c for films obtained with PLA 4032D are comparable and no significant increase of crystallinity could be detected, thus suggesting that the shoulder observed in the endothermic area could not be attributed to a melt-recrystallization process.

Table I.A.4. *Thermal properties derived from the 2nd heating scan**

Films	**T_g (°C)**	**T_m (°C)**	**T_c (°C)**	**ΔH_m (J/g)**	**ΔH_c (J/g)**	**X_c (%)**
U	60.1 ± 0.1^{ab}	169.9 ± 0.2^{a}	112.0 ± 0.6^{a}	37.4	33.4	25.61
U/C10A	59.9 ± 0.1^{abc}	168.7 ± 0.7^{ab}	110.3 ± 0.6^{ab}	38.3	34.3	27.21
U/C20A	60.0 ± 0.2^{ab}	168.7 ± 0.5^{ab}	108.5 ± 1.4^{bc}	36.5	32.7	25.92
U/C30B	59.9 ± 0.4^{abc}	168.2 ± 0.5^{b}	116.3 ± 0.9^{d}	40.2	36.4	28.56
U/C93A	60.7 ± 0.4^{a}	168.7 ± 0.5^{ab}	107.6 ± 0.3^{c}	33.5	31.1	23.83
Z	59.2 ± 0.3^{cde}	155.1 ± 0.5^{cd}	128.4 ± 0.7^{ef}	3.4	3.1	2.69
Z/C10A	58.7 ± 0.4^{de}	155.3 ± 0.1^{cd}	129.7 ± 1.0^{efg}	3.6	3.3	2.53
Z/C20A	59.5 ± 0.2^{bcd}	155.7 ± 0.4^{c}	130.7 ± 0.3^{g}	8.8	8.3	6.27
Z/C30B	58.4 ± 0.5^{e}	154.7 ± 0.4^{cd}	130.5 ± 0.2^{fg}	8.1	7.8	5.71
Z/C93A	59.4 ± 0.2^{bcd}	154.2 ± 0.5^{c}	128.3 ± 0.4^{e}	16.6	16.3	12.00

*Values are mean ± standard deviation. Different superscripts in the same column indicate significant difference ($P < 0.05$) between treatments.

According to Di Lorenzo et al. (2011), a disordered modification of the α-form was recently proposed which was named α'. This new form is more unstable than the α-form and tends to be transformed to α-form upon heating. In their study on isothermal melt-crystallization conditions, Zhang et al. (2008) found that α'-form crystals are present at T_{cc} below 100°C, α- and α'-forms coexist at temperatures between 100 and 120°C, while α-form crystals are present at T_{cc} above 120°C. Consequently, the formation of a shoulder, which is a function of the process conditions and DSC heating program, can be attributed to the coexistence of *α*- and *α'*-form crystals (Tsai et al., 2010).

In our study, the shoulder was not detected in PLA 4042D , since only *α*-form crystals are present at crystallization temperatures around 130°C.

The addition of OMMTs slightly influenced the thermal events. T_m and T_{cc} were significantly ($P < 0.05$) affected by the PLA optical purity. In fact, a decrease in melting temperature by about 14°C and an increase of cold crystallization by about 20°C in films containing a higher D-isomer content were observed. This may probably be due to the perturbation of the PLA stereoregularity determined by the D-isomer, which causes a slower crystallization and a depression of T_m (Pluta, 2006). This depression may be explained by enthalpy or entropy depending on whether the optically impure units are respectively incorporated into, or rejected from, the crystal structure. Abe et al. (2005) found that D-isomer units were trapped in crystalline regions during the beginning of the crystallization process, but were excluded from the crystalline lamellae. The T_g was slightly affected by the addition of nanoclays. However, a general decrease in glass transition was observed. An increase in the glass transition was observed for the films U/C93A, Z/C20C and Z/C93A.The variation of T_g , due to the incorporation of nanoclays, has been explained by either restricted segmental motions at the organic-inorganic interface neighborhood of intercalated composition that lead to a slight increase of the glass transition (Lim et al., 2008; Pluta et al., 2002; Zhou and Xanthos, 2009),or by a plasticizing effect of the PLA matrix by the organic modifier of the organoclay which results in a small decrease in T_g (Pluta, 2006). The T_g was significantly affected by the difference in L-isomer content. However, the interaction between the polymer matrices and nanoclays was not significant and did not affect T_g.

The cristallinity was seen to slightly increase in the majority of nanocomposites. This can be explained by the effect of the nanoclays to acts as heterophase nucleating agents (Sanchez-Garcia and Lagaron, 2010). The lower content in nanoclay observed for Z/C10A and Z/C30B through TGA analysis resulted in a lower crystallinity percentage due to the reduced nucleating effect.

I.A.2.3.XRD

XRD measurements were carried out on nanoclays (powder) and nanocomposite films (Fig. I.A.5). The patterns of silicate powders, namely C10A, C20A, C30B and C93A, were characterized by diffraction peaks at $2\theta = 4.83$, $2\theta = 3.61$, $2\theta = 4.94$ and $2\theta = 3.49$, respectively, which correspond to a d-spacing (d_{001}) of 1.83 nm, 2.45 nm, 1.79 nm and 2.53 nm.

The addition of OMMTs to PLA matrices produced x-ray diffraction peaks in composites where the diffraction maximum was shifted to a lower 2θ angle when compared with OMMT powder**.** This suggests an interaction between polymer chains and nanoclays with a formation of nanocomposites characterized by intercalated structures and a partial preservation of OMMTs layered configuration (Rhim et al., 2009).

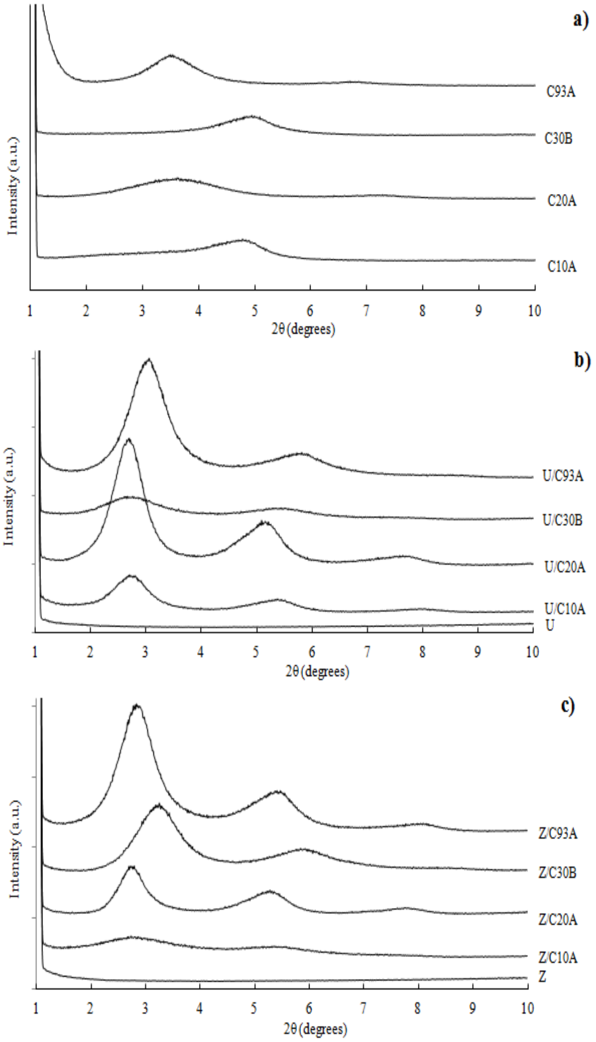


Figure I.A.5. *XRD patterns of (a) nanoclays, (b) PLA 4032D and (c) PLA 4042D series nanocomposites.*

PLA chains were inserted within the galleries of OMMTs, thereby increasing the basal spacing between layers (d_{001}) as shown in Table I.A.5. C30B had the greatest shift to lower 2θ angles of the diffraction maximum and the lowest intensity of peaks in the nanocomposites, which suggests a partial exfoliation of the clay **(**Zaidi et al., 2010). It was observed that L-isomer content had a slight effect on the nanocomposite structures, shifting the diffraction maximum of nanocomposites obtained, with the matrix containing the lower L-isomer, to lower 2θ angles However, further studies are necessary in order to validate this finding.

Table I.A.5. *Effect of the addition of OMMTs and polymer purity on the basal spacing in nanocomposite films.*

OMMT type	**OMMT powder**		**PLA 4032D composites**		**PLA 4042D composites**	
	2θ (°)	**d_{001} (nm)**	**2θ (°)**	**d_{001} (nm)**	**2θ (°)**	**d_{001} (nm)**
C10A	4.83	1.83	2.83	3.13	2.71	3.26
C20A	3.61	2.45	2.78	3.17	2.70	3.27
C30B	4.94	1.79	2.73	3.24	2.77	3.19
C93A	3.49	2.53	3.24	2.73	3.06	2.89

I.A.2.4.FTIR

In order to characterize the films produced, FTIR spectra, conducted within the relevant wavelenght fingerprint region, can be used by observing peaks corresponding to different vibrations associated with the functional groups involved. These vibrations are defined as stretching or bending vibrations which are dependent upon variations in bond length or angle, respectively.

Spectra for films obtained for PLA 4032D and PLA 4042D are shown in Fig. I.A.6. IR banding detected at about 1747 cm^{-1} could be attributed to C=O stretching, while the three bands visible in the range 1300-1500 cm^{-1} may be assigned to symetric and asymmetric deformational vibrations of C-H present in CH_3 of PLA (Pamula et al., 2001).

The peak at 1181 cm^{-1} can be attributed to C-O-C stretching of PLA (Rimoli et al, 1999; Zhang et al, 2005). The cooling process involved in the manufacturing of the films seems to be fast enough to prevent a rearrangement of chain polymer into a crystalline structure. This can explain the shoulders shown at wavenumbers in the range 1200-1210 cm^{-1} (Carrasco et al., 2010). The appearance of peaks at 1128, 1080 and 1043 cm^{-1} may correspond to C-O stretching vibrations (Pamula et al., 2001).

Finally, IR bands detected at 868 cm^{-1} and 755 cm^{-1} can be assigned, respectively, to the amorphous and crystalline phases of PLA (Gonçalves et al., 2010).

It is interesting to note that the appearance of two new peaks in the nanocomposite films were observed at around 520 and 627 cm^{-1}. These peaks may be attributed to the stretching of Al-O and bending of SI-O groups relating to the organoclays present in the films (Frankowski et al, 2007). The presence of Al-O and Si-O groups in nanocomposites confirm that nanoclays are not fully exfoliated in the matrix. Moreover, the width of Si-O peak can explain the degree of exfoliation of OMMTs. In fact, it is broad in case of their agglomeration, and become narrower as the degree of exfoliation increases. The width of the peak shown in Fig. I.A.6 around 520 cm^{-1} may also explain the intercalated structure originated by the incorporation of the nanoclay in the two different PLA matrices.

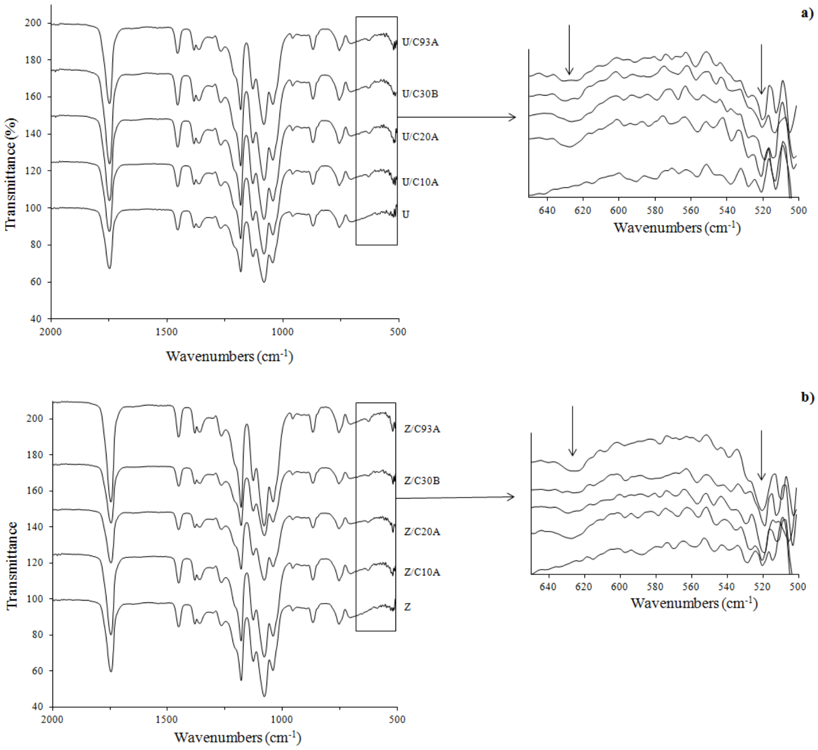


Figure I.A.6. *FTIR spectra of films manufactured with PLA 4032D (a) and PLA 4042D (b). The zoomed region of interest (500-650 nm) is shown alongside the spectra with peaks indicated by the two arrows.*

I.A.2.5. SEM

The morphology of the cross-section of the selected PLA films is shown in Fig. I.A.7. Nanostructured films U/C20A, U/C30B, U/C93A were chosen for their performance and compared with the control U. In particular, U/C20A and U/C30B showed the best results in the improvement of WVTR and OTR respectively, while 4032D/C93A performed a remarkable increase in *E* (Table I.A.7).

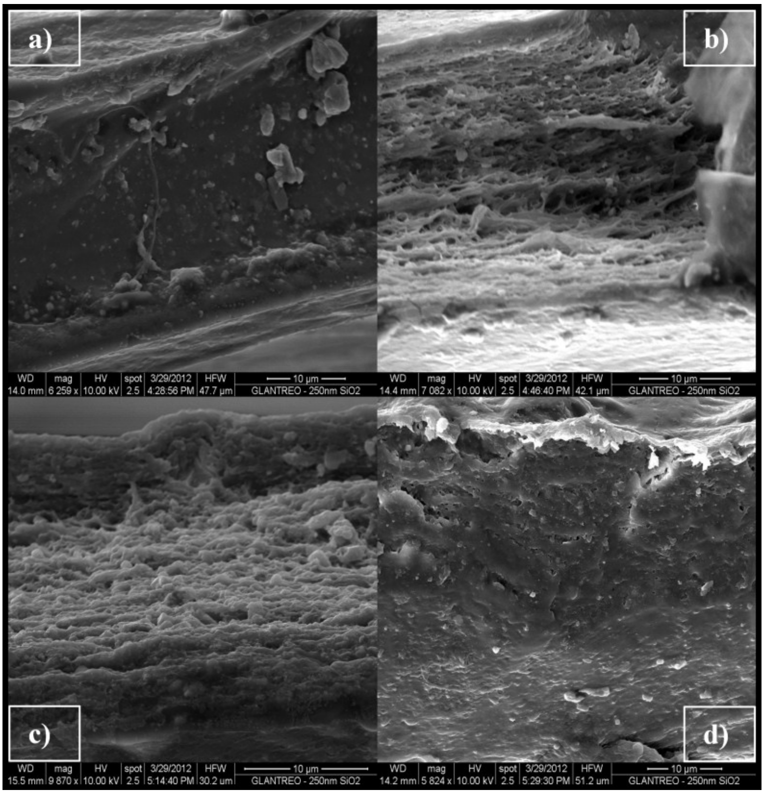


Figure I.A.7. *SEM cross-sectional images of a) control U and b) U/C20A, c) U/C30B and d) U/C93A nanocomposites.*

While no layers can be observed on the control (Fig. I.A.7a), the SEM image show a clearly visible separation in layers for the nanocomposite films U/C20A and 4032D/C30B (Fig. I.A.7b and c). These films reveal a stratified structure constituted by several parallel layers oriented along the tensile direction, due to the separations determined by the incorporation of the nanoclays in the polymer. Based on the barrier properties results, this stratified structure seems to have affected significantly the permeation of molecules through the polymer matrix, increasing the time of diffusion and thus decreasing their transmission rate. A similar separation in layers was already observed in nanocomposite films obtained by the incoporation of layered phyllosilicates into the matrix (Aulin et al., 2012). In agreement with Kumar et al. (2010), nanoclay C30B apparently dispersed more evenly than C20A, the latter showing the formation of small aggregates. Conversely, U/C93 image shows that the addition of the nanoclay produced an organization of the structure with not clear separation of layers (Fig. I.A.7d). While this structure apparently provided little improvement on the barrier properties, however, this structure affected significantly ($P < 0.05$) the E values, producing an increase of this mechanical property.

I.A.2.6. Dynamic frequency sweep test

The complex viscosity (η^*) was affected by the addition of nanoclays in both polymer matrices in function of frequency (ω), while it was not affected by the small difference in PLA L-isomer content. Therefore, nanocomposites containing the same nanoclay had a very similar behaviour independently from the polymer matrix used. The interaction between polymer and nanoclays led to an increase of the complex viscosity at low frequencies. The increase was in function of the OMMT-type, with nanocomposites containing C20A showing the highest values of complex viscosity and thus indicating the finest clay dispersion (Najafi et al., 2012). The higher the organoclay dispersion in the PLA matrix the larger the interface area between the components (Pluta, 2006). Fig. I.A.8 showed the rheological behaviour of the most relevant nanocomposites.

I.A.2.7. Appearance of films

Control PLA and nanocomposite films were flexible and free-standing, characterized by regular parallel lines visible along the machine direction of the films due to the use of the casting apparatus. Control films were smooth, transparent and colourless (Fig. I.A.9). Among the nanocomposite films, PLA nanocomposites obtained by blending of C93A using the two different grades of PLA were slightly opaque, smooth and characterized by an off-white appearance. All other nanocomposite PLA films were characterized by higher transparency. As shown in Table I.A.6, an increase of thickness was observed for nanocomposite films compared to their respective controls. This result was attributed to the addition of solid matter in the matrix and in line with previously reported observations (Rhim et al., 2009).

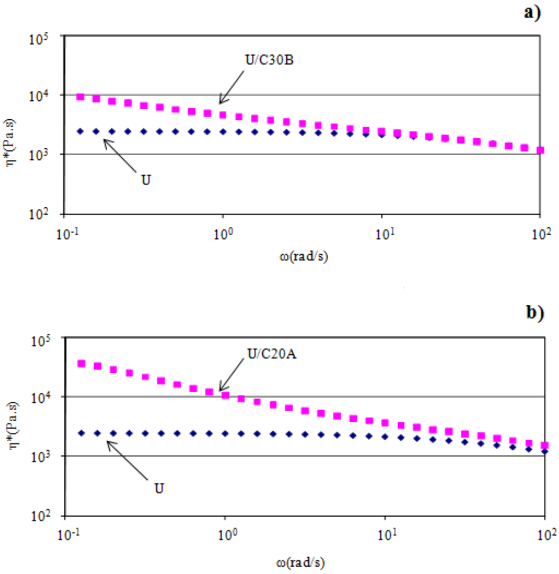


Figure I.A.8. *Frequency sweep test of selected materials: (a) U/C30B compared to U and (b) U/C20A compared to U.*

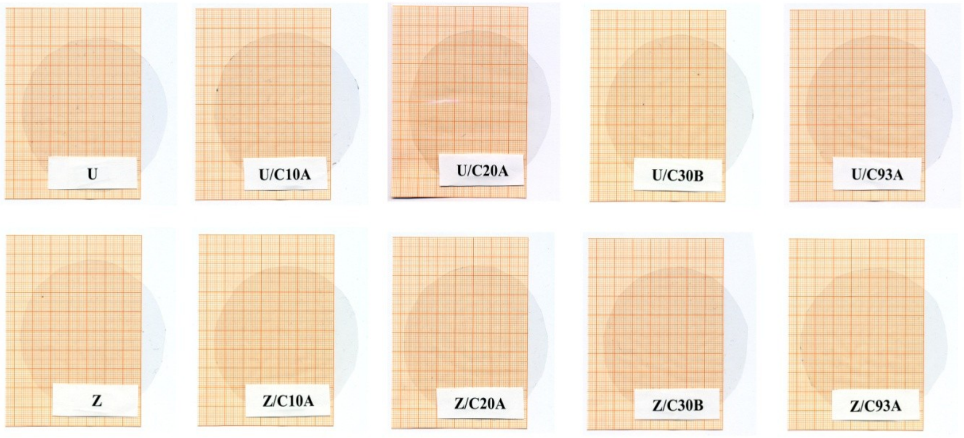


Figure I.A.9. *Images of the films.*

I.A.2.8 Transmittance and transparency

Results showed a general decrease in transmittance for nanocomposites at the wavelengths considered (Table I.A.6).

This seems to be related to the degree of dispersion in the polymer matrix. Interestingly, platelets of layered silicates were 1 nm thick and did not appear to affect the optically clarity of films in visible light for nanocomposites containing 4 % w/w OMMT (Sinha Ray et al., 2003; Zeng et al., 2005). It could also be explained by a presence of intercalated structures with a preserved pristine layered organization that hinders the passage of light, thereby causing an increased scattering of the light.

