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**EVALUATION OF THE PROCESSING CONDITIONS  
AND THE CONCENTRATION OF NANOCCLAY IN  
SOY BASED BIOPLASTICS**

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Keywords: Bioplastics, soy protein, dynamic mechanical thermal analysis, tensile strength test, water absorption

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# 1-Introduction

Plastic materials are the most commonly used materials due to their extraordinary properties (Plastic Europe, 2008), increasing the number and the new applications each day. Indeed, combining different polymers allows us to make materials properties without limits. The problem is that a big majority of plastics materials are petroleum-based and it is known that oil resources are not eternal and, on the other hand, a large part of this plastics are non recyclable and causes an accumulation of waste. In this context the substitution of petroleum-based plastics by bio-based plastics is seen such as a promising alternative (Alvarez-Chavez et al, 2012). However, nowadays, the challenge is divided in two parts. On the one hand, scientists have to develop technologies which allow us to produce those new materials and on the other hand, they have to adapt it for existing applications. One alternative has been the development of biodegradable materials from renewable resources (mainly proteins and polysaccharides). New researches are devoted to develop new protein-based materials, which were able to replace fossil-based polymer for high quality applications such as superabsorbent. Nowadays, bioplastic applications are limited mostly to food, medical or agriculture industry. However, it appears that more and more bioplastics replace conventional plastics. For example, biopolymers were made in order to replace PVC pipes, or making cell-phone coating.

The European bioplastics production had double in 2013 compared to 2010 for reach 509 000 tons and increases each year. (Matériaux Plastiques et composites, 2015). However, bioplastics production remains low compared to the 240 million tons of conventional plastic (Plastic Europe, 2008). Bioplastics are composed by a polymer matrix (polysaccharide, protein ...), a plasticizer (in order to reduce intermolecular forces among polymer chains, increasing mobility and reducing the glass transition) and some additives to improve the processability or properties of the final product. In this study, soy protein (polymer matrix), glycerin (plasticizer) and nanoclay (additive) were used. In fact, this product is known as nanobiocomposites.

Soy proteins, a co-product with soybean oil and it is one of the cheapest proteins in nature, shows superabsorbent properties due to the presence of hydrophilic amino acids. (Tian et al, 2012). Soy protein concentrates are suitable raw materials for the production of bioplastics, which has been demonstrated to be suitable for performing bioplastics exhibiting

a high water uptake (Liu and al, 2005; Song and al, 2011). In addition, lamellar nanofillers have been postulated to improve mechanical and barrier properties (Alexandre et al, 2000; Angellier-Coussy et al, 2013). Natural Montmorillonite (MMT- $\text{Na}^+$ ) is one clay minerals widely used in polymer science as filler (Peelman et al, 2013). It is widely available in the nature as micron-size tactoids, which consists on several hundred of individual platy particles held together by electrostatic forces, the gap between each layer is about 1 nm which stack together, by Van der Waals forces, to form the primary particles of the material (tactoids) (Kumar and al, 2010). The introduction of this materials leads to increase the water uptake capacity, while the mechanical properties of the hydrogel increase at the same time (especially strength and stiffness) (Bagheri Marandi and al, 2010). However, the efficient dispersion of nanoclays in biopolymer matrices is a key problem in bionanocomposite development, where exfoliation is the desirable arrangement for improving the properties of nanocomposites (Yang and al, 1999). The dispersion of these particles within the polymer structure is complex.

Most of the protein-based bioplastic properties can be easily controlled by adjusting different parameters such as the soy/plasticizer ratio, the quantity of filler or the molding time and temperature (Felix and al, 2013). However, the strength of the polymer is too low and these properties are really influenced by moisture absorption (Liu et al, 2005).

The overall objective of this work is to develop SPI/MMT nanocomposite plastic materials, plasticized with glycerol by using injection molding process. Nanoclay was incorporated with the intention of improving the water absorption. Rheological and tensile strength measurements have been carried out in order to evaluate the structure of bioplastics. Moreover, X-rays diffraction and microscopy have been assessed to analyze the nanoclay incorporation into the material and evaluate its influence on the structure.

## 2- BACKGROUND

### 2.1 PROTEIN

#### 2.1.1 INTRODUCTION

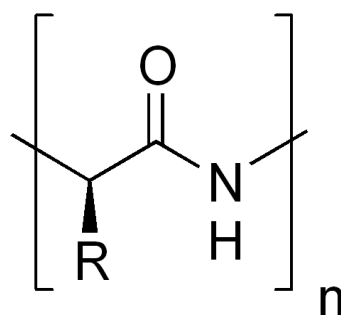
Proteins are described as a natural biopolymer. They are the most abundant biological macromolecules and its structure is a sequence of amino acid residues. Each protein properties depend on its amino acid sequence. The combination of twenty amino acids enables to create a huge quantity of different proteins which may acquire a multitude of different conformation and, consequently a wide variety of biological functions. Those functions can be bio catalyst (enzymes), structure function (collagen), regulation (insulin), defense (keratin), storage (egg albumin), hormones or transport protein (hemoglobin) (Mckee and Mckee, 2013).

#### 2.1.2 STRUCTURE

The properties of the protein depend on the structure of the macromolecule. There are 4 types of structure according to the scale measurement. (Amino acid sequence,  $\alpha$  and  $\beta$ -helix, polypeptide chain and assembled subunits).

##### 2.1.2.1 Primary structure

The primary structure, or sequence, of a protein corresponds to the linear sequence of amino acid residues. The interaction between residues and side chains determine the spatial structure (Mckee and Mckee, 2013).



*Figure 1: Amino acid involved in the primary structure of a protein. R represents the side chain of residue.*

##### 2.1.2.2 Secondary Structure

The secondary structure describes the local arrangement of the main chain of a protein. The secondary structures exist because the favorable refolding of the peptide chain is limited



and only some conformations are possible. Some conformations are significantly favored. The most commonly structure are  $\alpha$  and  $\beta$ -helix conformations.

### **2.1.2.3 Tertiary Structure**

The tertiary structure of a protein corresponds to the folding of the polypeptide chain in space. It is commonly called the three-dimensional structure. The three dimensional structure of a protein is closely related to its function. It means that the protein will lose its function if its structure is broken by the use of denaturing agents (Lehninger et al, 2005).

The tertiary structure depends on many interactions such as:

- Covalent interactions
- Electrostatic interactions
- Van Der Waals interactions

### **2.1.2.4 Quaternary Structure**

This structure referred to the association of at least two polypeptide chains with weak bonds like hydrogen bonds (no covalent bonds). It creates tangles bonds between different chains. This obtained product is called oligomer.

## **2.1.3 DENATURATION**

When the protein loses its biological function, it is denatured. Modifications of its structure may lead to denatured protein. It can happen with high level of temperature, pressure, surface tensions, shear stress and modification of pH or the presence of surfactants.