Table I.A.6. *Thickness, transmittance, transparency and total colour difference values for controls and nanostructured films**

Films	**Thickness (μm)**	**λ (nm) 280**	**λ (nm) 350**	**λ (nm) 400**	**λ (nm) 500**	**λ (nm) 600**	**λ (nm) 700**	**A_{600}/mm**	**ΔE***
U	41.6±0.8	83.8±1.2	87.5±1.1	88.8±1.2	90.0±1.0	91.0±0.9	91.0±0.8	1.0 ± 0.1^{a}	0.00
U/C10A	51.1±1.1	50.9±2.2	73.0±3.2	77.1±3.5	81.2±3.5	84.0±3.0	85.4±3.0	1.5 ± 0.3^{abc}	0.43±0.07
U/C20A	57.4±2.1	38.0±6.5	72.0±2.8	77.1±2.4	81.9±1.9	85.0±1.5	86.3±1.2	1.2 ± 0.1^{ab}	0.75±0.09
U/C30B	62.4±1.0	43.3±3.3	72.4±2.3	76.1±2.5	79.4±2.4	81.6±2.5	82.4±2.6	1.4 ± 0.2^{abc}	0.58±0.06
U/C93A	44.4±1.6	41.5±2.6	65.2±0.9	69.7±1.2	74.9±1.0	78.8±0.8	80.6±0.5	2.3 ± 0.1^{de}	0.21±0.02
Z	40.1±0.9	80.1±3.5	85.3±1.9	86.9±1.5	88.1±0.7	89.5±0.2	89.8±0.4	1.2 ± 0.1^{ab}	0.00
Z/C10A	47.5±2.8	46.1±3.2	71.9±1.4	76.3±1.2	81.1±1.1	84.1±0.8	85.5±0.8	1.6 ± 0.1^{bc}	0.55±0.07
Z/C20A	41.5±1.8	34.0±8.7	69.4±3.9	75.0±3.0	80.6±1.9	84.1±1.3	85.8±1.1	1.8 ± 0.1^{cd}	0.39±0.04
Z/C30B	61.8±2.3	51.8±2.4	72.8±1.6	75.9±1.5	79.4±1.6	82.0±1.5	82.4±1.6	1.4 ± 0.1^{abc}	0.85±0.13
Z/C93A	42.3±1.5	37.5±5.7	62.6±4.2	67.6±3.6	73.5±3.0	77.5±2.5	79.6±2.1	2.6 ± 0.3^{e}	0.27±0.05

*Values of thickness, transmittance and transparency are mean ± standard deviation. Total colour values are mean ± standard error. Different superscripts in the same column indicate significant difference (P <0.05) between treatments.

Transparency was significantly affected ($P < 0.05$) either by the addition of nanoclays or by PLA purity, but no significant interaction between PLA-OMMT was observed. Interestingly, nanocomposites obtained with PLA 4032D, which possesses the higher stereopurity and higher crystallinity, showed a lower decrease in transparency compared to those obtained with PLA 4042D. Nanocomposites showed a significant decrease in transparency compared to controls (Table I.A.6). While films obtained with C10A, C20A and C30B have comparable values in relation to the experimental error, the addition of C93A in the matrix led to the worst performance with a significant greater decrease in transparency ($P < 0.05$), which is in agreement with what has been reported in the appearance characterization section. Considering that the only difference between C20A and C93A lies in a methyl group chlorine anion in C20A and a hydrogen atom and bisulphate anion in C93A, it is believed that the different behaviours of films manufactured with C93A may be due to the bisulphate anion that characterizes this nanoclay. According to Gonçalves et al. (2010), the light yellow colour of PLA products may represent a drawback for food packaging applications, since a change of the degree of yellowness could affect the transparency. As displayed in Fig. I.A.10, nanocomposites (especially films containing C20A and C30B) showed an increase of *b** values which correspond to an increase in yellowness (Cruz-Romero et al, 2007) and thus leading to a slight decrease in transparency. C20A showed the best results in terms of barrier properties to UV radiation while retaining transparency to a significant extent. Its addition in the polymer matrix lead to a decrease in transmittance up to 55% at $\lambda = 280$ nm. This may be related to its best dispersion in PLA as discussed in section 3.4.

According to Sanchez-Garcia and Lagaron (2010), in packaging applications for certain types of foodstuffs, this behaviour would be of interest as a significant reduction in UV contact with the food, through the packaging, would be expected to reduce negative photo-oxidation reactions of food pigments and polyunsaturated fats

I.A.2.9. Colour

The effects of different types of OMMT and PLA L-isomer on CIE *L**, *a** and *b** values are shown in Fig. I.A.10.

The addition of nanoclays in both polymer matrices resulted in a slight decrease in *L** values and *a** values, and in an increase in *b** values for films. In general, PLA nanocomposites were darker, greener and yellower compared to control PLA, and these changes were dependent on the nanoclay-type added. The greater yellowness values observed concurred with visual observations. Δ*E** values (Table I.A.6) showed that OMMTs affected total colour differences but, according to the classification scale, the addition of organoclays in the two PLA matrices produced at most only a small colour difference. Similar changes in film colour *L**, *a** and *b** parameters were reported by Ataeefard and Moradian (2011). These authors obtained films by mixing polypropylene with Cloisite® 15A and proposed that the variation in colour parameters observed for nanocomposites was dependent upon the presence of nanoclays at the polymer surface which increased film surface roughness which affected measurement. Rhim et al. (2011) also reported a decrease in *L** values and an increase in *b** values and Δ*E** in films produced by blending agar with Cloisite® Na$^+$, 20A and 30B. The authors suggested that the differences in colour may be attributed to the degree of dispersion of the nanoclays in the polymer matrix which was in function of their surface hydrophobicity. Since the films were prepared under hydrophilic conditions, Rhim et al. (2011) concluded that the higher values for ΔE^* found in the agar/Cloisite® 20A nanocomposite films were due to incomplete dispersion of this OMMT.

The hydrophobicity of the silicates used in this work decreased in the following order: C20 > C93A > C10A > C30B.

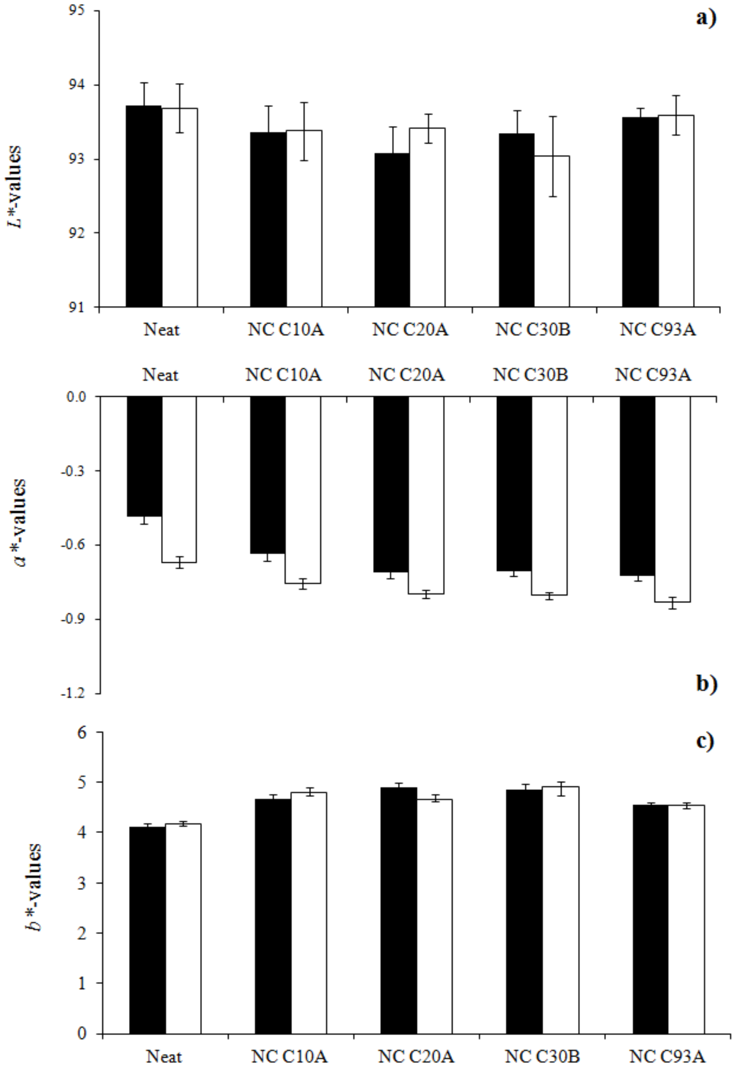


***Figure I.A.10**. Effect of PLA L-isomer content and OMMT type on Hunter L- (a), a- (b) and b- (c) values of films 4032D series (■) and 4042D series (□).*

Results generated in this study indicated that the degree of hydrophobicity associated with these silicates did not appear to be a factor in affecting film colour. Rather, it is believed that the difference in film colour observed in this work may be due to a variation in the surface properties of films determined by the addition of nanoclays as a function of the resin system used and chemical treatments of the clay.

Statistical analysis showed that while no significant difference in total colour was observed between the controls, a significant interaction ($P < 0.05$) between L-isomer content and nanoclays occurred. However, the classification scale based on ΔE^* indicates that films possessed very small, or no differences at all, in total colour.

I.A.2.10 Barrier properties

The addition of commercially available OMMT affected the OTR and WVTR of films; the OTR and WVTR decreased significantly ($P < 0.05$) (Figs. I.A.11 and I.A.12).

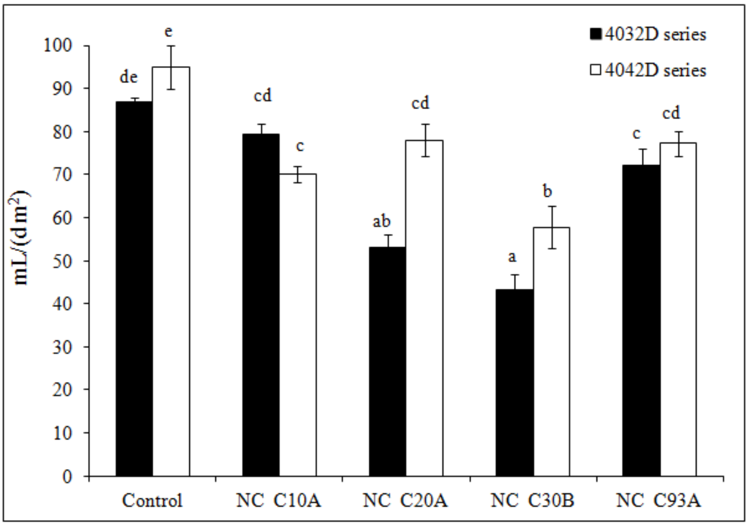


Figure I.A.11. *Effect of PLA L-isomer content and the addition of OMMT type on OTR of control and nanocomposite (NC) films.*

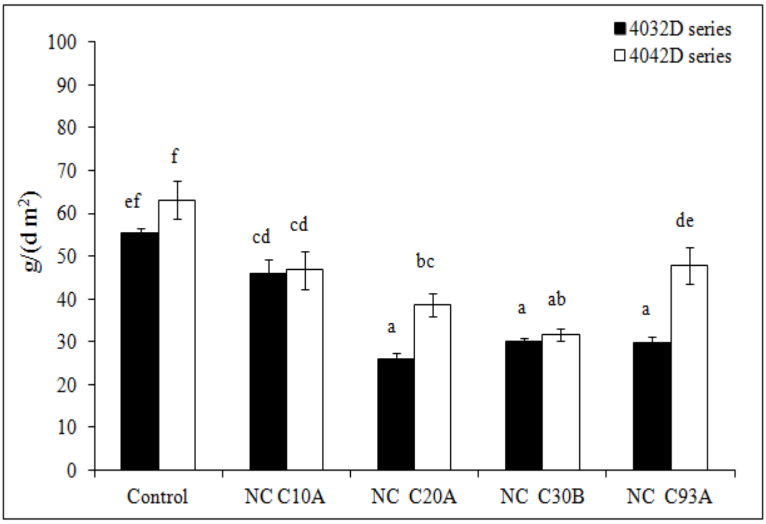


Figure I.A.12. *Effect of PLA L-isomer content and the addition of OMMT type on WVTR of control and nanocomposite (NC) films.*

The statistical analysis showed also a significant ($P < 0.05$) interaction between PLA and nanoclays. The best OTR performance was obtained when C30B was incorporated in PLA 4032D, leading to an improvement up to 50% when compared to the respective control. Considering the intercalated structure of the nanoclays occurring in the nanocomposites through the insertion of polymer chains between the clay platelets as previously observed, the improved barrier properties may be generally explained by the theory developed by Nielsen (1967), based on the tortuous pathway that a permeant has to do in order to diffuse across the polymer. The tortuosity is produced by the dispersion in the matrix of the layered silicates platelets, the latter consisting in a thickness of 1 nm and lateral dimensions as large as 10 µm. As a consequence, the longer diffusion pathway increases the time of diffusion, thus leading to a decrease of the permeability (Duncan, 2011; de Azeredo, 2009). According to Duncan (2011), barrier properties are not only influenced by tortuosity, but also by changes occurring to the polymer matrix at the interfacial regions. In case of favourable interactions between polymer and nanofiller, polymer strands close to each nanoparticle can be partially immobilized, thus working against the diffusion of permeants. For example, the Beall (2000) model focused in particular on the polymer–clay interface as an additional factor affecting permeability. It states that the polymer region around the clay that mainly affects diffusion is the constrained region, characterized by a lower free volume and therefore by a lower diffusion coefficient. Conversely, WVTR results showed that the lowest value was obtained when C20A was incorporated in PLA 4032D with an improvement up to 53%. However, no significant differences were observed when C30B and C93A were also incorporated in PLA 4032D. This may be explained not only by how favourable the interaction between clay and polymer is, but also by the effect of the degree of hydrophobicity of the organically modified nanoclay (C20 > C93A > C10A > C30B) that seemed to have affected the diffusion of water molecules through the polymer matrices (Rhim et al., 2009). The results also showed that barrier properties were also significantly affected ($P < 0.05$) by PLA L-isomer content. The L/D ratio of isomer content is known to have an important effect in barrier properties, even whether findings in literature are inconsistent about the effect of PLA stereochemical purity on permeation of gases (Almenar and Auras, 2010). Our results obtained in this study indicated that a better performance could be generally achieved when the polymer possessing the higher L-isomer content was used. This may be due by the higher percentage of cristallinity observed in the PLA 4032D matrix, given that cystallinity has an important impact on permeation as suggested by Lehermeier et al. (2001).

I.A.2.11. Mechanical properties

The analysis of variance showed that the incorporation of the different nanoclays in the polymer affected the mechanical properties of manufactured films depending on the type of OMMT used (Table I.A.7). The interaction between PLA and nanoclay type was significant ($P < 0.05$). The TS of PLA nanocomposites obtained in this study were comparable to those of conventional oil-based films such as high density polyethylene (HDPE), polypropylene (PP) and polystyrene (PS), possessing TS range values of 22-31, 31-38 and 45-83 MPa, respectively (Hernandez et al., 2000). Tensile properties of nanocomposite films were generally characterized by a decrease of TS (max 30.6 %) and YM (max 21.8 %) and an increase of E (max 157.8 %) compared to their respective controls. These values were in agreement with values reported by Martin and Avérous (2001). In general, the addition of organically modified layered silicate in a polymer matrix resulted in a significant improvement of YM and an increase of the TS. Moreover, the relationship between TS, filler/matrix adhesion and dispersion are more complex than for modulus so that few authors made no attempt to explain the results with quantitative models (Stretz et al., 2005; Zhaidi et al., 2009). This behaviour was explained by a high interfacial interaction and the formation of ionic bonds between the nanoclays and the host polymer whether a uniform dispersion occurs (Alexandre and Dubois, 2000; Pandey et al., 2005; Pavlidou and Papaspyrides, 2008; Sinha Ray and Bousmina, 2005).

Table I.A.7. *Effect of the addition of OMMTs and polymer purity on the mechanical properties of PLA controls and nanocomposite films**

Films	TS (MPa)	E (%)	YM (MPa)	PS (N/mm)	Log E (%)	Log PS (N/mm)
U	39.9±3.6^{f}	7.1±0.7	1765±135^{cd}	408.1±40.5	3.683^{e}	6.007^{de}
U/C10A	27.7±2.7^{a}	6.4±0.6	1562±159^{abc}	287.6±18.4	3.319^{a}	5.660^{b}
U/C20A	31.1±3.3^{abc}	7.4±0.8	1654±150^{bc}	389.5±34.1	3.431^{ab}	5.961^{cd}
U/C30B	34.7±3.4^{cde}	7.9±0.8	1731±125^{cd}	289.1±18.8	3.543^{bcd}	5.665^{b}
U/C93A	33.2±1.8^{bcd}	18.3±2.1	1381±124^{a}	284.6±25.2	3.501^{bc}	5.648^{b}
Z	39.2±3.9^{ef}	8.2±0.8	1655±151^{bc}	360.2±35.2	3.664^{de}	5.882^{c}
Z/C10A	27.7±2.0^{a}	9.9±1.0	1462±137^{ab}	283.4±16.4	3.319^{a}	5.645^{b}
Z/C20A	34.6±3.3^{cd}	8.7±1.0	1895±104^{d}	437.3±18.8	3.540^{bcd}	6.080^{e}
Z/C30B	28.8±2.8^{cde}	8.7±0.8	1373±164^{a}	193.5±18.1	3.356^{a}	5.261^{a}
Z/C93A	37.6±4.0^{def}	9.1±1.0	1688±180^{cd}	364.9±21.3	3.622^{cde}	5.898^{cd}

*Values are mean ± standard deviation.

The results obtained in our study suggested that the nanoclays may not be well incorporated and agglomeration through the polymer matrix could have occurred as previously reported by other authors (Rhim et al., 2009; Silverajah et al, 2012). Moreover, according to the observed reduction in stiffness and increase in elongation of the nanocomposite materials, the organomodified layered silicates added in the PLA matrices seemed to have a plasticizer-like effect on mechanical properties as previously reported by other authors on behaviour of plasticizers (Cuq et al., 1997; Gontard et al., 1993; McHugh and Krochta, 1994; Martin and Avérous, 2001). In the PLA 4032D series, the incorporation of C30B produced the lowest decrease in YM and TS. This behaviour may be attributed to the stronger interactions between polymer and C30B nanoclay compared to the other nanoclays used. This consideration is based on the fact that the higher polar behaviour characterizing the nanoclay C30B by the presence of hydroxyl ethyl groups (—CH_2—CH_2OH) appear to favour the formation of a greater number of hydrogen bonds with the polar groups present in the PLA chains. A great increase in elongation was observed when C93A was incorporated into the PLA 4032D matrix. Thellen et al. (2005)

reported greater elongations for nanocomposite films compared to plasticized control films. The different structures generated in function of the clay type addition as shown in the SEM cross-section of the films (Fig. 4) played a role in the tensile properties of nanocomposites, since stratified layers as seen for C30B and C20A probably provided better toughening effects compared to the structure originated by the incorporation of C93A. A different behaviour was observed in PLA 4042D series, particularly with the addition of nanoclays C20A and C93A, with the former providing the highest increase in YM (14.5 %) and the latter the less reduction in TS (4.1 %). It seemed that a higher D-isomer content in the matrix could have affected the interactions between nanofiller and host polymer, probably due to the different PLA sterical configuration that may favour the interaction with the organically modified nanoclay that possess longer carbon chains. In the PLA 4032D series, the addition of organoclays decreased significantly ($P < 0.05$) the normalized PS of films and the decrease in the PS was related to the clay-type. In this matrix, the incorporation of C20A produced the lower reduction in the PS. Nevertheless, a significant ($P < 0.05$) increase in the PS was observed when C20A and C93A were incorporated in the PLA 4042D matrix, confirming the increased toughness of the material observed due to the increased value of the YM. Therefore, it can be concluded that the PLA stereopurity affected significantly ($P < 0.05$) the mechanical properties. This findings are in agreement with Perego and Cella (2010), who found that a small increase in D-isomer content slightly decreased the YM and TS and produced an increased E. It was reported that the introduction of a small amount of amorphous impurity resulted in a lower crystallite dimensions (Auras et al., 2003). Results showed a contrasting behaviour in the nanocomposite films, which was dependant on the nanoclay type incorporated and the different interactions between polymer and nanofiller. The greatest significant decrease in TS, YM, E and PS was observed when C30B was added in PLA 4042D matrix (17.0, 20.7, 5.3 and 7.1 %, respectively). However, the addition of C93A in the same matrix increased significantly ($P < 0.05$) the YM to 22.2 %.

I.A.2.11. Contact angle

Contact angle values of each film are shown in Fig. I.A.13. The results in the contact angle of the PLA controls indicated that the surface is characterized by slight hydrophobicity. It has been reported that the water contact angle values for poly(D,L-lactide) without surface modification ranged from 69° to 96° (Cai et al.,2002; Yang et al., 2002; Zhu et al., 2002; Paragkumar et al., 2006). The different types of organoclays (Cloisite®) added into the two PLA polymer grades produced a small significant increase of the hydrophobicity and it was correlated to the nature of the nanoclay incorporated, therefore, correlated to the hydrophobicity degree characteristic of the organic modifiers as reported in section 3.1. Consequently, the highest and lowest contact angle (*θ)* values were obtained when nanoclays C20A and C30B were added in the two PLA matrices, respectively. The results indicated that a higher D-isomer content in the PLA polymer produced a slight but not statistically significant decrease in *θ* values.