## **2.2 SOY PROTEIN**

### **2.2.1 HISTORICAL PART**

Soy is consumed in Asia for over 5,000 years. It is one of the most popular cereals with wheat, rice and barley. Traditional Chinese medicine used it as sedative and antipyretic properties. Soy was introduced in Europe at the beginning of the XVII century. But, only from the XX century, scientists have discovered lots of application of soy. It is not only used for feeding (soy milk, soy lecithin, flour, meat substitute), but it can be used for anti-cancer effects, fertilizer (nitrogen catcher on the ground), soap and painting as well as manufacturing of bio-plastics, glues and textiles (Charvet, 2012).

### 2.2.2 SOY PROTEIN IN THE WORLD

The following table (Table 1) shows the big producers of soy in the world. The United States, Brazil and Argentina represent more than 80 % of the world production. Approximately 77% of soybean is genetically modified.

Country	Production (Tones)	Percentage
USA	90,609,800	34.6 %
Brazil	68,518,700	26.2 %
Argentina	52,677,400	20.1 %
China	15,083,204	5.8 %
India	9,810,000	3.8 %
Paraguay	7,460,440	2.9 %
Canada	4,345,300	1.7 %
Uruguay	1,816,800	0.7 %
Ukraine	1,680,200	0.6 %
Bolivia	1,637,000	0.6 %
Russia	1,222,370	0.5 %
<b>Total world</b>	<b>261,578,498</b>	<b>100 %</b>

*Table 1: Production in tones and percentage of world production (2010) all the numbers are in ton per a year (FAO, 2013)*

Production of soy increases each year, being one of the biggest sources of food, for both human and animal. Soy protein is found like a powder and is obtained by extruding the seeds as co-product from soybean oil production. The main problem is that it needs to be kept in a dry place because the protein has the ability to absorb a big quantity of moisture (FAO, 2013).

### 2.2.3 SOY PROTEIN USES

Soybeans are mainly used in food directly in the form of cereal or derivative as soy sauce or soy milk. Soybean contains a large amount of proteins, carbohydrates, lipids, vitamins A and B, phosphorus, potassium, calcium, magnesium, zinc and iron. Soy protein may serve as substitute to animal meat. Moreover it is also widely used in animal feed because of its high content of protein. Soy is also use for it cardiovascular properties and anti cancer effect. Nowadays, it is increasingly its used to develop bioplastics.

## 2.3 NANOCCLAY

### 2.3.1 GENERAL REVIEW

Nanoclay is available in nature as micron-size particles, which consists on several hundred of individual platy particles held together by electrostatic forces (the gap between

each layer is about 1nm which stack together) and by Van der Waals forces, to form the primary particles of the material (tactoids). When the particles interact with the polymer matrix at the molecular level, a number of physical and chemical properties are greatly improved. The nanoclay exhibits hydrophilic properties (Cousin, 2016). One example is the Montmorillonite in this work, the nanoparticles are obtained by grinding the Montmorillonite, a mineral found in nature.

## **2.3.2 NANOCCLAY PROPERTIES**

### **2.3.2.1 DECREASE OF PERMEABILITY**

Nanoparticles reduce the porosity of the resin because of their flat structure. In some cases, the use of nanoclay allows to obtain significant effect on the diffusion of water. If nanoclays are exfoliated, water absorption capacity is improved.

### **2.3.2.2 RESISTANCE TO CHEMICAL ATTACK**

The decrease of permeability increases its chemical stability from external attacks. The barrier effect to gases and liquids has a protective effect on the resin.

### **2.3.2.3 RIGIDITY**

Nanoclay has reinforcing properties and influences various mechanical properties such as tensile strength and flexural strength and curing.

### **2.3.2.4 THERMAL STABILITY**

The softening temperature increases in the presence of nanoclay. Nanoparticles create more rigid structures and increase the glass transition temperature of 10 ° to 20 ° C.

### **2.3.2.5 WATER ABSORPTION CAPACITY**

The introduction of this materials leads to increase the water uptake capacity, while the mechanical properties of the hydrogel increase at the same time (especially strength and stiffness) (Bagheri Marandi et al, 2010).

## 2.4 BIOPLASTICS

### 2.4.1 DEFINITIONS

According to the European Bioplastics, a plastic material is defined as a bioplastic if it is either bio-based, biodegradable, or features both properties (Bourg, 2012).

***Bio-based material:*** It is called bio-based when a material or product is partly (or entirely) derived from biomass (renewable source) such as soy, wheat or corn.

***Biodegradable:*** Biodegradation is a chemical process during which micro-organisms that are available in the environment convert materials into natural substances such as water, carbon dioxide, and compost.

***Plasticizer:*** Proteins are generally mixed with a plasticizer in order to reduce intermolecular forces among polymer chains, increasing mobility and reducing the glass transition.

***Additives:*** Additives are added in order to enhance some mechanical properties of bioplastics. Usually, nanoparticles or nanofibers are used as additives. It creates nanocomposites.

***Nanocomposites:*** It is a solid material composed of different materials. At least one of material dimension is less than 100 nanometers. A nanocomposite is generally the combination of a solid matrix with nanometer reinforcing phase, it create a material with different chemical and mechanical physical properties due to the structural and chemical differences.

### 2.4.2 BIOPLASTICS MANUFACTURING

#### 2.4.2.1 MATERIALS USED IN THE BIOPLASTIC MANUFACTURING

Usually, a bioplastic material (of the biodegradable group) consists of a polymeric network formed by a biodegradable macromolecular substance (in this case is a protein) and a plasticizer.

##### 2.4.2.1.1 NATURAL RAW MATERIALS

In recent years there has been a great interest to utilize renewable biomass in order to manufacture consumer goods which exhibit high-quality, cost-competitive and biodegradable, reducing the consumption and the dependence on petrochemical feedstock and diminishing

environmental pollution (Rosentrater et al, 2006, Felix, Martin-Alfonso et al, 2014). Proteins and polysaccharides have been postulated as renewable biomass to manufacture biopolymers for many years (De Graaf, 2000, Hernandez-Izquierdo et al, 2008). Polysaccharides are naturally extended, and are widely used for food industry. These compounds have been also used for bioplastics (e.g. starch and chitosan are good examples of polysaccharides used for this purpose). As regards proteins, they are a renewable and biodegradable resource with great potential to improve the quality and stability of a large range of food products by using a number of processing techniques (Romero, Cordobes et al, 2008, Jayasundera, Adhikari et al, 2009, Erni, Windhab et al, 2011). For a long time, proteins have been used to produce edible materials, but understanding of the precise physical and chemical mechanisms of protein interactions, they can be used to produce stable bioplastic materials (Hernandez-Izquierdo et al, 2008; Balaguer, Gomez-Estaca et al, 2011).

#### 2.4.2.1.2 PLACISTICZERS

Plasticizers are generally added to improve the processability of the protein network, as well as in order to modify the properties of the final structure, decreasing the glass transition and the brittleness. Usually, plasticizers consist of compounds which exhibit low-molecular weight, low volatility and that interact with the polymer chains producing swelling (Hernandez-Izquierdo et al, 2008). This type of compounds is widely used in polymer industries as additives. The primary role of such substances is to improve the flexibility and processability of polymers by lowering the second order transition temperature, the glass transition temperature (T<sub>g</sub>). The council of the IUPAC (International Union of Pure and Applied Chemistry) defined a plasticizer as “a substance or material incorporated in a material (usually a plastic or elastomer) to increase its flexibility, workability, or distensibility”. These substances reduce the tension of deformation, hardness, density, viscosity and electrostatic charge of a biopolymer, at the same time as increasing the polymer chain flexibility, resistance to fracture and dielectric constant. Other properties are also affected, such as degree of crystallinity, optical clarity, electric conductivity, fire behaviour and resistance to biological degradation (Vieira, da Silva et al, 2011). There are many plasticizer for protein-based bioplastics, such as: 1,4- Butanediol, DATEMa, Dibutyl, Glycerol, Lactic acid, Octanoic, Palmitic acid, Sorbitol, Sucrose and Water (Hernandez et al, 2008). For this study, Glycerol has been the chosen plasticizer. This is a widely used bioplastic, exhibiting hydrophilic properties with a low molecular weight, and high boiling point. Its high plasticizing effect has been attributed to the ease with which glycerol can insert

and position itself within the 3-dimensional biopolymer network (di Gioia and Guilbert, 1999).

#### **2.4.2.2 METHODS FOR PROTEIN BASED BIOPLASTICS MANUFACTURING**

Proteins offer a large range of possible physical and chemical interactions. This dual character is given because proteins can participate in non-covalent interactions such as ionic, hydrogen, and van der Waals bonding or in chemical reactions through covalent linkage (peptide and disulphide bonds). Usually, the formation of the protein network is divided in two main stages: Plasticization and protein interactions (Hernandez-Izquierdo and Krochta, 2008).

##### **2.4.2.2.1 PHYSICAL-CHEMICAL METHOD**

This method requires chemical agents in order to break sulfur bind, to disperse and solubilize the protein. Then it is dried. Thermal and moisture conditions influence a lot the structure of the obtained bioplastic.

##### **2.4.2.2.2 THERMOPLASTIC METHOD**

This method consists in mixing at the same time protein, plasticizers and additives in order to obtain homogenous dough. This process can be carried out in batch, semi-batch or continuous reactor (Perez Puyana, 2014).

##### **2.4.2.2.3 THERMOMECHANICAL METHOD**

The first part of this process is the same as thermoplastic method, but, the next step consists in molding the dough.

The conditions of injection molding need to be well-known, because if the temperature is too low, the glass transition is not reached and consequently, it will be so difficult to inject. If the pressure is too low, the mold will be not entirely full.

Molding process can be performed using thermoformed plastics process, injection or compression molding and extrusion.

**Thermoformed process:** firstly, the polymer is heated, tensioned on a frame and by air or vacuum pressure is stamped or pressed on the mold walls.

**Compression process:** The polymer is added as a powder directly in the mold, and then the mold is subject to high pressure and temperature. After a required time piece is recovered.

**Injection molding:** It has been carried out in this study. The injection process can be divided in two parts. The first one is the fusion of the polymer. In fact, this part is called pre injection. The material is heated at a predefined temperature for a necessary time in order to reduce the product viscosity and then facilitate the injection. The second one is injection in the mold. Once melted, a piston pushes the polymer material in a high temperature and pressure mold. When the temperature decreases, the material takes a rigid structure. This process is particularly used for thermoplastics and bioplastics (Gutierrez Solis, 2014).

**Extrusion process:** It is used for continuous process, the polymer is heated until it reaches the glass transition, and subsequently, the polymer is pushed thanks to a screw through an adequate form.

### 2.4.3 BIOPLASTICS PROPERTIES

Bioplastics exhibit the potentials of various properties of the materials, which some of the relevant are summarized below.

#### 2.4.3.1 MECHANICAL PROPERTIES

##### 2.4.3.1.1 RHEOLOGICAL PROPERTIES

Rheology studies the relationship between the external forces acting on a body and strain it produces. Since the movement of each particle forming the body is subjected to the laws of mechanics, rheology is based on physics branch (Pérez Puyana, 2014). Among the most important parameters used to define this relationship are the following:

- Stress ( $\tau$ ): is defined as the ratio between a force (F) and an area (A).
- Strain ( $\gamma$ ) or deformation rate ( $\dot{\gamma}$ ) is related to the velocity gradient produced in response to shear stress. The relationship between these variables allows classifying the different types of materials found in nature, as shown in the following figure:

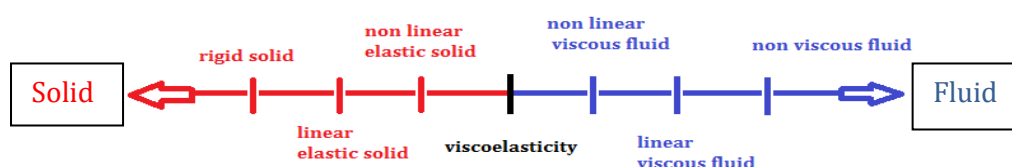


Figure 3: Classification of different materials as a function of their rheological behaviour

Bioplastics show viscoelastic behaviour which means that they have elastic and viscous behavior simultaneously. It is called viscoelastic materials. In the case, viscosity is a function of the stress applied or the strain rate and sometimes the time of deformation.

#### 2.4.3.1.2 TENSILE PROPERTIES

When tensile test are performed, the stress and strain at break are the most commonly used quantified properties for polymer materials (Gennadios, 2002). The stress / strain curves of these tests provide information on flexibility, strength and strain at break capacity of the material. It is useful for predicting the behavior during handling, use and storage bioplastics (Hernandez et al, 2008).

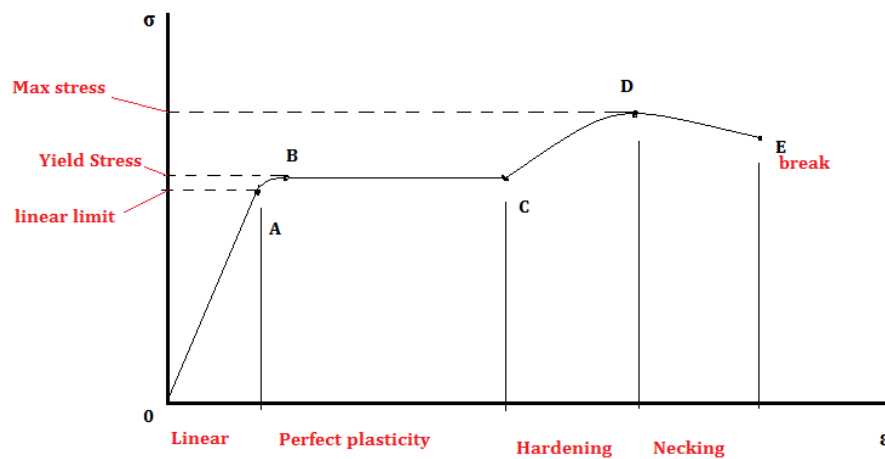


Figure 4: Tensile properties and different regions in stress ( $\sigma$ )-strain ( $\epsilon$ ) curves

The main parameters obtained from the stress-strain curves of Tensile tests are defined by ASTM 882 standard, below describe those of interest to this study:

- Maximum Stress ( $\sigma_{max}$ ): It is calculated by dividing the maximum charge before break by the original cross-sectional area of the specimen, expressed as a force per unit surface.
- Strain at break ( $\epsilon_{max}$ ): It is calculated by dividing the extension at the time of the break by the initial characteristic length of the specimen, it can be expressed in percentage
- Elastic modulus or Young's Modulus is obtained by using the tangent to the linear region and calculating the slope of the tangent line (Perez Puyana, 2014).