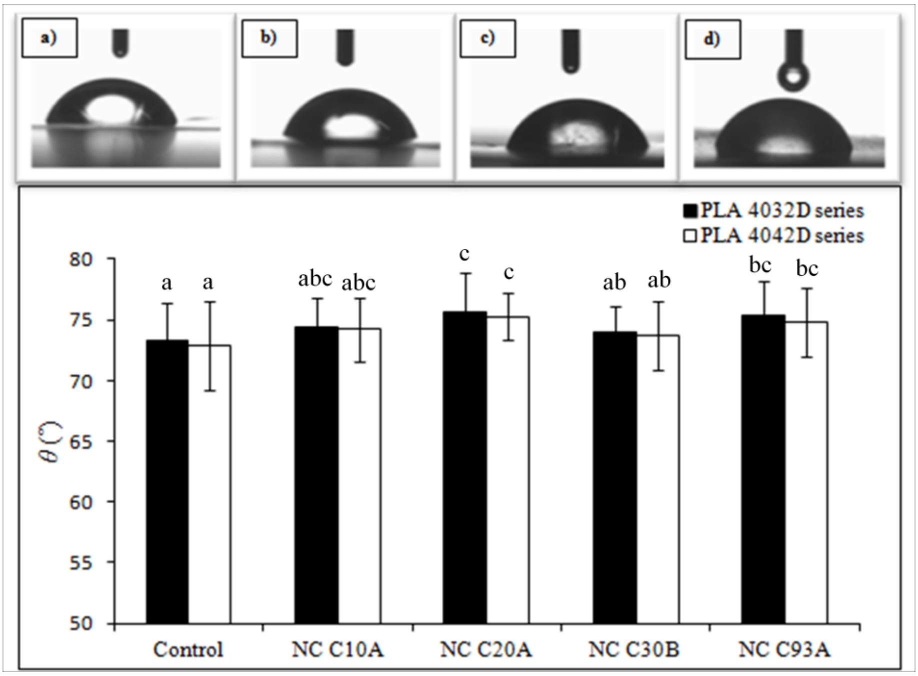


Fig. I.A.13. *Effects of PLA L-isomer content and the addition of OMMT type on the contact angle values of control and nanocomposite (NC) films. Selected images of the water drop are shown for films a) U, b) U/C20A, c) U/C30B and d) U/C93A.*

I.A.2.12. Antimicrobial properties

The qualitative results obtained through the agar disk diffusion methodology in order to evaluate the potential antimicrobial activity of nanostructured films in comparison with the unfilled films are shown in Fig. I.A.14.

The images showed that all the nanocomposite films as well as the unfilled PLA had no antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas Fluorescens*, since there was no inhibition zone around the testing films. On the contrary, a clear radial inhibition halo was observed around the streptomycin-impregnated disks for all the bacteria tested. These findings are consistent with other studies (Rhim et at., 2009; Shameli at al. 2010). Rhim et al. prepared nanostrucuted PLA films using a casting method and chloroform as solvent. They mixed the PLA polymer matrix with three different types of Cloisite®, obtaining nanocomposite films with intercalated structures when PLA was incorporated by organically modified clays Cloisite® 20A and Cloisite® 30B. Even if they previously reported a strong antimicrobial activity of organically modified powders, especially Cloisite® 30B, for both Gram-positive and Gram-negative bacteria with small amount of clay, in this study no antimicrobial action was provided against *Escherichia coli* and *Staphylococcus aureus* by nanostructured materials.

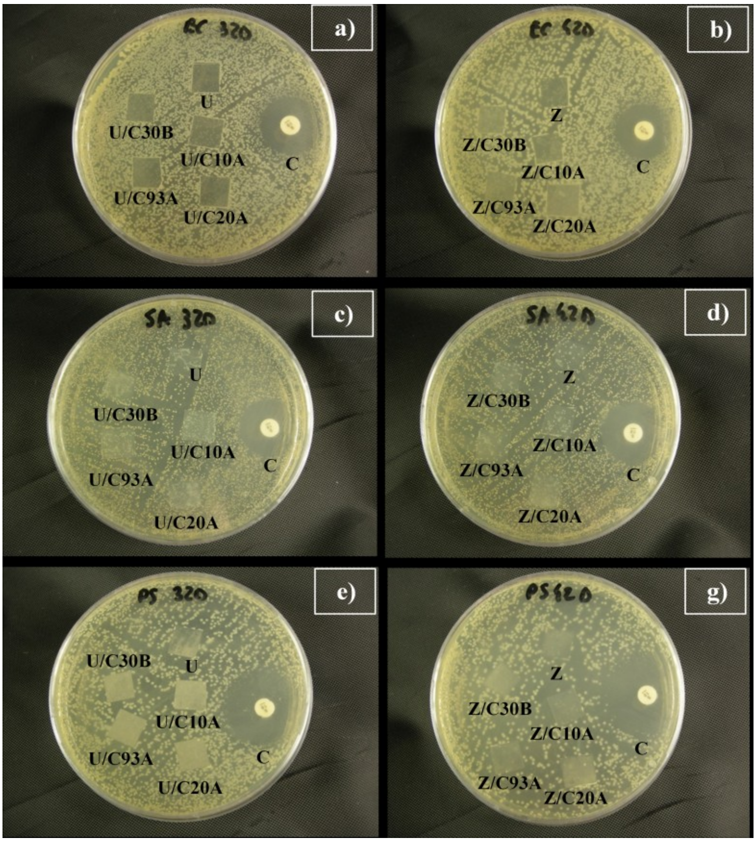


Figure I.A.14. *Evaluation of the antimicrobial activity of nanocomposite and unfilled PLA by comparison of inhibition halo for PLA 4032D and 4042D series again8889st Escherichia coli (a and b), Staphylococcus aureus (c and d) and Pseudomonas Fluorescens (e and g).*

I.A.3. Conclusions

The incorporation of nanoclays in two PLA matrices consisting of a different L-isomer content resulted in the formation of nanocomposites characterized by intercalated structures and a partial preservation of OMMTs layered configuration independently from the organic modifier and the PLA matrix used, as shown by the XRD patterns. The thermal analysis indicated that the addition of nanoclays slightly affected the thermal properties of the films. The dynamic frequency sweep test showed that C20 had the best dispersion among the nanoclays used. The organoclays had an effect on the trasparency and the colour of films, by decreasing the former and ascribing the nanocomposites to a darker, greener and yellower appearance as a function of the Cloisite® used. C20A films also showed the best results in terms of UV barrier properties. The nanoclay type and the L-isomer content affected the OTR and WVTR. The incorporation of C30B improved the OTR by 50% and C20A improved the WVTR by 53%. However, this improvement does not seem enough to compete with conventional oil-based packaging such as polyethylene (high barrier to water vapour) and EVOH (High barrier to oxygen). The organically modified nanoclay used as filler also affected the mechanical properties of the nanocomposite films compared to PLA control samples, reducing the TS and slighlty increasing the E, with the nanocomposite U/C93A showing the greatest E. Nanocomposite films obtained with the PLA matrix possessing the L-isomer content showed greater E, probably due to the lower crystallinity as observed in thermal analysis. The surface properties of nanocomposites were influenced by the degree of hydrophobicity of the organic modifier. No antimicrobial activity was observed in the nanocomposites against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas Fluorescens*. In summary, the nanocomposites were shown to have improved functional properties and consequently, may be employed by the food manufacturing and agricultural sector.

In conclusion, a high barrier coating is needed to challenge the barrier properties of conventional oil-based materials.

PART I

SECTION B

EFFECT OF NANOCLAY-TYPE ON THE PROPERTIES OF PLA-BASED NANOCOMPOSITE FILMS MADE BY SOLVENT CASTING

I.B.1. Materials and methods

I.B.1.1. Materials

PLA 4032D pellets with 98.5 % L-isomer lactide content were used as the polyester matrix (NatureWorks® LLC, Blair, NE, USA). Three types of nano-sized Cloisite®, C20A, C30B and C93A (Southern Clay Products Inc., Gonzales, TX, USA), were purchased and used as fillers. The characteristics of the organoclays used are reported in Table 2.1 of Part I - Section A. DCM (ACS for Analysis, ≥ 99.5%, Carlo Erba Reagenti, Milano, Italy) was used as solvent for both nanoclays and PLA.

I.B.1.2. Preparation of PLA/OMMT nanocomposites

Before processing, both PLA pellets and nanoclays were dryed overnight in a vacuum dryer at 75˚C and subsequently placed in desiccators for 1 h, due to their hygroscopic nature. PLA and PLA-based nanocomposite films were then prepared using the solvent casting method as follows:

- PLA: 5.00 g of PLA pellets were dissolved in 100 mL of DCM and stirred under a fume hood at room temperature at 300 rpm. During this step, the sample has been covered in order to slow down the solvent evaporation. Successively, it was placed in a ice batch to avoid the overheating of the solution and sonicated (UP200S Ultrasonic processor, sonotrode Micro tip S2, Hielscher, Berlin, Germany) for 3 min (setting paremeters: Cycle =1; Amplitude =100%). Temperature was then set at 9±1˚C.

- PLA/OMMT: 4.80 g of PLA pellets were dissolved in 60 mL of DCM stirred at the same conditions of pure PLA. In a different beacker, 0.20 g of Cloisite® were dissolved in 40 mL of DCM in the same stirring consitions to allow the nanoclay to be swollen. Both samples were covered to slow down the solvent evaporation. The two solutions were sonicated separately as for pure PLA. The nanoclay solution was successively mixed with the PLA solution and sonicated again. Temperature was then set at 9±1˚C.

The dissolved solutions were poured onto a leveled teflon sheet (Tardivello srl, Udine, Italy) and spread evenly with Bird-type film applicator (cut depth 1.25 mm). The solvent was allowed to evaporate at room temperature for 24 h. Films were covered during this step to slow down the evaporation of DCM and to obtain a more homogeneous surface. The resultant films were successively peeled off from the teflon surface and vacuum dried at 50°C for 5 h in order to remove the remaining DCM and prevent the solvent from acting as a plasticizers (Rhim et al., 2006). Films were prepared according to the procedure shown in Fig. I.B.1.

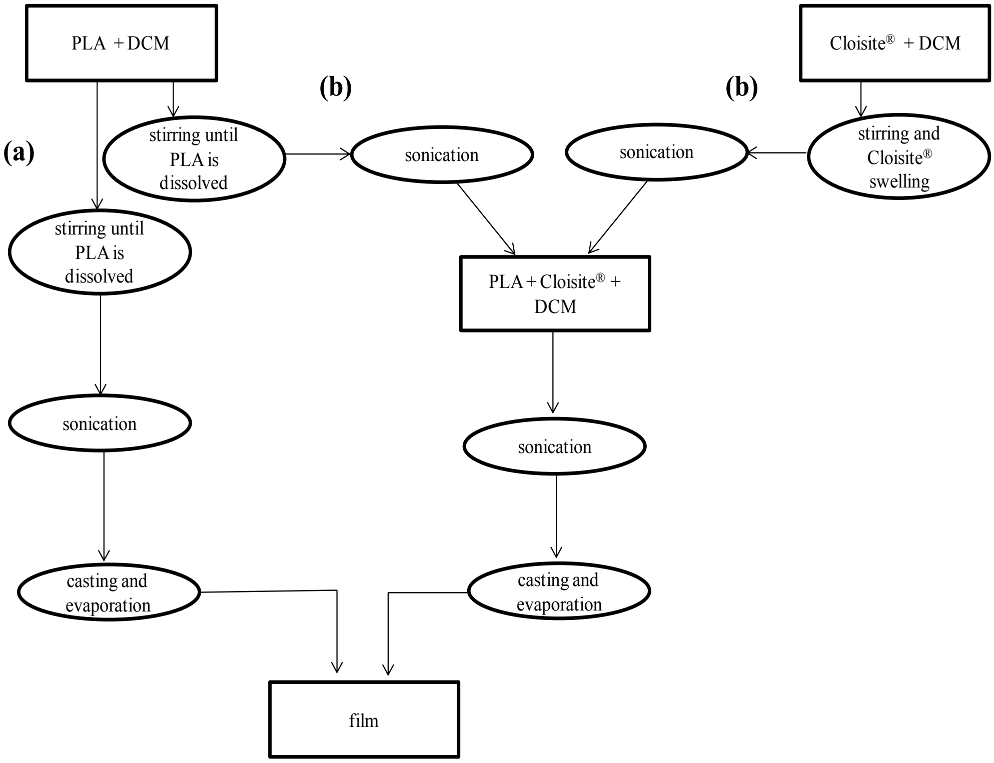


Figure I.B.1. *Scheme of the main steps for the preparation of (a) films obtained by pure PLA and b) nanostructured films obtained by the addition of nanoclays in PLA matrix.*

I.B.1.3. Characterization

I.B.1.3.1. Film thickness and conditioning

Film thickness was measured using a micrometer 345F Digital Thickness Gauge (Elcometer, Manchester, UK) which was previously calibrated with 23.8 μm and 51.5 μm standards.

Before measurement, films were preconditioned for at least 48 hours in an environmental chamber set at 23°C and 53 % R.H.

I.B.1.3.1. Structural analysis

Investigation on the structure of nanoclays and nanocomposite films were carried out in a XRD instrument (X'Pert Pro, PANalytical, Almelo, Netherlands) as detailed in the section I.A.1.3.3.

I.B.1.3.2. WVTR

WVTR of films was calculated using a gravimetric method according to the ASTM E-96 standard method. Circular-shaped film samples (7 x 7 cm) were mounted across the test cup (Payne Permeability Cup 1003, Sheen Instruments, Redhill, UK) opening. Distilled water was pipetted in cups (100 % R.H.) and the cups were sealed using vacuum silicone grease. The cups were successively placed in a equilibrating chamber set at 23°C and 22 % R.H., the latter obtained thorough a saturated solution of potassium acetate. The weight of test cells were monitored every 2 h intervals over a 24 h period of time. WVTR was determined from the slope ($R^2 > 0.99$) obtained by the regression analysis of the data of weight loss as a function of time, once the steady state was reached. Results are based on three replicates.

I.B.1.3.3. Contact angle

Contact angle measurements were carried out using a sessile drop method on a goniometer EasyDrop (Krüss Gmbh, Hamburg, Germany). The images acquired were elaborated with the image analysis software Drop Shape Analysis (Krüss Gmbh, Hamburg, Germany).

A 10 µL droplet of distilled water was deposited with a precision syringe (needle diameter = 0.5 mm) on the surface of films. Three solvents with different surface tensions were used (Table I.B.1.).

Table I.B.1. *Surface tensions of the solvent used.*

Solvent	**Surface tension** ($mN\ m^{-1}$)	**Dispersed component** ($mN\ m^{-1}$)	**Polar component** ($mN\ m^{-1}$)
Water	72.8	21.8	51.0
Diiodomethane	50.8	49.5	1.3
1- Bromonaphtalene	44.6	44.6	-

The analysis was carried out on both sides of films, in order to evaluate the effect of the different exposure during the solvent evaporation step. The two surfaces were identified as:

- A = surface exposed to the air,
- B = surface in contact with the teflon support.

The contact angle was monitored as follows:

- *Contact angle at time zero (t_0)*: measurements were carried out on the first image acquired after the deposition of the drop. In this case, results obtained from each side are based on the mean of tirty replicates.

- *Contact angle over time:* measurements were carried out on images acquired every 5 s, for a overall time of 60 s from the initial deposition of the drop. For both surfaces, results are based on a men of three repicates.

I.B.I.3.4. Surface energy determination

The surface energy (*SE*) of films was calculated on both sides based on the contact angles obtained at t_0. Values were obtained using the following methods:

- State equation (EOS),
- Extended Fowkes,
- Wu

I.B.1.3.5. Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the effect of the addition of OMMTs in the PLA polymer matrix. Tukey's HSD test was used to compare treatment means when significant differences were found with the ANOVA. The significance level was always set to 0.05. All the analyses were carried out using the software R v2.13.1 (R Foundation dor Statistical Computing, Wien, Austria) with the graphical interface R Commander.

I.B.2. Results and Discussion

I.B.2.1. Solvent evaporation

The control of the solvent evaporation was a key factor in obtaining films possessing good optical properties and homogeneous surface. In fact, too high evaporation rates due to the low boiling point of DCM (around 40˚C at 1 atm) affected negatively the appearance of films, leading to the formation of rougher surfaces. As a consequence, it was necessary to control the evaporation rate through the realization of a saturated chamber by covering the films during the evaporation, thus allowing to reduce its rate.

I.B.2.2. XRD

XRD measurements carried out on films prepared with unfilled PLA and through addition of nanoclays are shown in Fig. I.B.2.

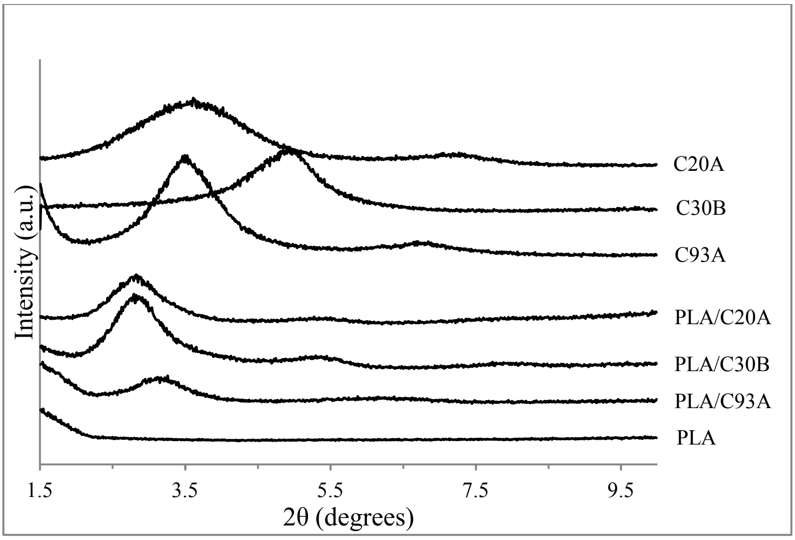


Figure I.B.2. *XRD patterns of nanclays, unfilled PLA and nanocomposites.*

As reported in the section I.A.2.3, the pattern of each silicate powder was characterized by a diffraction peak corresponding to a specific 2θ in function of the nature of the nanoclay (Table I.B.2).

Table I.B.2. *Effect of the addition of OMMTs on the basal spacing in nanocomposite films.*

OMMT type	OMMT powder		Film	
	2θ (°)	d_{001} (nm)	2θ (°)	d_{001} (nm)
C20A	3.61	2.45	2.78	3.17
C30B	4.95	1.79	2.73	3.24
C93A	3.49	2.53	3.24	2.73

The addition of OMMTs into the PLA matrix also produced patterns characterized by the presence of diffraction peaks. The comparison between those of unfilled PLA, pure nanoclays and films suggested that the structure derived by the interation between layered silicates and PLA. In particular, a shift to a lower 2θ angle of the diffraction peak of the different types of Cloisite® was observed in all the composite materials, thus corresponding to an increase of the basal spacing between layers. The d_{001} values obtained from the three different formulations (PLA/C20A, PLA/C30B and PLA/C93A) were 3.2, 3.2 and 2.9 nm respectively. These values were consistent with the results obtained by Pavlidou and Papaspyrides (2008) and they were compatible with the formation of nanocomposite materials possessing an intercalated structure. The increase of the interlayer distance was explained by the insertion of PLA chains between the platelets of the clays, with a partial preservation of the regular structure of the nanoclays. The greater basal spacing showed by the films PLA/C20A and PLA/C30B suggested a slight better intercalation in comparison with PLA/C93A. The pure PLA showed no diffraction peaks. As it can be observed in the present study, the d_{001} values reported in nanocomposite films obtained by extrusion and solvent casting, using DCM as a solvent, were very similar under the manufacturing conditions used.

In literature, Ogata et al. (1997) observed the formation of a tactoid structure in films obtained by addition of nanoclays in PLA through the use of a solvent casting technique. Rhim et al. (2009) obtained nanocomposite films with intercalated structures by incorporating C20A and C30B in PLA. Unlike the present study, their experimental plan consisted in the realization of films using a different solvent and the use of two cycles of sonication, taking 30 min to complete the process. Krikorian and Pochan (2003) observed an exfoliated structure in

PLA/C30B nanocomposite films prepared by solvent casting (DCM). However, they used greater amounts of solvent in relation to the polymer and two cycles of sonication (60 min). These findings explained the strong relationship between the process setting conditions and the resulting structure obtained. Moreover, several authors produced PLA/OMMT nanocomposite materials using the melt processing technique, reporting either intercalate or exfoliated structures, in function of the nature of the organic modifier used, the process conditions and the nanoclay amount (Kubies et al., 2006; Pluta et al., 2006; Zhou and Xanthos, 2008; Krishnamachari et al., 2009; Nieddu et al., 2009). This means that it is fundamental to optimize all the process conditions, in order to tailor materials possessing specific requirements in function of the system used, independently from the technique used for the preparation of nanocomposite materials.

I.B.2.3. WVTR

Results of the WVTR of nanocomposite materials compared to the unfilled PLA are shown in Table I.B.3.

Table I.B.3. *WVTR values measured for unfilled and nanostructured PLA films.*

Film	**WVTR** ($g\ m^{-2}\ d^{-1}$)	**ΔWVTR** (%)
PLA	134±13.0^{a}	-
PLA/C20A	90±16.4^{b}	-33
PLA/C30B	93±13.1^{b}	-31
PLA/C93A	111±5.7^{c}	-17

The value obtained by the pure PLA film was comparable with that reported by Rhim et al. (2009) and much lower if compared to that observed by Rhim et al (2006). In particular, the latter was obtained by measuring films characterized by a certain content of residual solvent (chloroform), thus acting as a plasticizer and decreasing the barrier properties against water vapour molecules. This means that the technique used in the present study to remove the solvent from the films was effective, even if the treatment time was shorter in comparison with other operative methods reported in literature (Krikorian and Pochan, 2003; Rhim et al., 2009).