### 2.4.3.2 THERMAL PROPERTIES

It is important to study the thermal transition of materials in order to predict the behavior of materials. Differential scanning calorimetry (DSC) and Dynamic Mechanical Thermal Analysis (DMTA) are the most commonly used test. (Hernandez et al, 2008).

#### 2.4.3.2.1 Differential scanning calorimetry (DSC)

This technique measures the heat flow and the temperature associated with the phase transition as a function of the time and temperature. The results provide qualitative and quantitative information on the chemical and physical changes that produce an exothermic or endothermic reaction. The energy supplied to compensate for the difference of temperature between the sample and a reference is recorded (phase change, crystallization, fusion reactions). In these graphs, it is possible to measure the quantity of heat  $\Delta H$ , the calorific capacity also the glass transition temperature. Moreover, it is possible to observe if there is thermal degradation of the materials (Perez Puyana, 2014).

#### 2.4.3.2.2 Dynamic mechanical and thermal analysis (DMTA)

This test consists of applying an oscillating strain of the sample in bending conditions while it is subjected to a heating cycle. The sinusoidal response is measured and related to the imposed strain. It is possible to simultaneously measure the viscoelastic properties of the sample. If the material behaves like a solid, the resulting stress is proportional to the amplitude of the deformation, and consequently stress and strain are in phase. If the sample behaves like a fluid, the force is proportional to the strain rate. In this case, the force signal has a 90 ° offset in phase with the strain.

Viscous and elastic responses can be related to the properties through the stress-strain relationship or modules.

**E'** (**elastic modulus**): capacity of the material to stock elastic energy.

**E''** (**viscous modulus**): capacity of the material to dissipate energy.

**Tan  $\delta$**  (**loss tangent**): the loss factor indicates the ability of a viscoelastic material to dissipate mechanical energy into heat.

$$\text{Tan } \delta = \frac{E''}{E'}$$

The loss tangent represents the ratio of the viscous component and elastic material. This property is an indicator of how the material loses energy due to molecular rearrangement and or internal friction.

Such test, where different geometries may be used, is to apply an oscillation to the sample at a predetermined frequency, while temperature sweep is performed. So you can get the rheological response of the material in a wide temperature range (Jerez et al, 2007).

#### **2.4.3.3 BARRIER PROPERTIES**

The requirements concerning permeability of the product depend on the final use. In general, plastics are relatively impermeable to small molecules such as gases, water vapor, organic vapor and some liquid. It provides a range of mass transfer characteristics for grouping the materials according to their ability as a barrier (Félix, 2015).

Permeability is defined as a steady-state property that describes the extent to which a permeating substance dissolves and then the rate at which the permeate diffuses through a film. To achieve this, usually the driving force is related to the difference in concentration of both sides of the film.

##### *Water vapor permeability (WVP)*

Protein films have quite high WVP compared to edible waxes. Thus, protein film exhibits WVP values two to four orders of magnitude greater than that of Low-density polyethylene (LDPE). Usually, higher plasticizer concentrations involve an increase of WVP (Krochta 2002).

##### *Oxygen permeability (OP)*

At low to intermediate relative humidity, protein films have values of oxygen permeability that are lower than those of the polyethylene-based plastics (which are not good oxygen barriers), and are comparable to as polyesters. The low OP of protein films would appear to make them useful for coatings and pouches for oxygen-sensitive products (Krochta, 2002).

#### **2.4.3.4 MICROSTRUCTURAL AND OPTICAL PROPERTIES**

Regarding to optical properties, some bioplastics have excellent properties and are easily moldable. Refractive index, transparency, transmission, brightness or color and behavior in the infrared spectrum are the most interesting properties. Bioplastics which do not contain additives or impurities are generally quite translucent, although this property is strongly influenced by the crystallinity of the material. Amorphous bioplastics are transparent,

while those crystalline were opaque (Gonzalez-Gutierrez et al, 2011). It is possible to use different techniques such as AFM, SEM or even Confocal Microscopy in order to obtain images illustrating the bioplastic microstructure.

#### **2.4.4 BIOPLASTICS USES**

Bioplastics have various applications. In fact, they are especially used for food industry such as articles of packaging (edible films and coatings aim: it reduces the effect of moisture, oxygen, and can protect from microbes). They are also used in agriculture as clips, or biodegradable mulching (reduce the costs of collecting). Moreover, it is useful to produce disposable items. Another field of bioplastics is hygienic and cosmetic products such as cotton swab or diaper as well as medicine. In the automotive sector, bioplastics are used for tires manufacturing. Indeed, biocompatible plastics are used as sutures, staples and pins implants. (Club bioplastiques, 2012)

The overall objective is to achieve the replacement of existing synthetic, non-biodegradable products and make material with relevant water absorption at the lowest cost possible (Félix, 2015).

## 3-MATERIAL AND METHODS

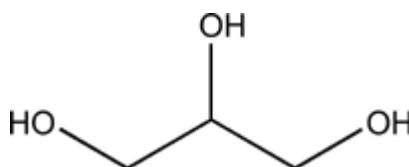
### 3.1 MATERIALS

#### 3.1.1 SOY PROTEIN

Soy Protein Isolated (SPI) Supro 500E was supplied by Protein Technologies International (Leper, Belgium). The protein content was determined in quadruplicate as % N x 6.25 using a LECO CHNS-932 nitrogen micro analyser (Leco Corporation, St. Joseph, MI, USA) being  $91 \pm 0.5$  wt.%. Glycerol (GL), used as plasticizer, was purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

#### 3.1.2 GLYCERIN

Glycerin, used as plasticizer, is an alcohol with three hydroxyl groups on a chain of three carbon atoms.



*Figure 5: glycerin*

Glycerin comes from Applichem Panreac. This is pure, with pharma grade, ( $C_3H_8O_3$ ,  $M = 92.10 \text{ g.mol}^{-1}$ )

#### 3.1.3 NANOCCLAY

The nanoparticle introduced was Cloisite®  $Na^+$ (MMT- $Na^+$ ). It was obtained from Montmorillonites, and manufactured by Southern Clay Products, Inc. (USA)

### 3.2 METHODS

#### 3.2.1 MIXING STAGE

Bioplastics were manufactured by a thermo-mechanical procedure including two stages. Firstly, blends containing 50 wt.% protein/glycerin, with different concentration of nanoclay: 3, 6 and 9%. Then those were mixed in a two-blade counter-rotating batch mixer, Haake Polylab QC (ThermoScientific, Germany).

The mixing conditions were 25°C and 50 rpm for 60 min and the torque and temperature were monitored during mixing to obtain a dough-like blend. After the first stage, the second stage consists on selecting a suitable time mixing by analyzing torque and temperature profile. Indeed, the blend needs to be well mixed, but cross linking reactions must to be avoided. After that new blends are performed using the selected time for each sample.



Figure 6: HAAKE POLYLAB Mixer (ThermoScientific, Germany)

After mixing, homogenous dough was obtained. During this stage, Specific Mechanical Energy, which is the ratio between energy and mass, was studied.

$$SME = \frac{\omega}{m} \int_0^{t_{mix}} M(t) dt \quad (I)$$

Where  $\omega$  (rad.s<sup>-1</sup>),  $m$  (g),  $M(t)$  (N·m) and  $t_{mix}$  (s) are the mixing speed is the sample mass, the torque and the mixing time, respectively.

### 3.2.2 INJECTION STAGE

After mixing stage, blends were subsequently processed by injection molding, using a MiniJet Piston Injection Molding System (Thermo Haake, Germany) to obtain bioplastic specimen. The most suitable processing variables, such as injection temperature and pressure, as well as residence time in the pre-injection mixing chamber, were selected after performing temperature ramp and time sweep tests to the dough-like materials.

Two moulds were used to prepare two different specimens: a 60×10×1 mm rectangular-shaped specimen for dynamic mechanical analysis (DMA) experiments, water absorption capacity and a dumb-bell-type specimen for tensile properties of plastics. The

device has a temperature and pressure controller for injection and polymerization (one for pre-injection and one in the mold).



*Figure 7: Haake Mini Jet piston injection molding system (ThermoScientific, Germany)*

### **3.2.3 DOUGH CHARACTERIZATION**

#### **3.2.3.1 X-ray Diffraction (XRD)**

XRD studies of the composites probes were carried out using a D8 Discover (BRUKE, Massachusetts, USA) (40 kV, 30 mA) equipped with Cu K $\alpha$  radiation ( $\lambda=0.1516$  nm). The scanning range ( $2\theta$ ) was from 2 to 30°, and the step size was 0.05°. Different crystalline phases were visualized, which may indicate systems whose microstructure could be different. X-rays were carried out on the dough after the mixing step.

#### **3.2.3.2 Water imbibing capacity (WIC)**

WIC of soy protein concentrate was determined by using a modification of the Baumann apparatus (Torgersen and Toledo, 1977). This device consists of a funnel connected to a horizontal capillary. Sample (50 mg) was dusted on a wetted filter paper which was fastened to a glass filter placed on top of the funnel filled with water. The apparatus was kept at 20 °C. The uptake of water by the sample at equilibrium was read in the graduated capillary and expressed as milliliters of water imbibed per gram of isolated. Determinations were performed at least in triplicate.

### 3.2.3.3 Dynamic Mechanical Thermal Analysis for compression

Those experiments were carried out by using a RSA3 (TA Instruments, New Castle, USA) connected to a Chiller in order to regulate the temperature.

An oscillating compression was applied to the sample while being subjected to a thermal cycle. The resultant sinusoidal force is measured and correlated with the generated distortion. It allows us to measure viscoelastic properties of the sample in a temperature range. With this information, it is possible to determine if the product behaves rather as a solid or as a fluid. Then data of different viscoelastic modulus  $E'$ ,  $E''$  and also  $\tan \delta$  were obtained

Many experiments can be carried out with this equipment. Dynamic temperature ramp for compression test was performed to characterize the dough and flexion for bioplastic. The temperature range for dough was 0 to 150°C with a frequency of 1 Hz and a heating rate of 3°C/min. Figure 9 represents compression geometry which comprises two cylindrical plates of 8mm.



Figure 8: RSA3 Rheometer (TA Instruments, USA)



Figure 9: Compression geometry

## 3.2.4 BIOPLASTICS CHARACTERIZATION

### 3.2.4.1 TENSILE STRENGTH MEASUREMENTS

Tensile tests were made by using the insight 10 kN Electromechanical Testing System (MTS, Eden Prairie, MN, USA).

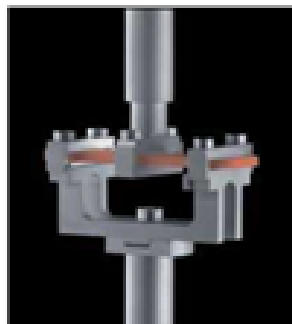


*Figure 10: Electromechanical Testing System (MTS, EDEN PRAIRIE, MN, USA)*

This equipment consists of a rectangular frame with a load cell attached to a cross member, a base and two vertical columns. The load cell head moves vertically between the two columns. Stress-strain curves were obtained from at least five duplicates for each product using dumb bell probes and an extensional rate of  $10 \text{ mm} \times \text{min}^{-1}$  at room temperature. The equipment is able to detect the material break. By analyzing the curves, it is possible to determine the strain at break (strain observed at the break of material), the maximum stress (stress observed for strain at break) and Young's Modulus (elastic modulus).

#### **3.2.4.2 DYNAMIC MECHANICAL THERMAL ANALYSIS**

Rheological characterization of bioplastics were carried out with RSA 3 (TA instrument), the same used for dough characterization. However, three bending point geometry was used (Figure 11).



*Figure 11: Three bending point geometry*

The following tests have been performed



**Strain sweep test:** A 0.01% to 10% strain was used to determine the linear viscoelastic range at a frequency of 1 Hz and at 20 °C and 80°C

**Frequency sweep test:** A frequency of 0.02 to 20 Hz at room temperature was used to study the behaviour of the polymer.

**Temperature ramp:** It was carried out from 20 to 140 ° C and at 3°C/min below the critical strain for each temperature calculated in the previous strain test (strain sweep test) in order to perform measurement under the liner viscoelastic region.

### 3.2.4.3 WATER UPTAKE CAPACITY

Water uptake capacity of bioplastics was determined following the ASTM D570 norm (ASTM D570-98, Standard test Method for Water Absorption of Plastics) (ASTM, 2001) using at least three 60×10×1 mm specimens immersed in distillate water for 24 h at room temperature.

The first step consists in drying in an oven the probes for four hours at 50°C and weight ( $w_1$ ). Then, the probes are immerses in distilled water for 24h at room temperature. Their weight for 24h is noted  $w_2$ . Finally, probes are dried again overnight and reweight ( $w_4$ ). After that, water uptake capacity is calculated according to:

$$\text{Water uptake}_{24h} \% = \frac{w_1 - w_2}{w_1} \cdot 100 \quad (\text{II})$$

Measurement of those parameters was a little bit difficult to perform because the material containing nanoclay dissolved in water because of the hydrophilic properties of nanoclay. In fact, it was not possible to take off the sample and to weight it. That is why a modified method was chosen. The samples were placed in a freezer for 24h and then freeze drying was performed using a Freeze-Dryer BETA 1-8 LD Plus Series (CHRIST, Germany). Because of the problem described above, it was not possible to determine the water uptake at 2h. On the other hand, without nanoclay, the SPI sample has not dissolved.