The nanostructuration of PLA with different types of nanoclays resulted in an enhancement of the barrier properties of films. In particular, the addition of Cloisite® 20A in the PLA polymer matrix determined a reduction of 33% in comparison with the unfilled polymer. This can be explained mainly by the physical barrier caused by the organoclays dispersed in the matrix interfering with the diffusion pathway of permeants. The water vapour molecules were forced to navigate around impenetrable particles/platelets thus increasing the mean gas diffusion lenght (Duncan et al., 2011). The effectiveness of the nanostructuration in increasing the barrier to the water vapour molecules depended on the characteristic of the organic modifier used in the Cloisite®. Kumar et al (2010) stated that the permeability depended on the degree of hydrophobicity of the organic cation adsorbed on the surface of the nanoclay. However, since the organic modifiers of the different OMMTs used possessed a degree of hydrophobilicy that follows the order C20 > C93A > C30B (Lim et al., 2009), this consideration can not be applied in the present study. In fact, the WVTR results were only partially consistent with the degree of hydrophobilicty of the nanoclays used, with the film PLA/C20A providing the best performance. This in full agreement with the discussion in section I.A.2.10. The interactions between nanoclays and polymer and the lamellar morphology of the Closite® are the main factor affecting the permeability of the nanocomposite films. The results showed higher values of WVTR than the same materials obtained thorugh extrusion process. This is in agreement with Rhim et al. (2006), who found a similar behaviour on pure PLA films prepared with thermocompression and solvent casting (chloroform). They explained the lower barrier observed in films obtained with solvent casting with the incomplete evaporation of the solvent, causing a plasticizing effect and thus increasing the permeability to water vapour molecules.

I.B.2.4. Contact angle

I.B.2.4.1. Contact angle at t_0

Results obtained by the contact angle measurements at t_0 in function of film characteristics and solvent used are reported in Fig.I.B.3. The values of θ ranged from 72.5° to 80.3°, thus showing that both pure and nanostructured PLA possessed tendentially hydrophobic surface properties. As described in section I.A.1.3.10 according to Vogler at al. (1998) the discrimination between hydrophilic and hydrophobic behaviour is provided by the value of $\theta = 65°$.

The addition of nanoclays produced a slight increase in contact angle, which was in function of the degree of hydrophobicity of their organic modifier. As a consequence, the most hydrophobic nanocoposite film was PLA/C20A. The same behaviour was observed for the nanostructured films obtained by melt extrusion (I.A.2.11). The comparison between contact angle and WVTR results suggests a lack of correlation between the surface properties of films and their barrier properties to water. This confims the considerations made in section I.A.2.10, where the hydrophobicity of the organic modifiers only partially affected the WVTR.

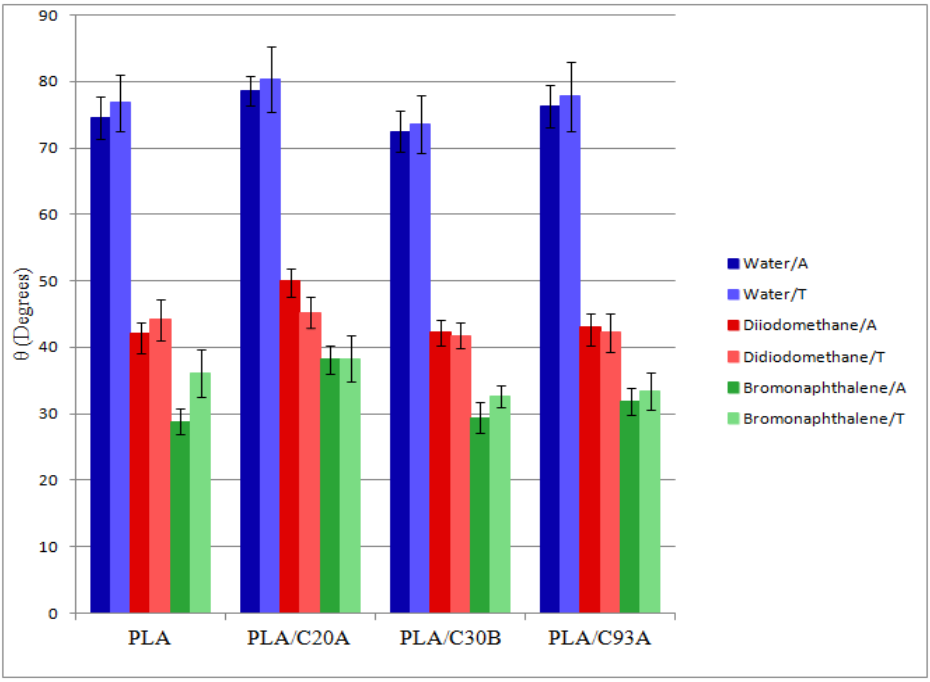


Figure I.B.3. *Average values of θ at t_0 of control PLA and nanocomposites obtained for the three solvents using the static sessile drop method. (A) and (B) are referred to the surface exposed to air and teflon respectively during the preparation of the films.*

Values of *θ* obtained by films in function of the solvents used showed a different affinity between film and solvent. The contact angle decreased with the increase of the solvent hydrophobicity, indicating that the affinity between film and solvent followed the order: water < didiodomethane < 1-bromonaphthalene. The greater affinity for the most hydrophobic solvent confirmed the tendentially hydrophobic nature of all the PLA films, in particular in relation to the nanocomposite films. This non-polar liquid can wet the surface of PLA films, because PLA chains possess hydrophobic groups sush as CH_3. The images acquired for the determination of the contact angle values of the pure PLA are shown in Fig. I.B.4. Among the three solvents, the drop of water tends to minimize the surface in contact with the film, due to the lower affinity between the two phases.

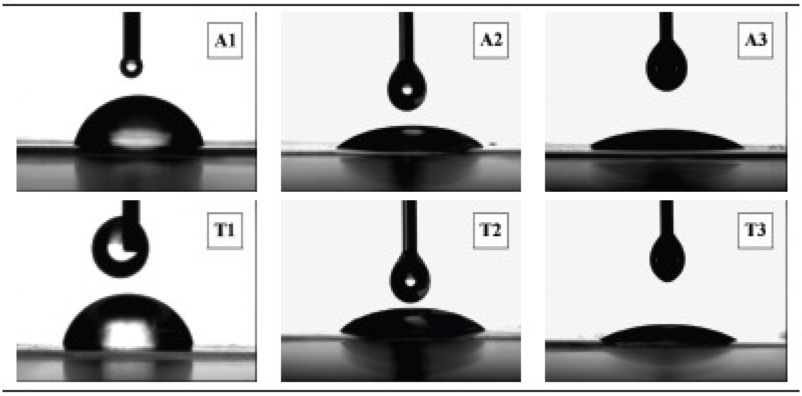


Figure I.B.4. *Images of the drop profile deposited on film made of pure PLA for the measurement of θ at t_0. Letters and numbers are referred to the type of surface (A: air, T: teflon) and solvent (1: water, 2: diiodomethane, 3: 1-bromonaphthalene) respectively.*

The comparison between the values of θ at t_0 of the two sides of the film did not show a significant effect in relation with the different exposure (air and teflon). However, higher θ values were observed in tests carried out with water and 1-bromonaphthalene for the surface in contact with teflon. This slight variation may be attributed to two factors as reported in literature. The first was the potential difference in the conformation of polymer chains on the surface of films due to the different exposure of the two sides during the solvent evaporation (Paragkumar et al., 2006). The second factor is the potential difference in the surface topography of the film due to the roughness of the support. Erbil et al (2003) reported an increase of θ values using water as solvent with the increase of the surface roughness of the testing materials. However, this possible explanation may be further investigated.

I.B.2.4.2. Contact angle over time

The observation of θ in the liquid and solid phases allows to understand either the potential interactions between them or the modifications occurring in the system. In fact, phenomena such as evaporation, absorption and swelling are influenced by the solvent and the testing film (Karbowiak et al., 2006).

An ehample of the values of θ over time obtained for the 4032D films with the three solvents are shown in Fig. I.B.5.

The trend of θ over time was similar for all the films tested. However, while no significant variations were observed for θ measured using water and diiodomethane showing no interactions between the solvent and the testing film (the slight decrease in θ over time related to water and didiodomethane can be attributed to the evaporation of the solvent itself), a different behaviour was reported for the θ measured with 1-bromonaphthalene.

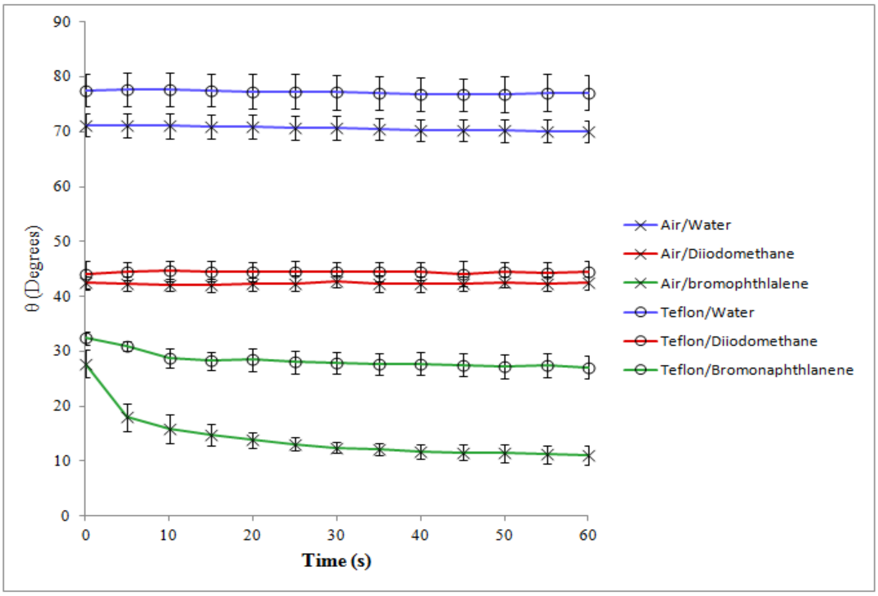


***Figure I.B.5**. Values of contact angle in function of time for a) control, b) PLA 4032D/C20A, c) PLA 4032D/C30B and d) PLA 4032D/C93A. (A) and (T) are related to the surface exposed to air and teflon respectively.*

In fact, a significant decrease in θ was observed from 0 to 5 s, in particular for the surface in contact with air. However, no variations due to absorption, swelling of the film or solvent evaporation can be observed. In Fig. I.B.6. the images of the drop formed by 1-bromonaphthalene monitored over time in control PLA and nanocomposite PLA/C20A are displayed.

The different behaviour of the contact angle observed using 1-bromonaphthalene can be explained by a greater affinity between this solvent and the materials tested. In fact, a progessive increase of the contact surface solid/liquid determined by the deposition of the drop on the film surface was observed over 60 s. Such affinity is due to the contribution of the polar and dispersive components of the surface tension related to 1-bromonaphthalene (Table I.B.1).

I.B.2.4.3. Surface energy

Values of SE^{TOT}, SE^{D} and SE^{P}, calculated according to the average contact angle at t_0, are reported in Table I.B.4. using three different methods. Since the values of θ measured on the two sides of films at t_0 didn't show significant differences, the calculation of the surface energy was carried out without considering any distinction between the two different surfaces.

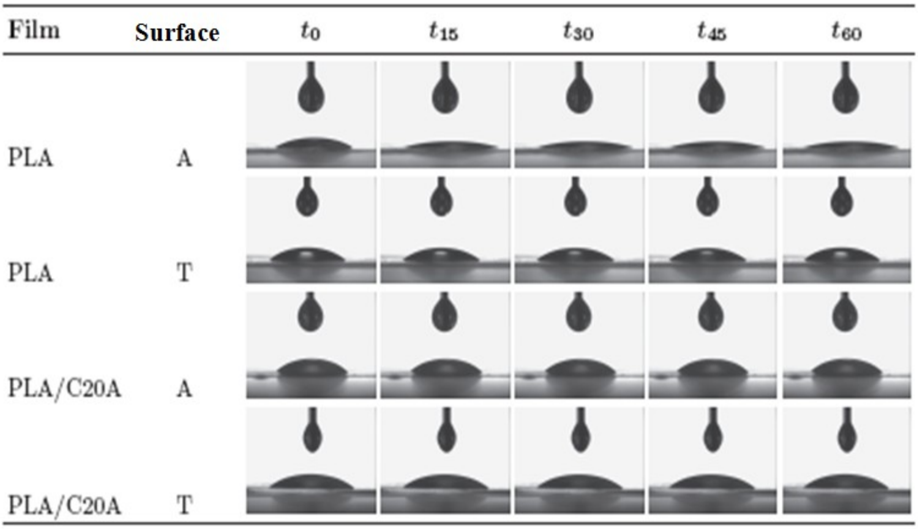


Figure I.B.6. *Images of the 1-bromonaphthalene drop profile over time of nanocomposite PLA/C20A and control film. Letter refer to the different exposure of the film (A: air; T: teflon) during the preparation.*

The SE^{TOT} reported for all the films was slight different depending on the method used. However, the comparison among the films showed that the addition of nanoclays produced a small variation in the SE^{TOT} of nanocomposites, which was in function of the different values of θ previously obtained at t_0 (Fig. I.B.3). The difference betweeen the values of surface energy in the films is probably due to differences in terms of conformation and surface molecular orientation (Fahs et al, 2010). Higher values of SE^{TOT} were observed with the decrease of the contact angle, that is with the increase of the hydrophilicity of the film surface. In particular, the lowest values were observed for PLA/C20A, while the highest were provided by PLA/C30B.

Among the three methods used for the determination of SE^{TOT}, *Extendes Fowkes* and *Wu* allowed to calculate SE^{D} and SE^{P} of the materials surface energy. Independently from the method used, values related to SE^{D} and SE^{P} were characterized by the same behaviour. In the nanocomposites, SE^{P} and SE^{D} decreased and increased respectively with an increase of the organic modifier hydrophobicity. The addition of the most hydrophobic nanoclay (Cloisite® 20A) produced the greatest decrease in SE^{P}l, due to the hydrophobic groups of the nanoclay organic modifier. Conversely, in the nanocomposite obtained with the less hydrophobic nanoclay (PLA/C30B), an increase of SE^D and SE^P was observed. The higher values of SE^P can be explained by the –OH groups constituting the organic modifier of Cloisite® 30B.

To date no studies on the characterization of the surface energy on nanocomposite materials obtained by the incorporation of layered silicates in a PLA polymer matrix were found in literature. However, the values of SE^{TOT}, SE^D and SE^P observed in the present study are very close to those reported by Meiron and Saguy (2007) obtained on plastic films made of LLDPE and PET. Morover, the surface energy values of pure PLA are very similar (41.65 N/m, obtained by averaging the values from the three methods) with those reported by Auras (2004) for 98% L-lactide (42 N/m).

According to Karbowiak et al (2006) the study of the surface properties thorough contact angle analysis and surface energy are critical in the food packaging industry, especially for processes which use the interaction between two phases, such as coating operations, spreding of inks (determination of the printing quality of inks) and adhesives on various substrates.

Table I.B.4. *Energy surface values of unfilled and nanocomposite PLA films calculated with three different methods (Equation of State, Extended Fowkes and Wu).* SE^{TOT} , SE^D *and* SE^P *are the total energy surface, dispersive component and polar component respectively.*

Method	**Film**	SE^{TOT} (mN m^{-1})	SE^D (mN m^{-1})	SE^P (mN m^{-1})
Equation of State (EOS)	PLA	37.22±2.64	-	-
Equation of State (EOS)	PLA/C20A	36.44±0.74	-	-
Equation of State (EOS)	PLA/C30B	39.47±0.60	-	-
Equation of State (EOS)	PLA/C93A	38.37±1.08	-	-
Extended Fowkes	PLA	40.68±2.53	34.58±0.92	6.11±1.60
Extended Fowkes	PLA/C20A	39.47±2.53	34.76±1.05	4.71±1.49
Extended Fowkes	PLA/C30B	44.11±2.36	37.50±0.73	6.61±1.63
Extended Fowkes	PLA/C93A	42.22±2.46	37.18±0.81	5.04±1.65
Wu	PLA	47.06±2.60	35.95±0.81	11.11±1.79
Wu	PLA/C20A	45.45±2.59	36.01±0.92	9.44±1.68
Wu	PLA/C30B	50.47±2.40	38.67±0.65	11.80±1.75
Wu	PLA/C93A	48.31±2.63	38.25±0.71	10.07±1.91

I.B.3. Conclusions

Unfilled and nanocomposite PLA-based films were prepared thorugh a solvent-casting technique using dichloromethane as solvent.

The addition of organomodified montmorillonite in a PLA polymer matrix affected the structural, barrier and surface properties of the resulting nanocomposite films. The results obtained in the present study suggested that:

- as observed from the XRD patterns, the procedure used for the preparation of films allowed to obtain nanocomposites with an intercalated structure, independently from the nanoclay type used;

- nanocomposite films were characterized by an improvement of the WVTR barrier properties. This enhancement is due to the “tortuous” pathway that increased the mean gas diffusion length, which is influenced by the quality of the dispersion of the nanoclays and the interfacial interactions polymer/nanoclay. However, the degree of hydrophobicity of the organic modifier seemed to have only a partial effect on the diffusion of water vapour molecules across the film;

- the different nature of the nanoclays affected the surface properties of the films in function of degree of hydrophobicity of the organic modifier.

Based on the method of film preparation used and the analysis carried out, it was observed that Cloisite® 20A was the nanofiller which mostly affected the properties of nanofillers, in particular the barrier to water vapour molecules. Cloisite® 93A was the less effective in enhancing the PLA properties, probably due to a poor interaction with this polymer.

The comparison between the two techniques used in Part I (extrusion and solvent casting) showed that both of them can be used to produce nancocomposite films. In particular, intercalation of PLA chains between the galleries of the nanoclays were observed for all films produced. While the surface properties of nanocomposites were found to be related more to the degree of hydrophobicity of the nanoclays than the process used for the production of the films, WVTR showed lower values in the samples obtained through extrusion. This suggests that the solvent used can persist as a residue in the film, thus acting as a plasticizer and raising also issues on safety. Moreover, extrusion is the proccess mostly used for the production of conventional plastic. As a consequence, it seems more feasible that films obtained in this study through this technique, would be reproduced commercially.

PART II

EFFECT OF HIGH PRESSURE ON THE OXYGEN-BARRIERPROPERTIES OF A BIO-SOURCED FOOD PACKAGING FILM

II.1. Introduction

Gelatin-based films have been largely investigated due to the easy availability, biodegradability, relatively low cost and excellent film forming properties of this material. (Eastoe and Leach, 1997; Arvanitoyannis et al., 1997a; Arvanitoyannis et al., 1997b; Cao et al., 2007; Núñez-Flores et al., 2012; Rivero et al., 2009; Wang et al., 2009). The large use of gelatin in the world is also proved by the recent increase in its global demand, reaching an annual output of nearly 326,000 tons (See et al., 2010).

Gelatin is obtained from renewable resources and thus possesses the advantages described in section 1.3.1. Gelatin has broad applications in the food, pharmaceutical, cosmetic and packaging industries. It is able to produce clear, flexible and strong films through the use of a casting process, after dissolution in water and in presence of suitable plasticizers (Gennadios et al, 1994). As food packaging, gelatin films are mainly deposited as coatings to extend the shelf-life of perishable foods, especially protecting them from oxygen, light, and moisture exchange (Farris et al., 2009). In fact, gelatin is known to produce films with excellent gas barrier properties (Giménez at al., 2009). Permeability to gas is very important for food packaging films. In particular, oxygen is responsible for many deteriorative processes occurring in packaged foods that lead to undesiderable modifications of organoleptic and nutritional characteristics. For instance, changes in colour, degradation of vitamin C and development of rancid flavours originated from oxidative reactions involving lipid oxidation, along with the growth of aerobic micro-organisms that promotes several enzyme catalyzed reactions, are all responsible for the reduction of shelf-life in foodstuffs.

However, films of proteins are highly sensitive to moisture and exhibit poor water vapour barriers (Guilbert et al., 1996 ; Wang et al., 2007).

II.1.1. Structure of collagen and gelatin

Collagen consists of three polypeptide strands supercoiled into right-handed triple helix by hydrogen bonds, forming units (tropocollagen) of around 300 nm long and 1.5 nm in diameter (Farris, 2009). According to Tanzer (1973), these units are able to pack together side-by-side, through aldol crosslinks between the amino acid constituting the polypeptide chains, and resulting in a stabilized structure as shown in Fig. II.1.

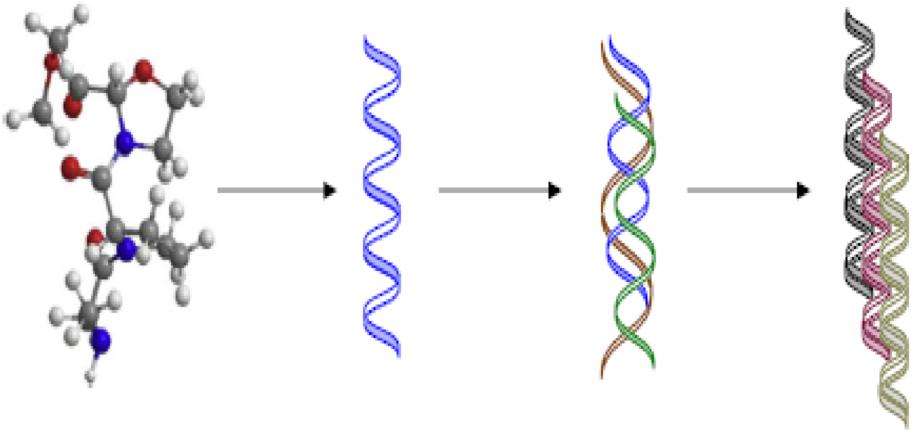
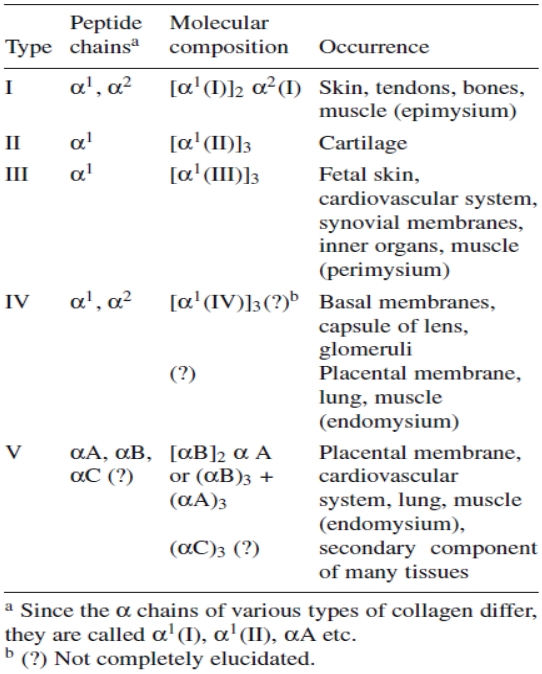


Figure II.1. *Sequential steps in association of peptide chains to form collagen (Farris et al, 2009).*

The three peptide chains constituting the triple helix can be identical or differerent, thus generating different types of collagen in function of their structure as shown in Table II.1 (Belitz et al., 2009).