### 3.2.4.4 Confocal Microscopy

Selected samples were analyzed by means of Confocal microscope Zeiss LSM 7 DUO (Germany), using the reflection mode and the target water. Microscopy was performed to a solution of 9% nanoclay bioplastic and 9% nanoclay in water used as reference. Both samples

were put 24 hours before the test. This test gives a perfect indication of the integration of nanoclay inside the dough.

#### **3.2.4.5 Scanning Electron Microscopy**

Before performing SEM, the samples were placed in a freezer at  $-80^{\circ}\text{C}$  for 1-2 h and then lyophilized to remove all traces of water.

After that, sample cube of 2-3 mm were cut and treated by Osmium vapor at 1% of concentration for 8h. Then, microscopy examination has been assessed with a JEOL JSM 6460 LV (USA) scanning electron microscope with secondary electron detector at an acceleration voltage of 20 kV.

## 4- RESULTS AND DISCUSSION

### 4.1 MIXING STAGE

This stage consists on mixing all the ingredients in order to obtain a material called dough. Then the analysis is important because it is used to find the best conditions for subsequent injection.

#### 4.1.1 PRELIMINARY STUDY

An analysis of mixing torque and temperature profiles allows obtaining necessary information to choose an optimal mixing time for each concentration of nanoclay. In this study, the ratio between soy protein and glycerin was fixed at 1:1. All the samples were processed at 25 °C for 60 min.

Soy-protein (polymer)	Glycerin (plasticizer)	Nanoclay
50% (30g)	50% (30g)	0% (SPI)
		3 % (1.8g)
		6% (3.6g)
		9% (5.4g)

Table 2: SPI/GL systems studied at different nanoclay concentrations

The following figures show torque and temperature values recorded as a function of mixing time.

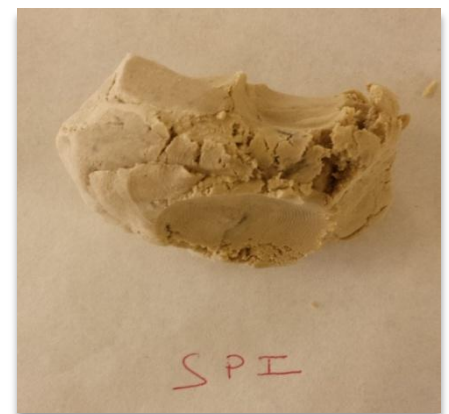
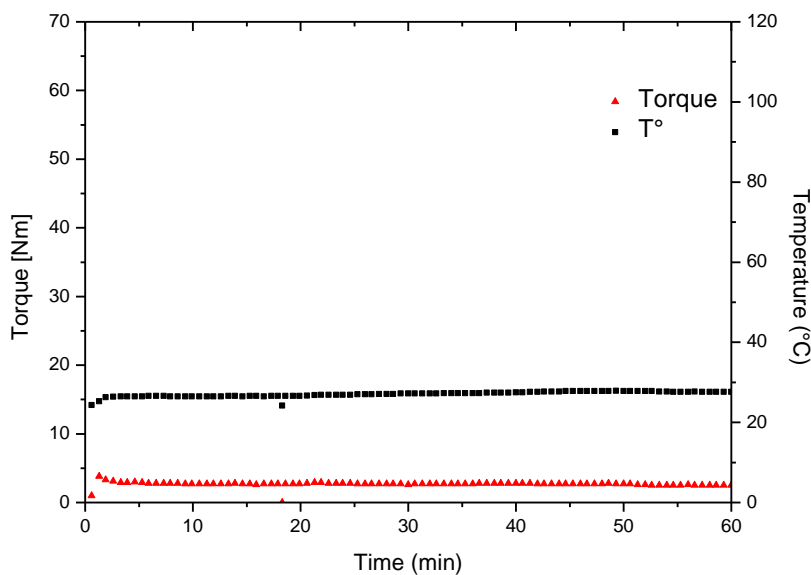


Figure 12: Torque and temperature profiles as a function of mixing time for SPI/GL (left) and SPI/GL dough mixed for 60 min (right)

Figure 12 shows that the torque and temperature remain constant throughout the experiment, when the additive is absent. During the mixing process, there are no cross linking reactions. The sample with 3% of nanoclay is quite similar (Figure 13).

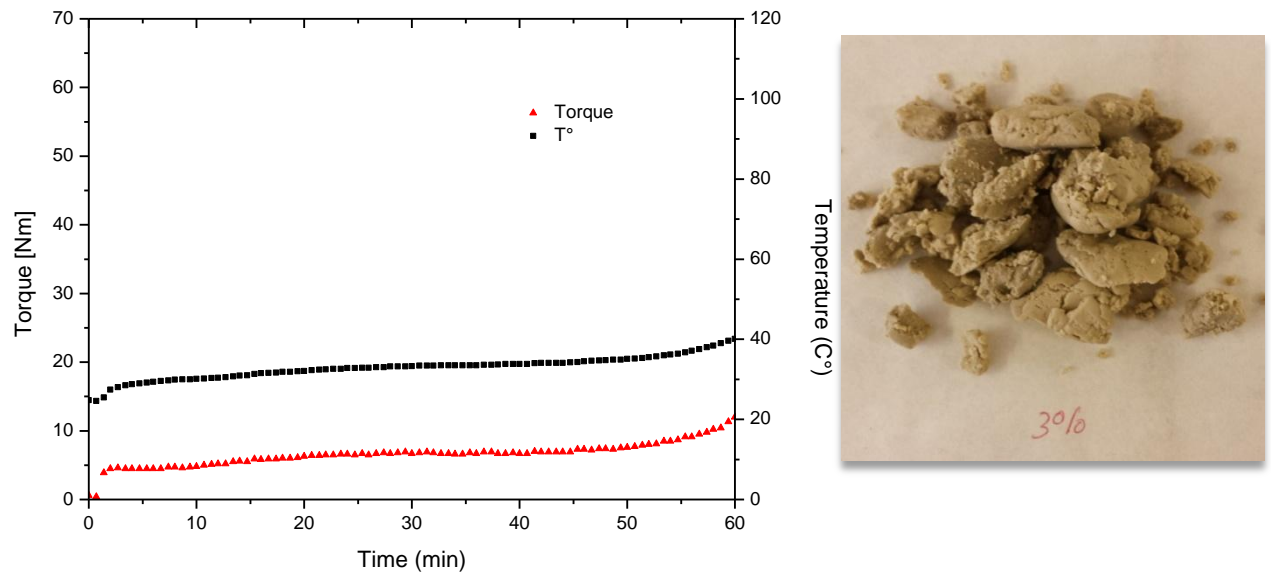


Figure 13: Torque and temperature profiles as a function of mixing time for 3% nanoclay (left) and dough mixed for 60 min with 3% nanoclay(right)

In this case, it is possible to observe a little increase of temperature and torque after mixing for 55 min. But the temperature stays much below 70°C, so this phenomenon cannot influence the structure of the matrix. The results are different for the sample with 6% and 9% of nanoclay (Figures 14 and 15).