Table II.1. *Different types of collagen.*



Type	Peptide chains[a]	Molecular composition	Occurrence
I	α^1, α^2	$[\alpha^1(\mathrm{I})]_2\ \alpha^2(\mathrm{I})$	Skin, tendons, bones, muscle (epimysium)
II	α^1	$[\alpha^1(\mathrm{II})]_3$	Cartilage
III	α^1	$[\alpha^1(\mathrm{III})]_3$	Fetal skin, cardiovascular system, synovial membranes, inner organs, muscle (perimysium)
IV	α^1, α^2	$[\alpha^1(\mathrm{IV})]_3$(?)[b]	Basal membranes, capsule of lens, glomeruli
		(?)	Placental membrane, lung, muscle (endomysium)
V	αA, αB, αC (?)	$[\alpha B]_2\ \alpha A$ or $(\alpha B)_3 + (\alpha A)_3$	Placental membrane, cardiovascular system, lung, muscle (endomysium),
		$(\alpha C)_3$ (?)	secondary component of many tissues

[a] Since the α chains of various types of collagen differ, they are called $\alpha^1(\mathrm{I})$, $\alpha^1(\mathrm{II})$, αA etc.

[b] (?) Not completely elucidated.

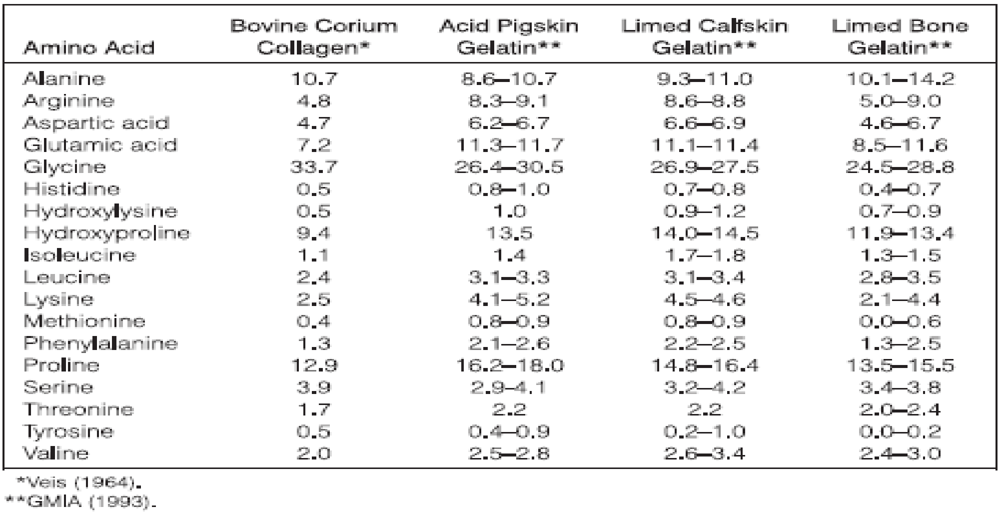
The collagen triple helix organization can be dissociated thermally or chemically by partial hydrolysis and forming gelatin. Gelatin is produced in the world mainly from pigskin (46%), bovine hide (29.4%) and bones (23.1%) (Gómez-Guillén et al., 2009). According to Arvanitoyannis (2002) the production of gelatin for commercial purposes is generally carried out through two different processes :

1) Gelatin produced by acid process (Type A gelatin) involves the soaking of skin or bone in a dilute acid followed by extraction at acid pH.

2) Collagen in hide or demineralized bone is partly depolymerized by breaking down the covalent cross-links and the resulting collagenous material is solubilized at near neutral pH at temperatures of 60-90˚C (Type B gelatin).

Gelatin is characterized by a unique sequence of amino acids, with a high level of glycine, proline and hydroxyproline (Table II.2.). In particular, PSG possesses a content of proline and hydroxyproline of around 30%, which is considerably higher if compared with that provided by fish gelatins (Karim & Bhat, 2009). The amount of proline and hydroxyproline is important in consideration of their effects on gelling properties of the different sources of gelatin (Gómez-Guillén et al., 2011). In particular, these amino acids are key factors in thermal stability, due to steric restrictions imposed by their pyrrolidine rings and the hydrogen bonds formed between these and other amino acids residues (Sikorski et al., 1984; Te Nijenhuis, 1997).

Table II.2. *Amino acid composition of collagen and gelatin reported as Residues per 100 total residues (Arvanitoyannis, 2002).*



Amino Acid	Bovine Corium Collagen*	Acid Pigskin Gelatin**	Limed Calfskin Gelatin**	Limed Bone Gelatin**
Alanine	10.7	8.6–10.7	9.3–11.0	10.1–14.2
Arginine	4.8	8.3–9.1	8.6–8.8	5.0–9.0
Aspartic acid	4.7	6.2–6.7	6.6–6.9	4.6–6.7
Glutamic acid	7.2	11.3–11.7	11.1–11.4	8.5–11.6
Glycine	33.7	26.4–30.5	26.9–27.5	24.5–28.8
Histidine	0.5	0.8–1.0	0.7–0.8	0.4–0.7
Hydroxylysine	0.5	1.0	0.9–1.2	0.7–0.9
Hydroxyproline	9.4	13.5	14.0–14.5	11.9–13.4
Isoleucine	1.1	1.4	1.7–1.8	1.3–1.5
Leucine	2.4	3.1–3.3	3.1–3.4	2.8–3.5
Lysine	2.5	4.1–5.2	4.5–4.6	2.1–4.4
Methionine	0.4	0.8–0.9	0.8–0.9	0.0–0.6
Phenylalanine	1.3	2.1–2.6	2.2–2.5	1.3–2.5
Proline	12.9	16.2–18.0	14.8–16.4	13.5–15.5
Serine	3.9	2.9–4.1	3.2–4.2	3.4–3.8
Threonine	1.7	2.2	2.2	2.0–2.4
Tyrosine	0.5	0.4–0.9	0.2–1.0	0.0–0.2
Valine	2.0	2.5–2.8	2.6–3.4	2.4–3.0

*Veis (1964).
**GMIA (1993).

II.1.2. Functional properties of collagen and gelatin

Due to their nature, collagen and gelatin possess hydration properties auch as swelling and solubility. However, the most important characteristics of collagen and gelatin can be divided into two different groups (Gómez-Guillén et al., 2011).

1) Gelling behaviour (gel formation, texturizing, thickening and water binding capacity;

2) Surface behaviour (emulsion, foam formation, adhesion, cohesion, film formic capacity, etc);

II.1.2.1. Gelling behaviour of gelatin

The factors that mostly affect the gelling behaviour of a system depend on its temperature along with the structure and the molecular weight of the materials constituting it. Collagen and gelatin have different gelation mechanism. In the case of gelatin for example, the gelation process is associated to a decrease in temperature of the solutions below 30°C, which determines the reverse coil-to-helix transition (Gómez-Guillén et al., 2011). The helices produced are similar to the collagen triple-helix without reaching an equilibrium. The gelation process for gelatin is thermo-reversible, where gelatin gels melt as a consequence of an appropriate increase in temperature.

The dissolution of gelatin in acqueous solutions determines a cross-linking phenomena between the chains, which results in a formation of matrices able to swell and constituting gelatin hydrogels. The ability to form hydrogels with the absorption of a great amount of water, along with the bio-source origin, make the gelatin an interesting material to be used in the field of food packaging. According to Farris et al. (2009), the properties of gelatin hydrogels are related to the pH of the system which influence the degree of electrostatic associations and ionic interactions in the gelling systems.

The incorporation of gas such as air in the matrix may lead to a weakening of the structure of gelatin gels, thus worsening the mechanical properties and also providing an opaque white appearance (Zúñiga and Aguilera, 2009).

II.1.2.2. Surface properties

The surface properties of gelatin are related to the presence of charged groups in the protein side chains. Gelatins with a relatively high isolectric point (pI ≥ 7.0) are suitable for creating oil-in-water emulsions with a postive charge over a wider range of pH values than is possible with conventional protein emulsifiers (Dickinson and Lopez, 2001). The emulsion ability of gelatin derived from fish is generally lower than that obtained from mammalians. Another factor to be considered in selecting an appropriate type of gelatin for emulsion purposes is the gel firmness. The higher the gel firmness, the firmer the gel-like protective sheath is around the oil droplets (Schrieber and Gareis, 2007). For instance, tuna fish gelatin possesses a lower emulsion activity index compared to pig skin gelatin at the same protein concentration (Aewsiri et al, 2008).

In general, gelatin has suitable foaming properties because it is able to decrease the surface tension at the liquid/air interface, since it increases the viscosity of the acqueous phase (Schrieber and Gareis, 2007). A greater viscosity is also related to a better foam stability (Jongjareonrak et al., 2010).

II.1.2.3. Film forming properties

Over the last years, gelatin has been widely studied due to its film forming ability and the properties to preserve food by acting as a barrier to oxygen and light (Arvanitoyannis, 2002).

According to Gómez-Guillén et al. (2009), the physical and structural properties of gelatin are influenced by the molecular weight distribution and amino acid composition. These characteristics are also determinant in the mechanical and barrier properties of the resulting films. For example, films obtained from tuna gelatin showed poorer mechanical properties than those produced from bovine hide gelatin. In particular, the breaking deformation values were found to be 10 times higher (Gómez-Estaca et al., 2009). This is probably due to a lower amount of amino acids such as proline and hydroxyproline. In fact, the pyrrolidine rings of these amino acids could affect the deformability of gelatin films due to the conformational constraints imposed.

Films produced from low-molecular weight gelatin are characterized by a higher plasticizer/biopolymer molar ratio, that makes them more plasticized (Thomazine et al., 2005). Moreover, a predominance of low-molecular weight fragments in a gelatin-based preparation determines weaker and more deformable films. This can be explained by protein heat degradation during the water extraction step (Muyonga et al., 2004a) or the evaporation step (Cavalho et al., 2008).

According to Muyonga et al. (2004b) and Zhang et al. (2007), the water vapour barrier and mechanical properties of films made from warm-water fish species (Nile perch or channel catfish) are comparable to those of films obtained from mammalian gelatin. They present also lower barrier properties than films obtained from cold-water fish gelatin (Avena-Bustillos et al., 2006). This can be explained by the difference in the amino acids composition that characterizes gelatins. In fact, cold-water fish gelatins possess higher amounts of hydrophobic amino acids and lower level of hydroxyproline. Nur Hunani et al. (2012) investigated the properties of different types of gelatin films from solution. The types of gelatin used derived from beef, pork and fish skin sources. The authors assessed the different composition of the gelatin sources through the use of FTIR spectroscopy and found that the different gelatin sources showed some changes in the wavenumber of the peaks related to amide-A, amide I and glycerol, suggesting that the film forming ability can be influenced not only by amino acid composition, distribution and polarity, but also by ionic cross-links between amino and carboxyl groups, hydrogen bonding and intramolecular and intermolecular disulfide bonds. The highest gas and water vapour barrier properties were provided by fish gelatin films, while films made of pork gelatin skin showed the highest tensile strength. They concluded that gelatin, irrespective of source and/or concentration used, demostrated itself to be an effective film-forming material which can be used for commercial usage. However, gelatin is a hygroscopic material and this may represent a problem when in contact with the surface of food characterized by high moisture content. In this case, gelatin films tend to swell or dissolve, thus decreasing the barrier properties. As a consequence, new approaches to manufacturing gelatin-based films will need to be investigated in order to improve the barrier properties of the resulting films.

At low to intermediate relative humidity, protein films possess oxygen barrier properties which are higher than those of the polyethylenes and approaching those of the best oxygen barriers such as ethylene-vinyl alcohol copolymer (EVOH) (Krochta, 2002). This means that protein films would appear suitable for coatings and pouches for oxygen-sensitive products.

II.1.2.4. Production of gelatin-based films

Biodegradable films obtained from natural biopolymers such as starch, gelatin, whey protein and fish proteins are thin, transparent and flexible (Bergo and Sobral, 2007). Such films are generally obtained through three steps: solubilization, heating and dehydration of a filmogenic solution, which is the denaturation of the triple-helix present in the collagen.

In order to form a film or coating, the protein is first dissolved in the solvent (water or acqueous ethanol). Most film-forming proteins are soluble in water, with the exception of corn zein, wheat gluten, sorghum kafirin and keratin (Krochta, 2002). Successively, heating is necessary because the bonds are denaturated by the additional heat which changes the three-dimensional structure of protein, exposing sulfydryl groups and hydrophobic side chains. A futher pH adjustment can be done to enhance film formation.

The film properties of the raw material need to be altered by the introduction of chemical cross-links. This can be carried out by using in formulation plasticizers such as glycerol and/or sorbitol which decrease the brittleness of the resulting films. Successively, a degassing step is introduced to eliminate bubble formation in the final film and coating.

Finally, for films obtained through the use of a solvent casting method, the protein films are formed by applying the prepared formulation to the desired casting or product surface and allowing the solvent to evaporate. In this case, the formulation needs to be spread evenly on the surface from where films are pealed off after drying.

II.1.3. HPP

HP treatments have been increasingly used for the preservation and processing of food over the last years. This alternative technology is used to preserve a variety of food materials with reduced thermal impact. The technology has been found to inactivate spoilage and pathogenic bacteria at room or modest temperatures, increasing shelf-life, by denaturing proteins, solidifying lipids and breaking bio-membranes. In particular, it induces a change in the molar volume of proteins that affects their conformation by acting on intra- and inter-molecular bonds (Heremans, 1995). This process depends on the pressure applied as well as on other parameters such as: temperature, holding time and molecular size of the protein (Nguyen and Balasubramaniam, 2011). HP treatments applied on different gelatins gels showed also an effect on the gel strength properties depending on the nature of the collagen used and, in cartain cases, a stabilization of gels formed from gelatin through the formation of new H-bonds was observed (Montero et al., 2002). Research on the application of HP in the food packaging area is in general related to the compatibility of this technology with existing ready-to-use packaging materials, but it is hardly seen as a method to improve the characteristics of films during the film-realization steps. In fact, effects of HP treatment on synthetic packaging film properties such as seal integrity, mechanical properties plus oxygen and water permeability, and its impact on migration of volatile compounds from packaging materials to foodstuffs have been recently reported (Bull et al., 2010; Dobiáš et al., 2004; Eisenbrand, 2005; Galatto, 2008; Rivas-Cañedo et al., 2009). Results obtained from these studies showed that HP treatments up to 600MPa affected the functional properties of tested films in function of temperature and time, determining in the majority of samples an increase in the permeance to oxygen and water vapour and a loss of sealability along with a significant change in the migration of compounds from plastic materials. To our knowledge, there are only a few studies that have assessed the effects of HP treatment on the resulting food packaging films, with particular reference to bio-sourced solutions or gelled systems (Bi et al., 2004; Montero et al., 2002).

II.1.3.1. Engineering concepts of HPP

The principles that govern HPP assume that a sample, which experience HP in a vessel, follows the isostatic rule regardless the size or shape of the food (Norton and Sun, 2008). The isostatic principle states that pressure is istantaneously and uniforly transmitted throughout a sample whether the sample is in direct contact with the pressure medium or hermetically sealed in a flexible package. As a consequence, the time necessary for HPP should be independent of the sample size, which is in contrast to thermal processing (Rastogi et al., 2007).

The application of HP affects food chemistry and microbiology by following the Le Chatelier's principle. The principle states that a system responds in a way that tend to minimize disturbance when its equilibrium is disturbed (Puling, 1964). This means that phenomena such as phase transition, chemical reactivity and chemical reaction that are associated with a decrease in volume are promoted by the application of HP.

The thermal-hydraulic processes occurring during the application of HP during either fluid or solid sytems can be highly influential on the efficiency and effectiveness of the process (Hartmann, 2002; Rademacher et al, 2002).

During the phases of compression/decompression related to HPP, the internal energy of the HP system undergoes some changes, where heat transfer between the internal system and its boundaries occurs. The uniformity of processing by preserving the high temperature level within the package throughout the pressurisation phase can be improved through the use of low thermal conductive material.

II.1.3.2. HPP equipment

A typical HP apparatus consists of a pressure vessel and a pressure-generating device, where a packaged substance is loaded into the vessel and the top is closed (Doona and Feeherry, 2007). Generally, water is the pressure medium which is pumped from the bottom onto the vessel and, when the desired pressure is reached, the pumping is stopped, the valves are closed and the pressure is mainteined without further need for energy input. HP needs to be applied on all regions of the package in an isostatic manner, so that the treatment can be uniform. The pressure in an isostatic process is transmitted in an uniform way throughout the pressure medium and the substance contained in the package (Fig. II.2.). The application of HPP treatments generates an increase of the temperature of the packaged sample through a process known as adiabatic heating (Norton and Sun, 2008).

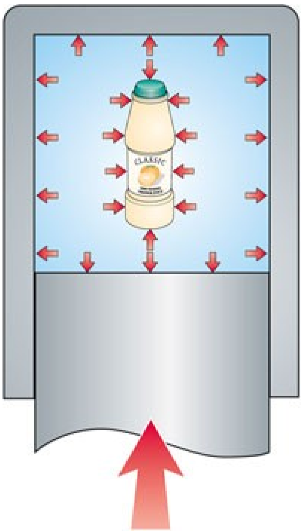


Figure II.2. *Example of uniform hydrostatic pressure application on packaged food.*

In case that such sample is food, shelf-stable pressure-treated food needs an application of HPP in conjunction with heat in order to kill resistant spores. HPP is generally a batch process where the capacities of the pressure vessels used for commercial food production consist of 35-350 L. When liquids such as fruit juices are treated, the vessel is filled with the liquid, the latter acting as the pressure transmission fluid. After the treatment, the liquid can be transferred to an aseptic filling line. A series of these vessels can work in a staggered sequence for an overall semi-continuous system. Many HP system developers couple a number of pressure systems so that most of the energy stored in a pressurized vessel can be successively used to pressurized a second vessel, thus saving energy and process time (Van den Berg et al., 2001).

Since foods decrease in volume when a pressure is applied, HPP requires airtight packages that can withstand a change in volume due to the compressibility of the product (Hugas et al., 2002). For this reason, the packaging used for HPP has to be characterized by a certain degree of flexibility, since it must resist up to a 15% reduction in volume and return to its original volume without loss of seal integrity or barrier properties. Moreover, vacuum-packed products are ideally suited for HPP (Norton and Sun, 2008).

II.2. Experimental

II.2.1. Materials

PSG Bloom 180 (average molecular weight 1 x 10^5 Da) was used as the film matrix (Healan Ingredientes Ltd, York, UK). Glycerol (Cahill May Roberts Ltd, Dublin, Ireland) was used as plasticizer. A 0.1 M NaOH solution made up from NaOH pellets (Lab Pak Ltd., Filongley, UK) was used for the correction of the pH.

II.2.2. Preparation of films

The scheme of the preparation of films is shown in Fig. II.3.

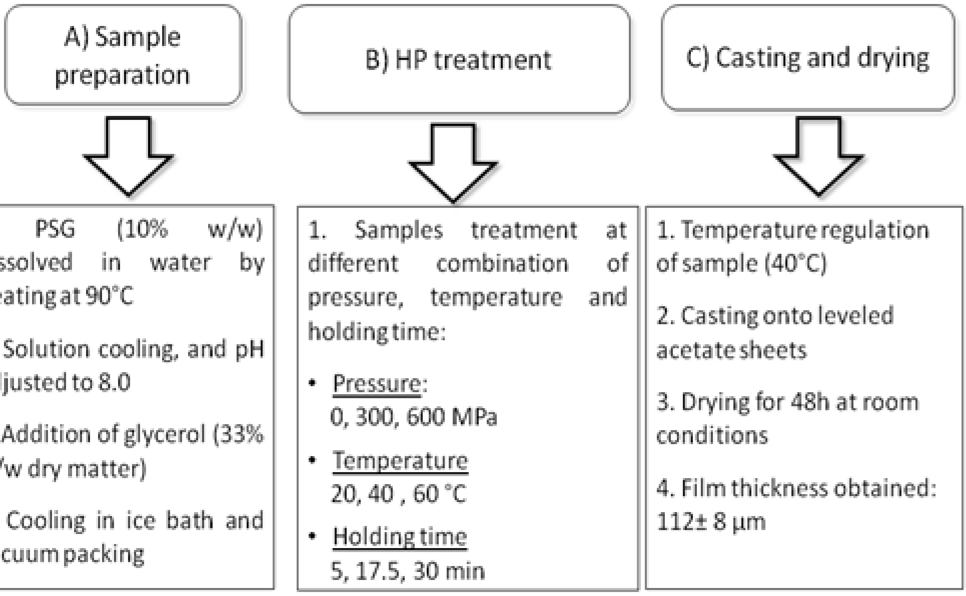


Figure II.3. *Steps of preparation of films.*

Dry PSG was dissolved in distilled water (10% w/w) by heating at 90°C in a water bath (SW23, Julabo USA INC., Allentown, PA, USA) for 30 min. Successively, the solution was cooled to 30°C and the pH adjusted to 8.0. After pH adjustment, glycerol was added under constant stirring at a concentration of 33% w/w dry matter. The solution was transferred into vacuum pack pouches, cooled in ice bath and vacuum packed. In accordance with the design of experiment detailed in section 2.5, samples were then treated at different combination of pressures, temperatures and holding time in a Stansted Fluid Power Iso-Lab 900 Power HP Food Processor (Stansted Fluid Power Ltd., Stansted, UK) and casted onto levelled acetate sheets using a Micron II film applicator (Gardco, FL, USA) after temperature regulation (40˚C).

II.2.3. Film thickness and conditioning

Film thickness was measured using a digital micrometer (Käfer Digital Thickness gauge, Käfer Messuhrenfabrik GmbH & Co., Villingen-Schwenningen, Germany) with an accuracy of 0.001 mm. Before measurement, films were preconditioned for at least 48 hours in an environmental chamber set at 23°C and 50% relative humidity.

II.2.4. OTR

The OTR determination was carried out as described in section I.A.1.3.8.

II.2.5. DoE and statistical analysis

The experiments were carried out according to a Box-Behnken design (BBD) . This statistical experimental design is an independent, rotable quadratic design where variable combinations are at the midpoints of the edges of the variable space and at the center (Prakash Maran et al., 2013). In the present study, the effects of independent variables such as pressure (0, 300 and 600 MPa), temperature (20, 40 and 60°C), and holding time (5, 17.5 and 30 min) on the response (OTR) were investigated. The experimental design consisted of 15 runs with three center points for the estimation of the pure error and was replicated three time (three blocks) (Table 1**).**

A second-order response surface was fitted to the experimental data. Optimal factor settings where then derived from the fitted surface.

All the analyses were done using Statistica 64 version 10 (StatSoft Inc, Tulsa, OK, USA). .

II.3. Results and discussion

The thickness of the films was shown in Fig. II.3. The data generated by the software for the optmimization process and the OTR response values related to each run are reported in Table II.3.

Table II.3. *The Box-Behnken design for the optimization of OTR and experimental results (mean over the three blocks).*

Runs	Pressure (MPa)	Temperature (°C)	Time (min)	Pressure P code	Temperature P code	Time P code	OTR (mL d^{-1} m^{-2})
1	0	20	17.50	-1	-1	0	2.90
2	300	40	17.50	0	0	0	3.60
3	600	40	30.00	1	0	1	2.65
4	300	60	5.00	0	1	-1	2.43
5	600	40	5.00	1	0	-1	3.05
6	300	60	30.00	0	1	1	3.76
7	300	40	17.50	0	0	0	3.64
8	600	60	17.50	1	1	0	3.91
9	0	40	5.00	-1	0	-1	2.74
10	0	40	30.00	-1	0	1	3.75
11	300	20	5.00	0	-1	-1	2.44
12	600	20	17.50	1	-1	0	2.63
13	0	60	17.50	-1	1	0	3.57
14	300	20	30.00	0	-1	1	2.57
15	300	40	17.50	0	0	0	3.14

The ANOVA table for the model is reported in Table II.4.

Table II.4. *ANOVA for the quadratic surface response. The R-squared value was 0.8059.*

Factor[3]	**SS**[1]	**DF**	**MS**[2]	***F*-Ratio**	***P*-Value**
A (L)	0.1908	1	0.1908	2.357	0.1343
A (Q)	0.0040	1	0.0040	0.0495	0.8252
B (L)	3.6504	1	3.6504	45.0903	0.0000
B (Q)	0.5740	1	0.5740	7.0901	0.0119
C (L)	1.6224	1	1.6224	20.0401	0.0001
C (Q)	2.0893	1	2.0893	25.8080	0.0000
A (L) · B (Q)	0.2730	1	0.2730	3.3722	0.0753
A (L) · C (L)	1.4770	1	1.4770	18.2442	0.0002
B (L) · C (L)	1.0860	1	1.0860	13.4146	0.0009
Blocks	0.2309	2	0.1154	1.4258	0.2547
Error	2.6716	33	0.0810		
Total	13.7611	44			

[1] *Sum of Squares*
[2] *Mean Square*
[3] *A=pressure; B=temperature; C=time; L=linear; Q=quadratic*

The *R*-squared value was 0.8059, indicating a good fit of the model. Temperature and Time had significant linear and quadratic effects, while pressure played a significant role interacting with time. Also the interaction between Temperature and Time was significant.

The fitted equation of the second-order response surface was:

$$\text{OTR (mL m}^{-2}\text{ d}^{-1}\text{)} = 1.32 + 0.00021 * \text{Pressure} + 0.036 * \text{Temperature} + 0.098 * \text{Time} + 2.1\text{E-}7 * \text{Pressure}^2 + 0.000025 * \text{Pressure} * \text{Temperature} - 0.000094 * \text{Pressure} * \text{Time} - 0.00057 * \text{Temperature}^2 + 0.0012 * \text{Temperature} * \text{Time} - 0.0028 * \text{Time}^2 \qquad \text{(Eq. II.2)}$$

where the values of the variables are specified in their original units. The model revealed that the quadratic Time and Temperature along with the interaction between Pressure and Time had a negative effect on the response variable.

The standardized effect of the three factors and their interactions on the optimization of OTR are shown in the Pareto chart of standardized effects (Fig. II.4).

This chart is characterized by bars related to each effect and possessing a different length in function of the decreasing order of significance. Each bar corresponds to a *t*-value, which is obtained by the absolute value of the estimated effects divided by the standard error (Zhu et al, 2012). The critical *t*-value which determines which effects are statistically significant, is represented by the vertical line. The effects characterized by a bar smaller than the critical *t*-value are not statistically significant and thus do not have a significant influence on the response variable. Bars beyond the vertical line are statistically significant at the selected significance level, which is set by defalut at 5%. In this case, OTR was affected by six significant main effects: Temperature (L and Q), Time (L and Q) and by the interactions Pressure (L)/Time (L) and Temperature (L)/Time (L). In particular, Temperature (L) was the effect with the major influence on OTR. Conversely, while pressure (L and Q) alone did not affect the response variable, its interaction with time affected significantly OTR.

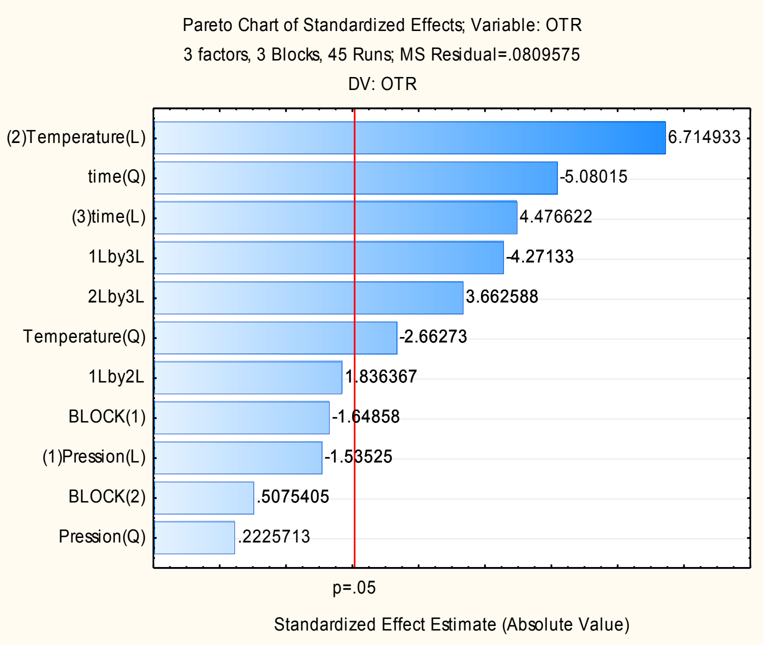


Figure. II.4. *Pareto chart of standardized effects on OTR. The vertical line is the critical value from the t distribution (5% significance level) The standardized effect of each term is reported alongside each bar.*

The influence of the main effects and their interections on OTR are displayed in the plots of Fig. II.5.

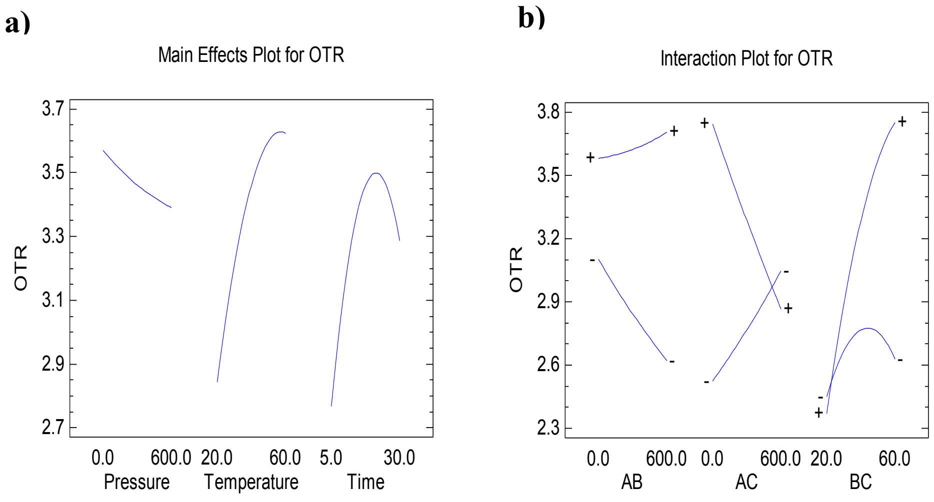


Figure. II.5. *Main effect plot (a) and interaction plot of the effects (b). The plots show average values of OTR corresponding to the different levels of the three factors (-=low, += high).*

The patterns shown in Fig. II.5a, represented the estimated variation in the response variable when each factor increases from its low level to its high level, maintaining the other effects constant. As it can be observed, temperature and time had the greatest impact on the modification of response, since a slight variation in these effects corresponded to a significant change in OTR. In particular, it is worth to mention the influence of Time, determining for low levels an increase and for high levels a decrease in OTR.

The intection plot (Fig. II.5b) indicates the interactions between the effects, with Pressure/Time and Temperature/Time being the significant interactions ($P < 0.05$) observed. In accordance with Hinkelmann and Kempthorne (2008), an *antidirectional interaction* between pressure and time was observed. Results indicate that at short time an increase of Pressure causes an increase in OTR, while the opposite happens for longer times.

This is an important finding, because it implies that the application of high pressure levels for a long time on the gelled solution can result in a film with improved barrier properties and thus an enhanced performance.

The optimal combination of the levels of the three factor is reported in Table II.5.

Table II.5. *Optimum values obtained by the combination of the effects pressure, temperature and time.*

Effect	**Pressure** (MPa)	**Temperature** (˚C)	**Time** (min)
Low	0.00	20.00	5.00
High	600.00	60.00	30.00
Optimum	600.00	20.00	30.00

The minimum predicted value was 1.83 mL m^{-2} d^{-1} and, according to the model, the best combination of the three factors involved the use of high level of HP (600 MPa) for 30 min, by holding the temperature at its low.

The response fitted surfaces are graphically represented in two dimensional contour plots (Fig. II.6), depicting the variation in the parameter modeled as the two effects move along the levels, holding constant the third factor at the optimum point predicted. In particular, in the diagram II.6a Time is kept constant (30 min) and variation of OTR is represented with respect to Temperature and Pressure. The plot clearly shows the absence of interaction between the two factors, because the isocurves are approximately parallel. The minimum is located in the lower right corner, characterized by high Pressure and low Temperature values. According to Fig. II.6b, even lower OTR values could be obtained by increasing the pressure above 600 MPa.

However, this part of the plot was outside the ranges of the effects studied and the applicability of the extimated model in this area need to be investigated. In line of principle, an increase of Pressure and Time and a reduction of Temperature could further reduce the value of OTR. This means that the OTR minimum predicted value is a global minimum, but not a critical point.

In theory, the decrease in OTR observed when HP is applied on PSG-based solutions for long time at room temperature may be explained by a denser polymeric matrix that affects the OTR by hindering the diffusion of oxygen molecules. In literature, according to Kulisiewicz et al. (2007), the application of HP stabilizes the helix junctions of the gelatin by promoting the formation of hydrogen bonds. In fact, it is well known that HP treatments can promote their formation due to their negative activation volume (Heremans & Smeller, 1998). Moreover, gels cured under elevated pressure possess more triple helix junction zones per molecular chain compared to the gels cured at normal conditions.

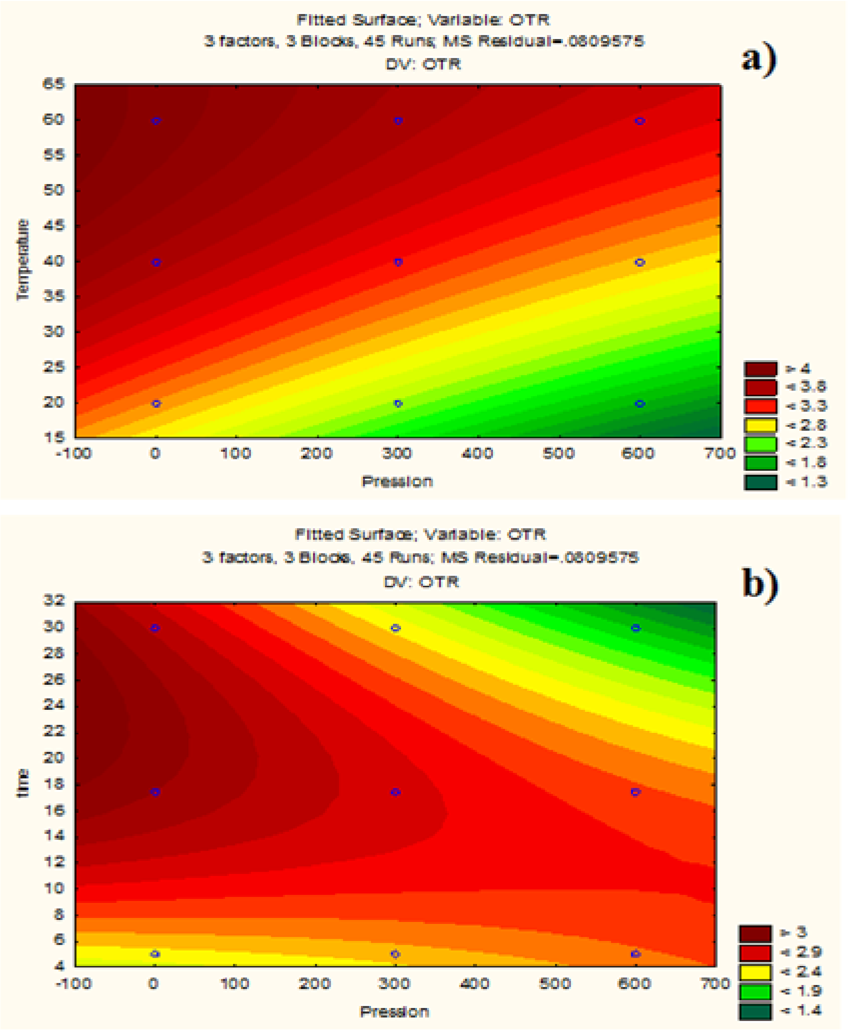


Figure II.6. *Minimization of the response variable through the best combination of the effects. The fitted surface response diagrams show the variation in OTR as the two effects move along the levels, holding constant the third factor at the optimum point. Pressure/Temperature at time = 30 min (a), Pressure/Time at temperature = 20 °C (b) are displayed.*

Longer time of HP treatment may favour this rection as deduced from the results obtained in this study, in which the longer the application of HP the lower the OTR values. According to Ku Kulisiewicz et al. (2009), it can be hypothesised that the triple helices formed under high pressure treatment possess a lower average length. However, a further investigation needs to be done on the physico-chemical characterizations of the PSG-based films produced in the current study. Nevertheless, further investigations are necessary to explain the decrease of the OTR by applying HP to the PSG solution.

II.4. Conclusions

The Box-Behnken design was successfully used in this study for the optimization of the response variable in function of pressure, temperature and time, with a coefficient of determination values (R-squared) of 80.59%. The results revealed that OTR was significantly affected from a statistically point of view by the three considered factors. Time and Temperature effects are relevant and sinergic, while Pressure plays a role only in raltion to Time: the application of high pressures for a long time seems to enhance in film-forming solutions, is a significant effect able to decrease the permeability of the resulting films and may be useful for the industrial commercial production of bio-sourced food packaging films such as edible films.

Further investigations are needed in order to find the optimal combination of the three factors, given that the study was not able to identify a critical point and only indicates that the minimum value of OTR is obtained on the boundary of the experimental space.

The good barrier properties to oxygen make PSG suitable to be used as a coating in a multi-layer structure. A possible combination with another material from renewable resources, possessing good sealing properties such as PLA, may be investigated in order to produce a multi-layer flexible film with improved barrier to oxygen performance.

PART III

REALIZATION OF BI-LAYER FILMS WITH IMPROVED BARRIER PROPERTIES

III.1. Introduction

The use of polymer-based structures for food packaging applications have been increasing over the last decades. Among the factors that contributed to the diffusion of these materials, the commercial appeal has been playing an important role, due to their ability to offer a wide range of tailor-made properties (Pilla, 2011). Moreover, polymer-based materials are low cost, production-friendly and can be converted into the required shape and size.

Several foodstuffs require appropriate atmospheric conditions to preserve their quality during storage. To date, there is an increasing number of foods which are being packed in protective atmosphere by using a selected mixture of gases, in order to ensure an optimum quality along with the consumer safety. For this reasons, food packages need to be selected for their barrier properties in fuction of the food application and the gas composition inside the package (Weber et al., 2000). The gas mixture used for MAP (Modified Atmosphere Packaging) are based mainly on a combination of oxygen, carbon dioxide and nitrogen (Pilla, 2011 p.133).

The conventional approach to manufacture food packaging flexible films in MAP application possessing high-barrier properties is through the realization of multi-layered films, based on a combination of different suitable materials to meet specific industry requirements. For oil-based polymers for instance, a often used multi-layer film in food packaging applications consists of external layers of materials with high water vapour barrier such as LDPE (low density polyethylene), combined with an inner layer with high gas barrier properties such as EVOH (Ethylene Vinyl Alcohol) (Weber et al, 2000).

A similar approach can be also used for bio-based materials to design materials with the required properties, since a number of biomaterials possessing appropriate oxygen and water vapour barrier are already available. However, while the oxygen permeability of biobased materials is quite similar to the large range of oil-based materials (Pilla, 2011), a major challenge is represented by barrier to water vapour (Weber at al, 2000). This can be explained by the hydrophilic nature of the majority of the bio-based polymers, which make them very sensitive to the moisture. For this reason, only very few bio-based materials can be used when a high water vapour material is required.

The ability of a packaging to maintain a specific gas composition is also given by the sealing properties of the materials used. The time of sealing depends on the characteristics of the flexible material and are strictly related to the sealing technique used (Piergiovanni and Limbo, 2010). The sealing techniques are classified into heat sealing and cold sealing. Heat sealing is a process characterized by the application of heat and pressure in order to melt two layers of heat

sealants and press together for a proper period of time to form a heat seal. Cold sealing is the seal betwen plastic interfaces attained without heat application (Lee et al, 2008). It is used when heat sealing is impossible or damages the packaging material and/or the food product.
The design of a multi-layer food packaging involves also the knowledge of the surface tension of the different polymers constituting the film. The characterization of the surface properties and in particular the surface tension components of the solids, are a very important factors in understanding the compatibilibility between materials, as adhesion between two polymers is related to interfacial energy (Hollander, 1995).

III.1.1 Industrial production technology of multi-layer materials

The industrial production of multi-layer food packaging flexible films involves the use of the following technology (Lee et al, 2008):

- *Coextrusion:* this technology is used for the realization of multi-layer structures for application where there is a favorable benefit/cost ratio and it is limited to combining polymers only. It involves the use of two or more extruders, in which the multi-layer structure is obtained through the merge and welding together in a feed block of the extrudates originated by the different extruders (Fig. III.1). Generally, the adhesion between different polymer layers can be improved by using a thin layer of adhesive resin such as maleic anhydride modified PE (polyethylene), PP (polypropylene), and EVA (ethylene vinyl acetate).

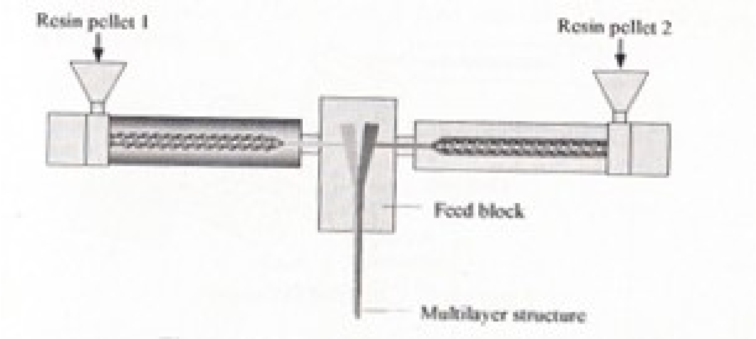


Figure III.1. *Image of the coextrusion process (Lee at al., 2008).*

- *Extrusion coating*: this technique involves the extrusion of a thin layer of molten plastic onto a large variety of substrates such as plastics, paper and metal foil. Firstly, the extrudate is drawn to the desired thickness in the melt state and coated ón the substrate. Successively, the polymer is then cooled on a set of chill rolls (Fig. III.2).