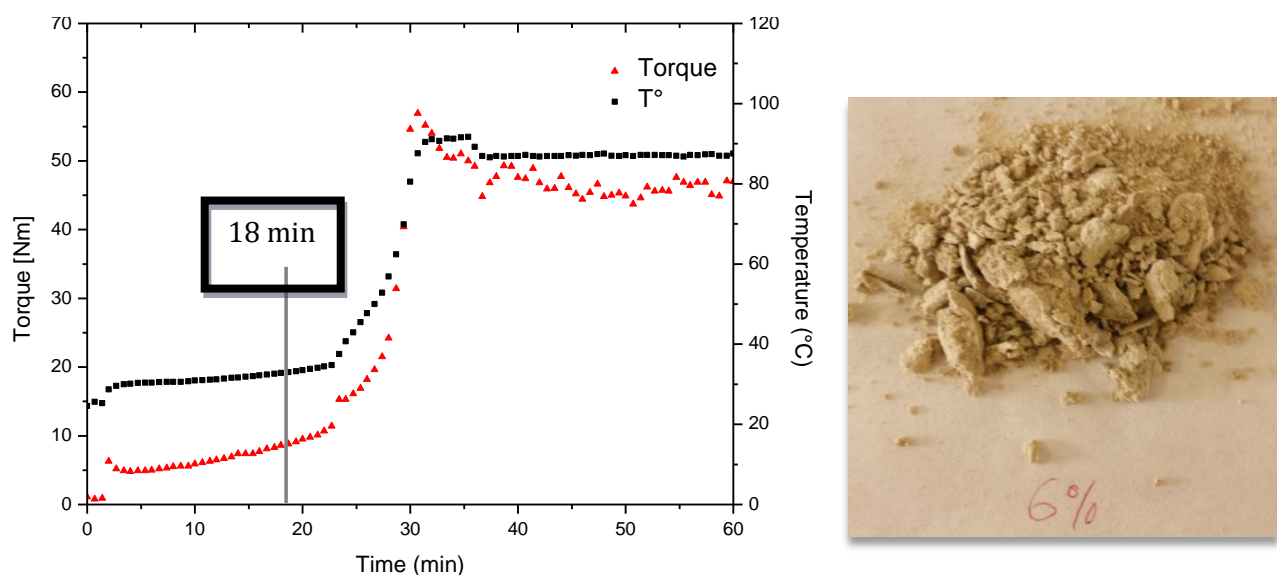


Figure 14: Torque and temperature profiles as a function of mixing time for 6% nanoclay (left) and dough mixed for 60 min with 6% nanoclay (right)

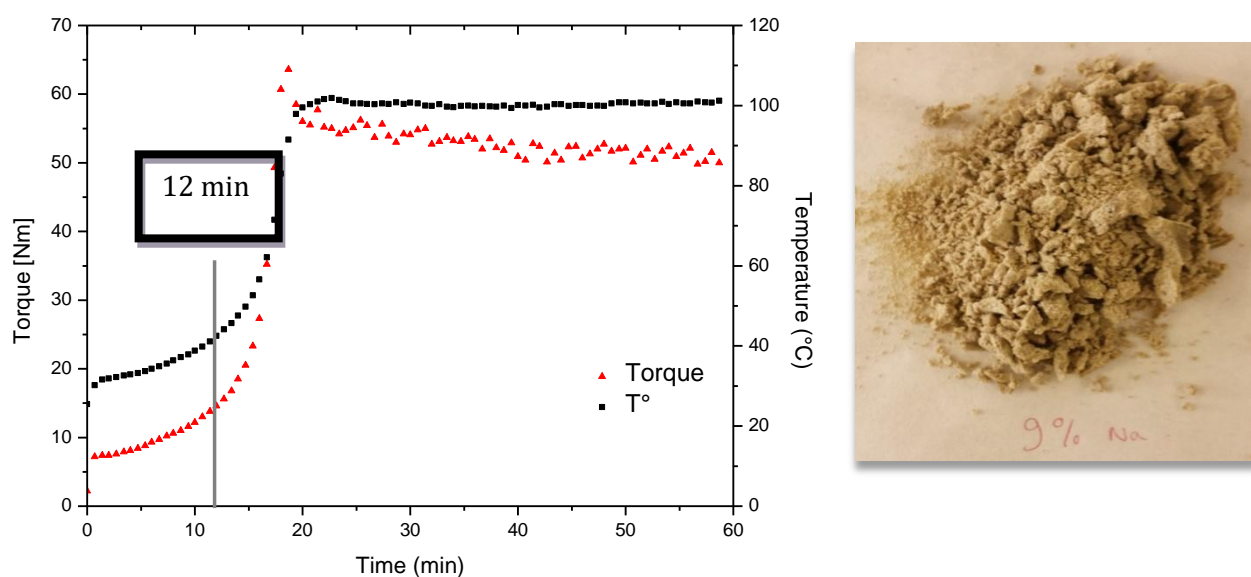


Figure 15: Torque and temperature profiles as a function of mixing time for 9% nanoclay (left) and dough mixed for 60 min with 9% nanoclay (right)

For both samples, there was a sudden increase in temperature and torque (18 min and 12 min for 6% and 9% nanoclay, respectively). This increase shows a structuring stage of the dough induced by mixing. Both increases in torque and temperature are anticipated as the concentration of nanoclay increases, as may be observed after comparing Fig. 14 and 15. This behaviour indicates that shear forces may induce protein cross-linking reactions that lead to material structuring. However, this structuring stage has to be avoided in order to facilitate the subsequent injection process. In fact, it was not possible to inject any of the doughs obtained after the maximum in torque.

#### 4.1.2 MIXING TIME CONDITIONS

With this information, it is possible to choose optimal mixing times. The parameter is selected allowing time long enough to have a fairly homogenous product, but short enough to avoid shear-induced cross-linking. The chosen time are given in the following table (Table 3).

	Mixing time
SPI	60 min
3% Nanoclay	30 min
3% Nanoclay	60 min
6% Nanoclay	18 min
9% Nanoclay	12 min

Table 3: Selected mixing time conditions for different systems

After this stage, the dough is placed in a freezer to avoid modification of its structure along time.

#### 4.1.3 MIXING ENERGY

As a consequence of the different torque profiles of torque, the specific mechanical energy of mixing is also different, as may observed in Figure 16.