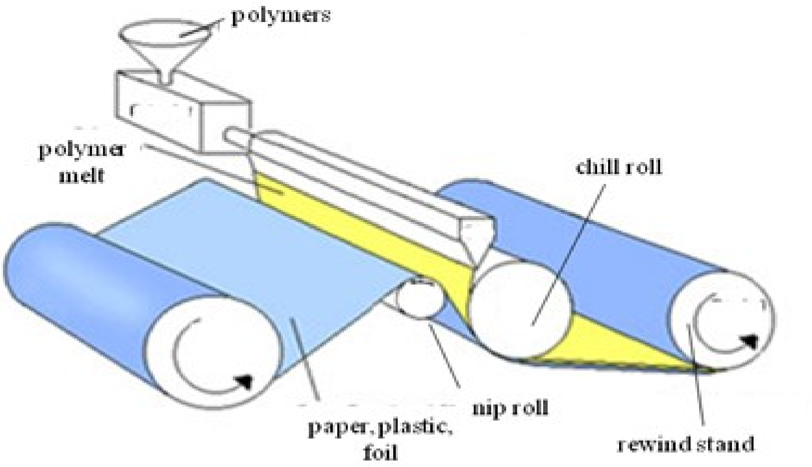


Figure III.2. *Extrusion coating process.*

In this technique, a good adhesion between the coating polymer and the substrate is required. Applications of such technology are coated paper cartion for milk and juices, coated aluminium foil and plastic structures which cannot be coextruded.

- *Extrusion lamination*: it involves the combination of two different substrates with a molten polymer, in which a large variety of materials possessing different thicknesses can be processed (Fig. III.3).

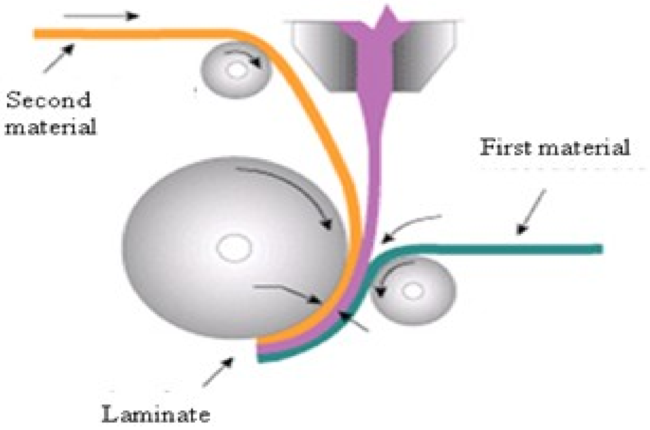


Figure III.3. Extrusion lamination.

Extrusion lamination is used for the processing of paper board, aluminium foil, OPP film, oriented PET film and metallized film. It is common in food packaging applications that require high barrier to moisture and gas, alomg with excellent print quality.

- *Adhesive lamination*: this process is similar to the extrusion lamination. However, unlike the latter technique where polymer melts are used, liquid adhesives are involved in the adhesive lamination. Liquid adhesives are coated onto a substrate, dried with heat and airflow, and successively laminated onto a second substrate through a heated compression nip (*dry bond lamination*) (Fig. III.4).

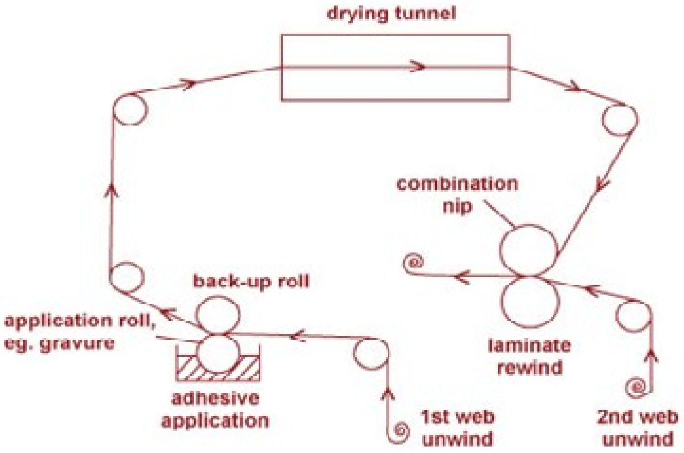


Figure III.4. *Adhesive lamination: dry lamination process.*

Adhesive lamination is useful for the realization of most flexible substrates.

Wet bond lamination differs from the dry bond lamination in the adhesive which is still wet. This occurs because webs are joined before the drying step.

- *Vacuum metallization*: a plastic substrate is coated with a very thin layer of metal (generally aluminium with a thickness ranging from 8 to 50 nm) under high vacuum conditions. This process is shown in (Fig. III.5).

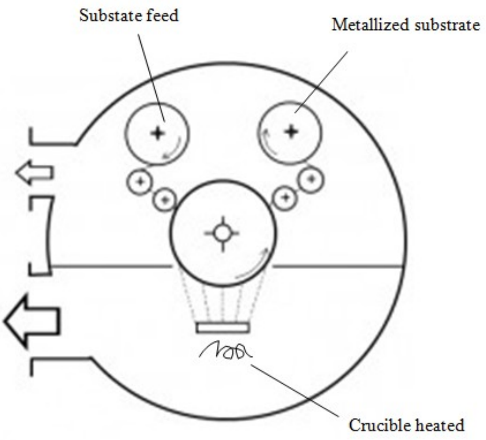


Figure III.5. *Vacuum metallization process.*

- Metallized plastic films are characterized by very a high barrier to gas, water vapour and light and are able to resist to flexing stress, thus preserving the barrier properties performance. The majority of metallized plastic films are based on PET (p*olyethylene terephthalate)*, PP and nylon.

Films manufactured using the techniques previously discussed rarely comply with the requirements needed for a food packaging material.

For this reason, their surface is often modified in order to improve the aesthetics and functionality of such films. In particular, surface modifications are able to enhance either the adhesion between the plastic materials of different layers, or the adhesion of inks on the substrates, coatings and adhesives used for the realization of multi-layer structures.

Among the most used techniques for the modification of the surface, the following can be mentioned (Piergiovanni and Limbo, 2010):

- *Corona discharge:* a partial degradation of the film is obtained through the decomposition of the ozone produced by the electrical discharges that ionize the air. This phenomenon is induced by the high difference of potential (3000 – 9000 V) applied between the electrode and the film.
- *Flame treatment*: The surface of the packaging material is modifiede through a brief exposure to a burner which flames onto the web guided by a cooling roll.
- *Plasma treatment*: in this case, not air but selected gases are ionized with electromagnetic waves to generate a plasma. This technique allow to modify the film surface under more controlled conditions.
- *Chemical treatment (priming)*: A very thin polymeric coating possessing polar properties is applied in order to imporve the adhesion.

Some examples of multi-layer structures are constituted by:

- PVDC *(*polyvinylidene chloride)/adhesive/nylon
- PP/adhesive/EVOH/adhesive/PP
- PP/adhesive/EVOH/adhesive/regrind PP/PP
- PA (polyamide)/adhesive/LDPE

- PET/adhesive/PP
- PP/adhesive/EVOH/adhesive/HDPE (high dendity polyethylene)

III.1.2. Production techniques of bi-layer materials for research purposes

Foulk and Bunn (2001) studied the physical and barrier properties of bi-layer protein films. They produced bi-layer protein films by three different preparation methods and compared the barrier and physical properties of such films to single layer films of the same base materials.

According to this study, bi-layer films were obtained by the following techniques:

- *liquid spread on dry film*: this method involved the formation of one protein film and, once dried, another layer was formed by pouring, spraying, or rolling, directly on top of the previously dried layer. The combined film was then allowed to dry. The pouring of the second layer was carried out according to Brandenburg et al. (1993) or Park et al. (1994). An electric paint sprayer moving at a constant speed while dispersing uniform amounts of solution was used for spraying a second layer onto the first one. The spraying technique allowed to form a very thin second layer, which would dry very quickly. However, it could not be used with viscous solution. Rolling was another option used for the realization of a second layer. Since it produced undesiderable air bubbles in certain materials, this technique needed to be used with care.
- *heat pressing dry films*: this technique involved the formation of two monolayer films individually and successively laminating these together using a solvent between them while applying a uniform pressure with the use of compression rolls. Films were coated on the side opposite to the surface in contact with the support (drying glass) during the single film formation.
- *solvent laminating dry fims*: it involved forming two monolayer films individually laminating them together using heat and pressure from a set of heated rolls. Films were coated as in the heat pressing dry films method, except no solvent was used.

However, these three methods used for film formation did not always produce films. Therefore, an appropriate technique has to be chosen in function of the materials used for the constitution of the bi-layer structure.

III.2. Materials and methods

III.2.1. Materials

The material used as a base layer was PLA U/C30B. This film showed the best barrier to oxygen as shown in Figs. I.A.11 and I.A.12.

The PSG-based material used as coating was prepared according to the regression equation (Eq. II.2). The factors were combined in order to obtain optimum values of films, possessing the lowest OTR (Table II.3).

III.2.2. Bi-layer film preparation

Two bi-layer films were prepared using film U/C30B as base material and using PSG-based materials as coatings (Fig. III.6).

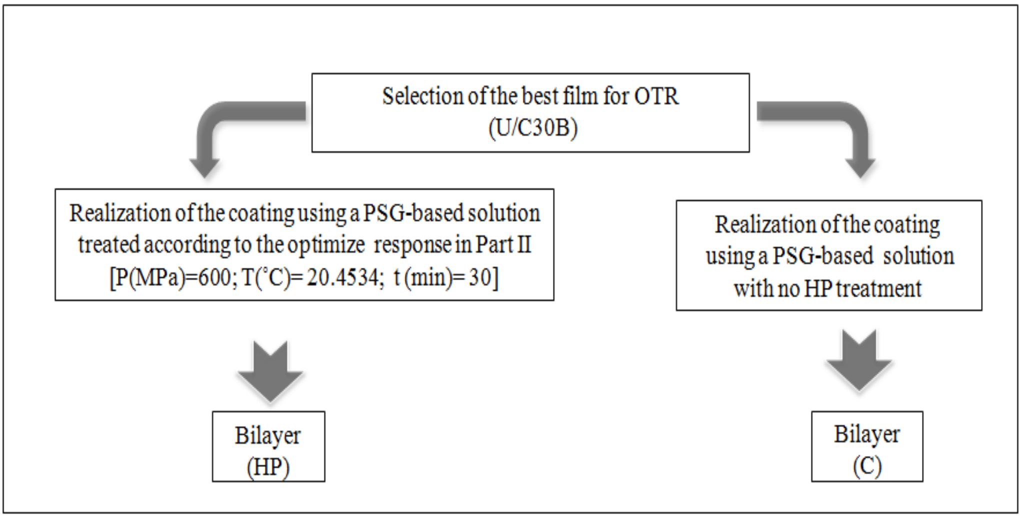


Figure III.6. *Preparation of the bi-layer using PSG-based material in accordance to the regression equation to minimize the OTR (Bilayer HP), and the bilayer control produced without high pressure (Bilayer C).*

- *<u>Bi-layer HP</u>*

  Dry PSG was dissolved in distilled water (10% w/w) by heating at 90°C in a water bath (SW23, Julabo USA INC., Allentown, PA, USA) for 30 min. Successively, the solution was cooled to 30°C and the pH adjusted to 8.0. After pH adjustment, glycerol was added under constant stirring at a concentration of 33% w/w dry matter. The solution was transferred into vacuum pack pouches, cooled in ice bath and vacuum packed and treated at the optimum combination of pressure (600 MPa), temperature (20 min) and holding time (30 min) in a Stansted Fluid Power Iso-Lab 900 Power HP Food Processor (Stansted Fluid Power Ltd., Stansted, UK). Successively, the solution was applied with a paint brush onto nanocomposite U/C30B film after temperature regulation (40˚C) and dried for 48 h in ambient conditions.

- *Bi-layer C*

  The control bilayer was produced following the steps as for bilayer HP but not treated with high pressure. Once the solution was transferred into vacuum pack pouches, cooled in ice bath and vacuum packed, the temperature was regulated (40˚C). Successively, it was applied with a paint brush onto nanocomposite U/C30B film and dried for 48 h in ambient conditions.

The technique used for the application of the coating (paint brush) was selected between the rolling and spraying, after pre-tests on the efficacy of the method used were carried out. The rolling of a coating onto the base layer was not successful due to the formation of many air bubbles which could affect the bi-layer performance. The spraying and the spreading with a Bird-type applicator of the PSG-based solution was not feasible due to the dishomogeinity of the solution which affected the efficacy of this technique. These drawbacks were already observed by Foulk and Bunn (2001) and reported in section III.1.2.

III.2.3. Efficacy evaluation of the coating technique

The efficacy of the coating application was previously visually evaluated by adding few drops of a Comassie Blue solution (1g/L) (R-250, BDH Biochemical Ltd, UK). The Commasie Blue solution was added under constant stirring before the application of the coating at 40˚C.
The coating solution was dried for 48 h in ambient conditions and successively the homogeneity of distribution and the potential presence of air bubbles evaluated.

III.2.4. Film thickness and conditioning

Film thickness was measured using a digital micrometer (Käfer Digital Thickness gauge, Käfer Messuhrenfabrik GmbH & Co., Villingen-Schwenningen, Germany) with an accuracy of 0.001

mm. Before measurement, films were preconditioned for at least 48 hours in an environmental chamber set at 23°C and 50% relative humidity.

III.2.5. Barrier properties

OTR was measured as previously described in section I.A.1.3.8. The O_2 content inside a known test cell volume chamber, flushed previously with nitrogen, was monitored non-invasively over a 24 h period with O_2 concentration recorded at 2 h intervals using an optical measuring system Optech^{TM} Platinum O_2 sensor device and disposable O_2 sensor stickers from Mocon (Minneapolis, USA). The cups were stored under controlled temperature and humidity as described in section 2.3 and a constant air velocity of 152 m/min maintained over the cups to ensure uniform air movement across cells. OTR was successively calculated considering the oxygen concentration, the volume of the test up chamber and the area of the film exposed for gas permeation using the equation (Eq. I.A.5).

WVTR of films was calculated according to the ASTM E-96 standard method as outlined by Wang et al. (2009) and described in section I.A.3.7. Distilled water (6 ml) was pippeted in each Perspex^{TM} circular test cup and each bilayer sample was mounted across the cup opening using vacuum silicone grease, with nanocomposite PLA side placed downwards to avoid the PSG-based coating to be damaged due to its high sensitivity to water. The film was secured in place and sealed into test cups with the four screws symmetrically located around the cup circumference. A constant air velocity of 152 m/min was maintained over the cups to ensure uniform movement of air across the WVP test cells. The weight loss of the test cells were monitored over a 24 h period with weight loss recorded every 2 h intervals. WVTR was determined from the slope obtained by the regression analysis of the data of weight loss as a function of time, once the steady state was reached. Both OTR and WVTR results were based on three replicates.

III.3. Results and discussion

III.3.1. Efficacy evaluation of the coating technique

The efficacy of the selected coating techniques is shown in Fig. III.7.

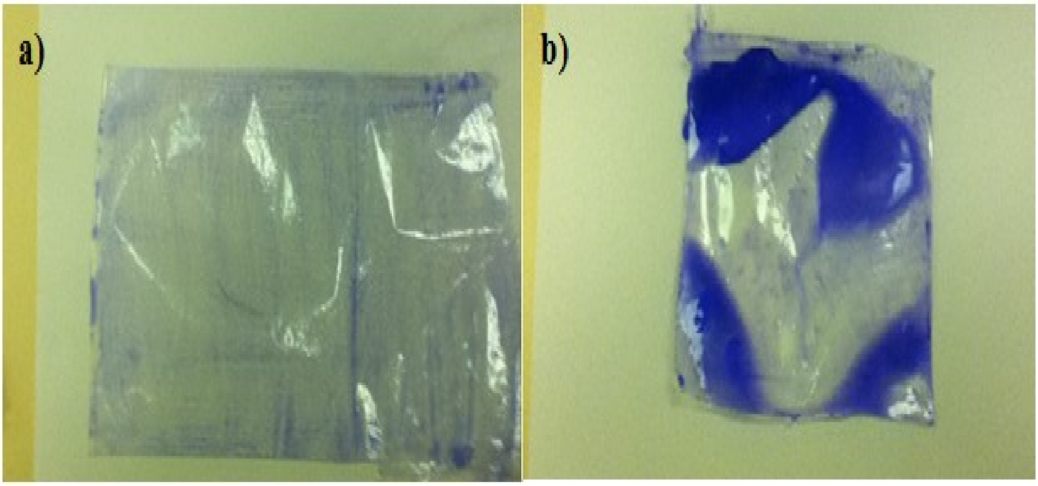


Figure III.7. *Efficacy of the different techniques of the coating application used: paint brush (a) and spraying (b).*

As it can be observed from the images, PSG-based gelatin solution was not evenly spread onto the surface of PLA U/C30B (Fig. III.9.b). This is explained by the waved surface that characterizes the base layer, thus forming aggregates of the coating solution in correspondance of valleys. The same behaviour was observed with the spreading of the solution through the use of a Bird-type applicator. On the contrary, when the coating solution was paint brushed onto the PLA-based surface, a more homogeneous spreading was observed, which was able to cover evenly the surface of the base layer. According to this visual evaluation, paint brushing the coating onto the surface pf the PLA-based surface was the best technique to form a bi-layer with improved barrier properties.

III.3.2. Appearance

The application of a PSG-based coating onto a base layer constituted by PLA containing a small amount of Cloisite® 30B, produced bio-based flexible free-standing films (Fig. III.8). The PLA-based layer was characterized by an off-white appearance, while the PSG-based coating by a yellowish an smooth surface.

The thickness measured for the bi-layer HP and bi-layer C was 117±5.4 and 117±7.9 respectively. The thickness of the PLA layer was 62.4±1.0 as reported in Fig. I.A.6.

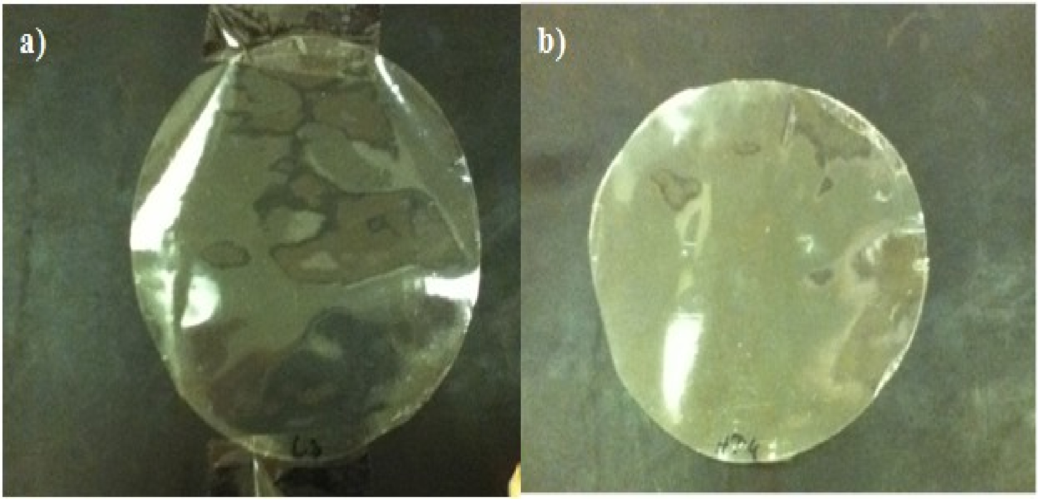


Figure III.8. *Images of bi-layer C (a) and bi-layer HP(b).*

III.3.3. OTR

The difference in the treatment of the PSG-based coating applied onto the surface of the base layer affected the OTR of the bi-layers (Fig. III.9).

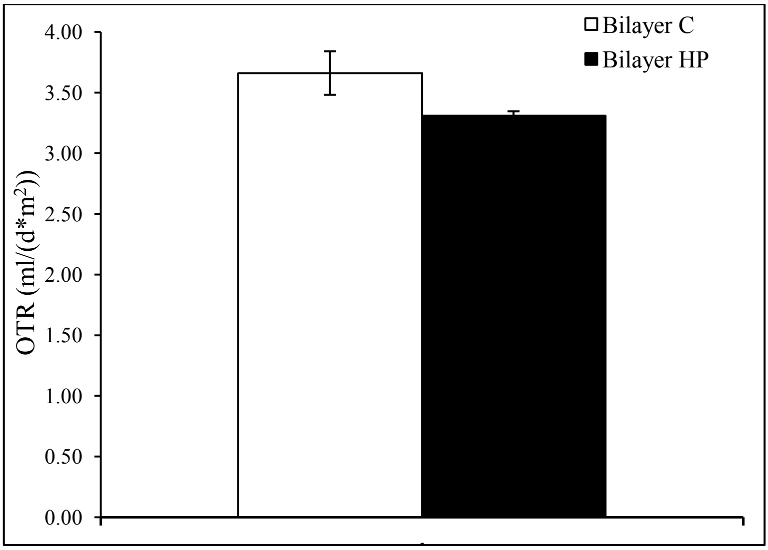


Figure III.9. *Effect of the different coating on the OTR of bilayers C and HP.*

The low values in OTR obtained for both bi-layers confirmed that the coating was evenly spread. The bi-layer HP showed OTR values slightly lower than bi-layer C (-9.59 %), indicating that processing the PSG-based solution according to the optimum settings had an effect also when it was applied onto the surface of a different material such as PLA. This behaviour was also observed in the study carrried out by Hong and Krochta (2006). However, they did not discuss the ralation between the adhesion on different materials and the variation in oxygen-barrier properties. Benmalek and Dunlop (1995) observed in their study that that barrier properties were related to the coating thickness, the roughness of the surface, and the contibution of polar and hydrogen bond to adhesion.

The values in permeability obtained for the bi-layers (19 and 21 ml*µm/m^{-2}*d^{-1}*kPa^{-1}) for bi-layer HP and C respectively) are similar to those (12-40 ml*µm/m^{-2}*d^{-1}*kPa^{-1}) previously reported for pig skin gelatin (Wang et al, 2009), whey protein films (Maté and Krochta, 1996; Sothornvit and Krochta, 2000) and PET films (Salame, 1986). According to Almenar and Auras (2010), PET is a high oxygen barrier polymer. An oxygen-barrier polymer is defined as a polymer having an OP values lower than 38.9 ml*µm/ m^{-2}*d^{-1}*kPa^{-1} at 23°C (Salame, 1986).

Hong and Krochta (2005) studied the oxygen permeability of a bilayer constituted by a base layer made of PE or PP coated with two types of coating solutions, WPI (whey protein isolate) and WPC (whey protein concentrate). However, Corona discharge as a surface pretreatment was applied in order to enhance to adhesion to the whey protein coatings, due to the notable difference in the degree of hydrophilicity. They found that the coating of PE and PP films reduced more then fourfold or one-order the oxygen permeability of the base materials. Similarly, the slight difference in the nature of the coating affected the barrier to oxygen.

The increase on oxygen-barrier properties on the bi-layer HP compared to the base material is shown in Table III.1. The decrease in OTR of the bilayer compared to PLA U/C30B was significant since it decrease from 43.36 to 3.31 mL/d^{-1}*m^{-2}. This suggested that the PSG-based coating can be considered as a potential material for multi-layer bio-based food packaging films, designed to improve the oxygen barrier properties of PLA films.

Table III.1. *OTR of the bi-layer HP in relation to the base material and the coating measured separately.*

Film	Bi-layer HP	PLA U/C30B	Optimized PSG
OTR (mL/(d*m^2))	3.31	43.36	1.83

The relative humidity (RH) affects the oxygen-barrier properties of hydrophilic materials such as the pig skin gelatin. In, fact an exponential increase of oxygen permeability in function of the increase of RH was previously observed for similar materials such as WPI (McHugh and Krochta, 1994), casein (Tomasula et al, 2003) and egg white protein (Lim et al, 1998). As a consequence, the pig skin gelatin has to be kept at low-intermadiate moisture content to mantain the OTR values observed in this study, and thus to have appropriate oxygen-barrier properties in food packaging application at high RH (Hong and Krochta, 2006). This purpose can be achieved if the inner layer, which is sensitive to moisture, is inserted in layers of materials possessing good moisture-barrier properties (Osborn and Jenkins, 1992; Hernandez et al, 2000). A practical commercial example is constituted by EVOH, which is generally sandwiched in coextrusion or lamination with polymers with low permeability to water vapour. A similar approach can be used for pig skin gelatin films.

III.3.4. WVTR

The WVTR results of the two types of bi-layers are shown in Fig. III.10.

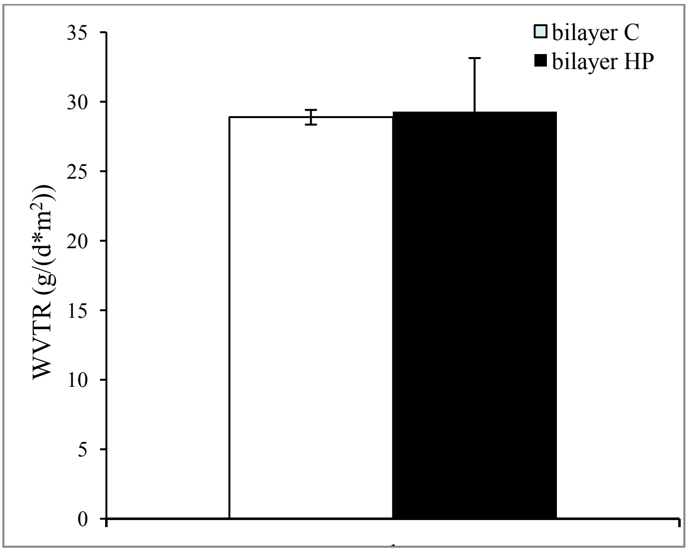


Figure III.10. *Effect of the different coating on the WVTR of bilayers C and HP.*

The WVTR values was very similar for both materials, indicating that the treatment at optimum conditions of the coating solution did not affect the barrier to moisture.

Rhim et al. (2007) studied PLA-coated SPI (soy protein isolate) films in order to evaluate the improvements on mechanical and water vapour properties of the resulting films. The coating

(PLA) was applied by dipping the base layer (SPI) in the coating solution and subsequntly allowed to dry. The resulting films possessed a barrier to water vapour similar to that of PLA films, improving the water vapour barrier of the SPI by 20- to 60-fold. The higher the PLA concentration, the closer the values to those of PLA.

The increase in water vapour barrier properties of SPI films coated with PLA was attributed to the hydrophobicity of PLA. They concluded that this material may be used for applications in packaging for foods with high water activity or to be used under high RH conditions.

The WVTR results of the bilayer HP, compared to those of the base layer and the PSG treated at the optimum conditions is shown in Table III.2.

Table III.1. *WVTR of the bi-layer HP in relation to the base material and the coating measured separately.*

Film	**Bi-layer HP**	**PLA U/C30B**	**Optimized PSG**
WVTR (g/(d*m^2))	29.98	30.15	63.47

As it can be observed, the WVTR of the bi-layer HP was very similar to that of the nanocomposite used as base material and less than 50% compared to that of the optimized gelatin, in agreement with the results of Rim et al. (2007) and confirming the potential applications in the food packaging field.

III.4. Conclusions

The application of a high oxygen barrier coating, made of a renewable resource such as the pig skin gelatin, onto the surface of a nacomposite PLA-based film produced a dramatic decrease in the oxygen-barrier of the PLA at the conditions used for testing. The final WVTR was very similar to that of the base layer, thus increasing by more than 50% the water vapour barrier to the PSG-based film. The technique used for the application of such coating affected significantly the success of the experimentation. The high-pressure treatment of the coating solution before the application affected the oxygen barrier properties, by decreasing the OTR of the bilayer by 9.59% compared to the bilayer control. The results obtained in the present study suggested that the bi-layer could be used for applications in packaging for foods with high water activity or to be used under high RH conditions.

PART IV

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

IV.1.General conclusions

- The incorporation of the nanoclays in the PLA matrix produced an intercalated structure in all nanocomposites, independently from the nature of the clay organic modifier, the L-isomer content of the polymer and the production technique used (extrusion and solvent casting).
- Films obtained by extrusion: the barrier properties were significantly affected by either the incorporation of nanoclays or the PLA optical purity.
- Films obtained by solvent casting: the barrier properties to water vapour were inferior than those of the extruded films, probably due to the residual solvent that remains in the samples, thus acting as a plasticizer.
- The high-pressure treatment on gel solution showed increasing oxygen barrier properties in function of time in the resulting films.
- The realization of a bilayer between PLA and PSG is feasible and has good barrier properties at the test conditions used for the measurements.

IV.2. Future perspectives

- An investigation using TEM (Transmission Electron Microscopy) may be useful in understanding the real degree of intercatation of the nanoclays in the PLA matrix and their dispersion.

- Further investigations are necessary to explain the decrease of the OTR by applying high-pressure to the PSG solution. For instance, the effects on the rheology of the solution and the structural, thermal and mechanical properties can be considered in future studies.

- Realization of multilayer films structure by co-extrusion, in order to obtain a nanocomposite PLA/PSG/nanocomposite PLA structure.

- Application on real foodstuffs stored at room temperature and sensitive to oxygen. The results obtained in the present study suggested that the bilayer could be used for applications in packaging for foods with high water activity or to be used under high RH conditions.

- Further investigations are necessary in order to evaluate the safety of the materials used.

PART V

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Glossary

DCM	Dichloromethane
DoE	Design of Experiment
DSC	Differential Scanning Calorimetry
E	Elongation at break
FTIR	Fourier Transform Infrared Spectroscopy
HP	High Pressure
HPP	High Pressure Processing
HSD	Honestly Significant Difference
LLDPE	Linear Low-Density Polyethylene
OMMT	Organically Modified Montmorillonites
OTR	Oxygen Transmission Rate
PET	Polyethylene Terephthalate
PLA	Poly(lactic acid)
PS	Puncture Strength
PSG	Pig Skin Gelatin
ROP	Ring Opening Polymerization
SE^{D}	Dispersive component
SE^{P}	Polar component
SE^{TOT}	Total Energy Surface
SEM	Scanning Electron Microscope
T_{cc}	Cold crystallization
T_{g}	Glass transition
TGA	Thermogravimetric Analysis
T_{m}	Melting
TS	Tensile Strength
UV	Ultraviolet
WVTR	Water Vapour Transmission Rate
X_{c}	Degree of crystallization
XRD	X-ray Diffraction
YM	Young’s Modulus
θ	Contact angle

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APPENDIX

PAPERS AND CONFERENCES’ ABSTRACTS

Journal of Food Engineering – accepted paper

Effect of nanoclay-type and PLA optical purity on the characteristics of PLA-based nanocomposite films

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KEYWORDS: poly(lactic) acid; montmorillonite; nanocomposites; renewable resources; packaging; structural properties.

Abstract

Nanocomposite films were produced by blending semicrystalline PLA with different types of OMMTs using extrusion. In order to study their effect on optical, structural and thermal properties, they were added at a fixed nominal concentration of 5 % w/w and compared with pure PLA films. The XRD measurements showed that the incorporation of small amounts of nanoclays resulted in a formation of nanocomposites, with the C30B demostrating the greatest compatibility with PLA. An increase in UV barrier properties was observed in nanocomposites, with Closite® C20A showing the best performance. The content of L-isomer in PLA significantly affected the thermal properties of the films, with a decrease in T_m of about 14°C and increase in T_c of about 20°C when the matrix, possessing a higher D-isomer content, was used. FTIR results showed a strong interaction between the nanoclays and the two different PLA matrices, due to the appearance of new peaks in nanocomposites films at around 520 and 627 cm^{-1}.

MATBIM 2012
2nd International meeting on Material/Bioproduct Interaction
22-25 April 2012, Dijon, France

EFFECTS OF NANOCLAYS TYPE AND POLYMER PURITY ON BARRIER AND THERMAL PROPERTIES OF BIO-SOURCED PACKAGING FILMS

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Introduction

Poly(lactic acid) (PLA) is a thermoplastic material with reasonably good optical, physical, mechanical, and barrier properties compared to existing petroleum-based polymers. It can be characterised by good processability, rigidity and clarity similar to polystyrene or polyethylene terephthalate, classified as GRAS (generally recognised as safe) and approved for use in all the applications pertaining to food, including the production of packaging materials (1-3). Over the years, many studies have been carried out as a means of improving the properties of PLA films. For example, orientation has been used to improve the mechanical properties of PLA. Similarly, new formulations and plasticizers, blending with other polymers, co-polymerisation, co-extrusion and lamination with more flexible and higher barrier polymers, coating, and structuration with fillers ranging from non-biodegradable to biodegradable materials have all been used to improve the properties of PLA-based packaging materials. Many studies have focused on PLA nanocomposites with organomodified layered montmorillonite (OMMT) for the benefits of the sinergism occurring between components used at specified dimensions (4), but only a relatively small number of them have considered a simultaneous effect of combining nanoclays and PLA of different degrees of purity. The objectives of this study were to investigate the combined effects of the addition of four types of OMMT possessing different chemical structures in two PLA matrices consisting of distinct contents of L-isomer, on the water vapour transmisison rate (WVTR), oxygen transmission rate (OTR) and thermal properties of the resulting manufactured films.

MATBIM 2012
2nd International meeting on Material/Bioproduct Interaction
22-25 April 2012, Dijon, France

EFFECTS OF NANOCLAYS TYPE AND POLYMER PURITY ON STRUCTURAL AND MECHANICAL PROPERTIES OF BIO-SOURCED PACKAGING FILMS

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Introduction

Nanocomposite polymer-phyllosilicate materials have many benefits. In particular they are characterized by better mechanical, barrier and thermal properties (1). Nevertheless, a blending of a polymer and nanoclay does not necessarily result in a nanocomposite material. When polymer and nanoclays are not compatible, the filler clay tends to agglomerate, causing a deterioration in the mechanical properties of the resulting films (2). However, when polymer chains penetrate between silicate layers, nanocomposite materials can be obtained (3).

The objectives of this research were to study the effects of four types of organically modified layered silicates (OMMT) in two PLA matrices consisting of distinct contents of L-isomer on the morphology and mechanical properties of the resulting composite materials.

BIOPOLPACK
2nd Congress on biodegradable polymer packaging
Milan (Italy), 10-11 May 2012

EFFECTS OF ORGANICALLY MODIFIED LAYERED SILICATES AND POLY(LACTIC ACID) PURITY ON STRUCTURAL AND MECHANICAL PROPERTIES

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The effects on structure and mechanical properties of the resulting nanocomposite materials manufactured by adding four types of organically modified layered silicates (OMMT), Cloisite® 10A (C10A), 20A (C20A), 30B (C30B) and 93A (C93A), in two poly(lactic acid) (PLA) matrices consisting of distinct contents of L-isomer (98.5 and 96% L-isomer lactide content, respectively) were investigated.

X-ray diffraction (XRD) results showed that the nanocomposites structure was affected by the nanoclay type and slightly influenced by the PLA L-isomer content. The addition of OMMTs to PLA matrices produced the shifting of diffraction maximum to a lower 2θ angle compared to control nanoclays. This suggested an interaction between polymer chains and nanoclays with a formation of nanocomposites characterized by intercalated structures and a partial preservation of OMMTs layered configuration. The nanocomposite obtained by blending C30B with 98.5% L-isomer content provided the best compatibility, due to the greatest shift to lower 2θ angles of the diffraction maximum and the best increase in the basal spacing between nanoclay layers (d001). Topographic images obtained with AFM showed an effect on nanocomposite films structure when OMMTs where incorporated in the matrix.

The addition of the OMMT type in the polymer and the different polymer purity affected the mechanical properties of manufactured films. In particular, a significant ($P < 0.05$) decrease in tensile strength (TS) and increase in elongation (E) was observed, with C93A providing the greatest E. The same behaviour was also observed when the lowest L-isomer content was used for the manufacturing of films. Overall, the blending of PLA with a small amount of OMMTs resulted in a formation of nanocomposites, where the positive interaction between nanoclays and PLA matrix improved the structure and mechanical properties of films with a general reduction in stiffness and increase in elasticity.

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Bio-based Food Packaging: Influence of Formulation and Processing on Functional Properties

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This PhD thesis research project aims to study and to set up a food bio-packaging PLA-based. In particular, the influence of formulation and process conditions on mechanical and physico-chemical properties of the film will be investigated. These properties will be compared with those of selected reference films for food products. In the second part of the project, the influence of the material on a food model system will be evaluated in comparison to the food packaging materials traditionally used.

Influenza della formulazione e delle condizioni di processo sulle proprietà funzionali di un imballaggio alimentare ottenuto da fonti rinnovabili

Il progetto di tesi di dottorato ha come obiettivo lo studio e la messa a punto di un biopackaging alimentare a base di PLA. In particolare, verrà approfondita l'influenza della formulazione e delle condizioni di processo sulle proprietà meccaniche e chimico-fisiche del film in oggetto, proprietà che saranno poste a confronto con film alimentari di riferimento. Nella seconda parte del progetto verrà valutata l'influenza del materiale su un sistema alimentare modello, rispetto ai materiali di imballaggio tradizionalmente usati.

1. State-of-the-Art

Over the last decades, plastics have been the most used material in the world, playing a central role in modern industrial economies (Carol *et al.*, 2004). Nevertheless, the growing reliance on oil-based polymers had raised several environmental and human health issues because of their persistence in the environment. Moreover, food packaging has recently been affected by significant changes in food distribution, due to the globalization of food supply and the growing consumer demand for better, more fresh and safer quality foods (Lopez-Rubio *et al.*, 2004).

This new approach has been a key factor in the development of alternative sustainable packagings, targeted to be "*beneficial, safe and healthy for individuals and communities throughout its life cycle*", at the same time meeting "*market criteria for both performances and cost*" and being "*sourced, manufactured, transported and recycled using renewable energy*" (www.sustainablepackaging.org). Examples of these packaging materials include bio-based polymers, bioplastic or biopolymer packaging products made from raw materials originating from agricultural or marine sources (Cha and Chinnan, 2004).

Generally, bio-based polymers or biopolymers are considered to be produced from renewable resources (Comstock *et al.*, 2004; Weber *et al.*, 2002; Petersen *et al.*, 1999).

Bio-based polymers are divided into three main categories depending on their origin and production: polymers directly extracted/removed from biomass, polymers produced by classical chemical synthesis using renewable bio-based monomers and polymers produced by microorganisms or genetically modified bacteria (Weber, 2000).

Recently, polymers like polyethylene, polypropylene, PVC or PET, but also high-performance polymers like polyamide or polyester have been totally or partially replaced by their renewable equivalents. Nevertheless, probably bio-based plastics will not be able to replace oil-based polymers in the coming years, because of low oil price, high production cost and restricted production capacity of biomass-based polymers that contains their technically possible growth in the near future.

According to Van Bailen and Poirier (2007), a recovery in the spread on the use of biopolymers in industrial and consumer product depends on economics, public acceptance and regulation. For instance, the recent strong increase in oil price has already allowed some biopolymers to be competitive with petrochemical plastics. However, public acceptance of the use of transgenic plants for non-food purposes is not assured, especially in the European Union (Gaskell *et al.*, 2006).

The most important biogenic sources of raw materials for industrial chemicals are oil plants (oil, fat, glycerol, celluloses), starch plants (starch, inulin, carbohydrates, celluloses), sugar beets and sugar cane (sucrose), wood (ligno-cellulose, cellulose), and waste and residues from agriculture and industry (biomass, fats, oils, whey, glycerol) (Wilke and Vorlop, 2004).

Polylactic acid (PLA) is a compostable polymer derived from renewable sources, in particular from starch and

Polylactic acid (PLA) is a compostable polymer derived from renewable sources, in particular from starch and sugar. During the last ten years, it has been used primarily for medical applications such as implant devices, tissue scaffolds and internal sutures, due to its high cost, low availability and limited molecular weight (Datta and Henry, 2006). Recently, since production cost has been lowered by new technologies and large-scale production, the use of PLA has been extended to other commodity areas such as packaging, textiles and composite materials (Drumright *et al.*, 2000; Garlotta, 2001). Because of its properties to be both derived from renewable resources and compostable, PLA has also been viewed as one of the solutions to reduce solid waste disposal problems (Lim *et al.*, 2008).

The building block of PLA, lactic acid (2-hydroxy propionic acid), can exist in optically active d- or l-enantiomers. Its good optical, physical, mechanical and barrier properties make it suitable to compete with the existing petroleum-based thermoplastics (Lim *et al.*, 2008).

Thus, this PhD thesis project will be directed to study and to set up a food packaging material PLA-based, evaluating its performance on a selected food model system.

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Bio-based Food Packaging: Influence of Formulation and Processing on Functional Properties

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The results on the barrier and thermal properties of food PLA-based bio-packaging manufactured as part of the first activity of the PhD research project are reported in this paper. In the first step, two grades of PLA at different cristallinity and four nanosized fillers at a fixed concentration were used for manufacturing neat and nanocomposite films using extrusion process. In the second step, thermal properties and water vapour permeability (WVP) of the films were investigated.

Influenza della formulazione e delle condizioni di processo sulle proprietà funzionali di un imballaggio alimentare ottenuto da fonti rinnovabili

In questo lavoro vengono presentati i risultati delle proprietá termiche e di barriera di un biopackaging alimentare a base di PLA, come parte della prima attivitá riguardante il progetto di tesi di dottorato. Inizialmente sono stati utilizzati due gradi di PLA a diversa grado di cristallinitá e quattro diversi nano filler a concentrazione fissa per ottenere films puri e nanocompositi attraverso un processo di estrusione. Successivamente, sono state approfondite le proprietá termiche e la permeabilitá al vapore acqueo dei films prodotti.

Key words: Poly(lactid acid), organoclay, water permeability, thermal properties, composite film.

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Bio-based Food Packaging: Influence of Formulation and Processing on Functional Properties

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The objectives of this research were to study the effects of four types of organically modified montmorillonite (OMMT) in two poly(lactic)acid (PLA) matrices consisting of distinct contents of L-isomer on the structural, thermal and mechanical properties of the resulting composite materials. The nanoclays were added at a fixed concentration of 4% (w/w) and films were obtained using an extrusion process. Results showed that the influence of the organic modifier was particularly significant in improving the barrier properties to water vapour and oxygen.

Influenza della formulazione e delle condizioni di processo sulle proprietà funzionali di un imballaggio alimentare ottenuto da fonti rinnovabili

Gli obiettivi di questa ricerca sono stati quelli di studiare gli effetti di quattro tipologie di montmorillonite modificata organicamente (OMMT) su due matrici di acido polilattico (PLA), a diverso contenuto di L-isomero, sulle proprietà strutturali, termiche e di barriera dei materiali compositi ottenuti. Le nanoargille sono state aggiunte ad una concentazione fissa pari al 4% (w/w) ed i film sono stati ottenuti attraverso un processo di estrusione. I risultati hanno evidenziato che l'influenza del modificante organico è stata particolarmente significativa nel migliorare le proprietà barriera al vapor d'acqua ed all'ossigeno.

Key words: Poly(lactid acid), organoclay, barrier properties, structural properties, nanocomposite.