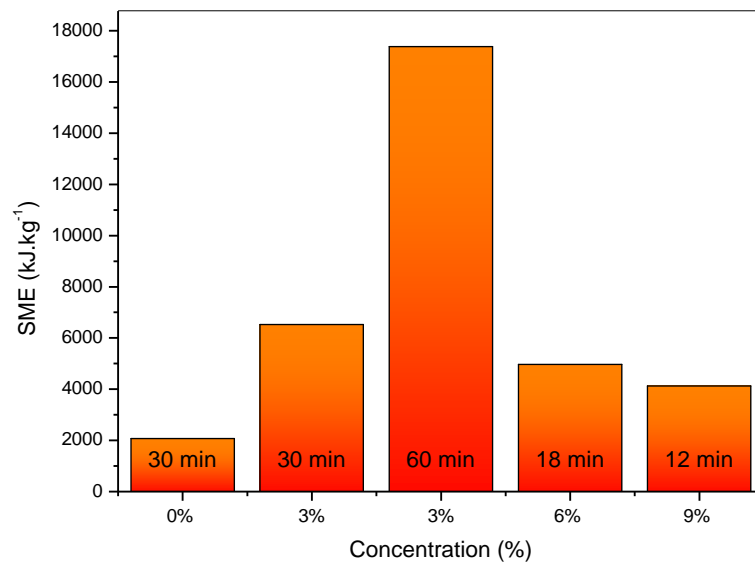


Figure 16: Specific mechanical energy for different dough as a function of nanoclay concentration at selected mixing times calculated by (I)

The quantity of energy needed is lowest for the dough mixed in absence of nanoclay since the torque required is moderate and there is no increase in temperature and torque. The addition of nanoclay up to 3% leads to a remarkable rise in the SME parameter. However, the energy needed decreases from 3% to 9% nanoclay, even though the increase in torque is more important because the mixing time is shorter. It is interesting to observe that for the same nanoclay concentration (3%) the energy needed is not a linear function of time and becomes much higher when the mixing time is doubled. This is probably a consequence of some cross-linking reactions taking place at long mixing time.

## 4.2 DOUGH CHARACTERIZATION

### 4.2.1 X-RAYS Diffraction (XRD)

Figures 17 A and B represent the results of X-rays diffraction of dough for different mixing time.

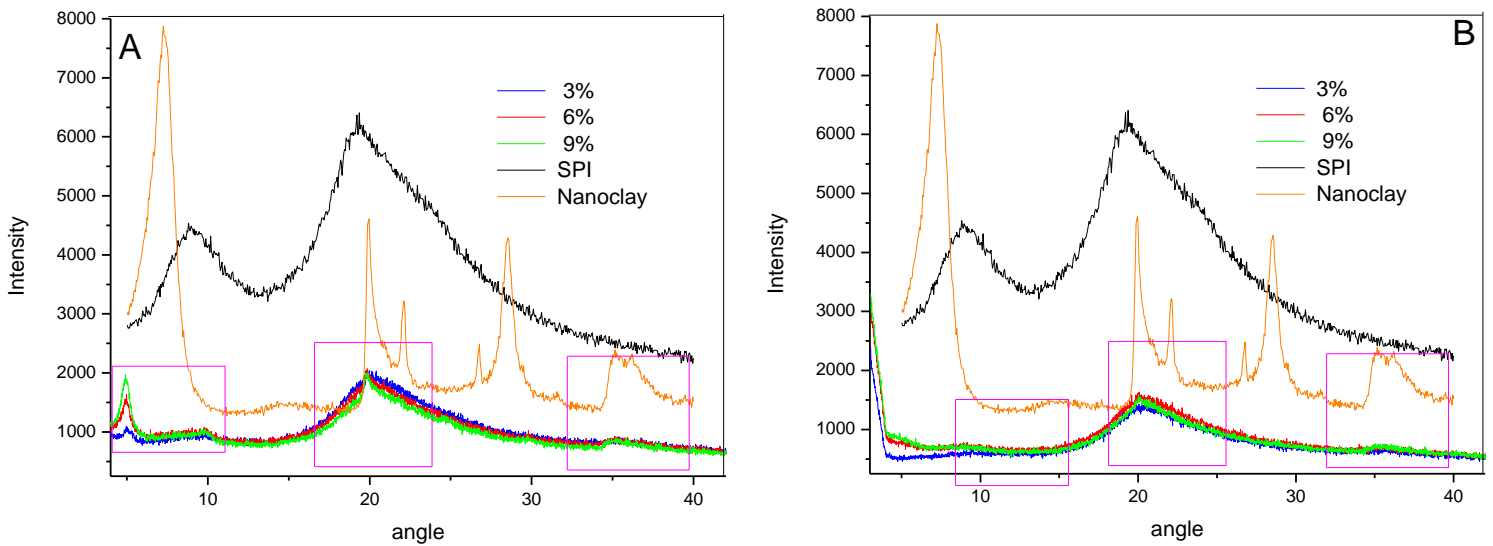


Figure 17: X-ray diffraction measurements for dough after (A) selected mixing time, (B) 60min of

Both profiles are similar. However, on Figure 17 A, the presence of the first peak reveals that nanoclay is less integrated than in the case of Figure 17 B where mixing time is longer and the peak tends to disappear. Furthermore, it may be noticed that the height of the first characteristics peak is lower for longer time. As a result, an increase in mixing time leads to improve the nanoclay integration. Unfortunately, as discussed in section 4.1.1, it is not possible to use this mixing time due to cross linking reaction.

### 4.2.2 WATER IMBIBING CAPACITY

These experiments have been assessed directly on the dough after mixing for 60 minutes. This allows us to analyse the absorption kinetics.

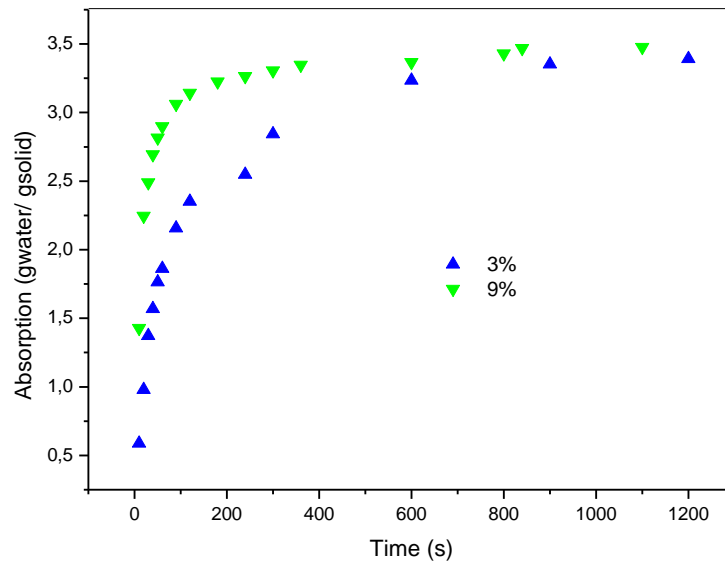


Figure 18: Kinetics of water absorption for 3 and 9% dough for 60 min as mixing time

Although the final absorption of water at equilibrium is the same for both doughs the absorption kinetics is different (Figure 18). Indeed, with 9% of nanoclay, the diffusion of water into the material is more difficult due to its closed structure. However, a big majority of particles are present at the surface of the material that is why it shows faster absorption. On the other hand, when water tends to diffuse inside the dough containing 3% of nanoclay, the absorption kinetic is slower although the final asymptotic value for water absorption is the same.

#### 4.2.3 DYNAMIC MECHANICAL TEMPERATURE ANALYSIS (DMTA)

Figure 19 shows the values of elastic and viscous moduli ( $E'$  and  $E''$ , respectively) from DMTA for different dough with different nanoclay content (0%, 3%, 6% and 9% of nanoclay) as a function of increasing temperature. The objective of the measurement is to select a suitable temperature to achieve a moderate viscosity in order to facilitate the injection to the mold. The stage was carried out with a temperature ramp from 0°C to 150 °C at 3°C/min as described in section 3.2.3.3.



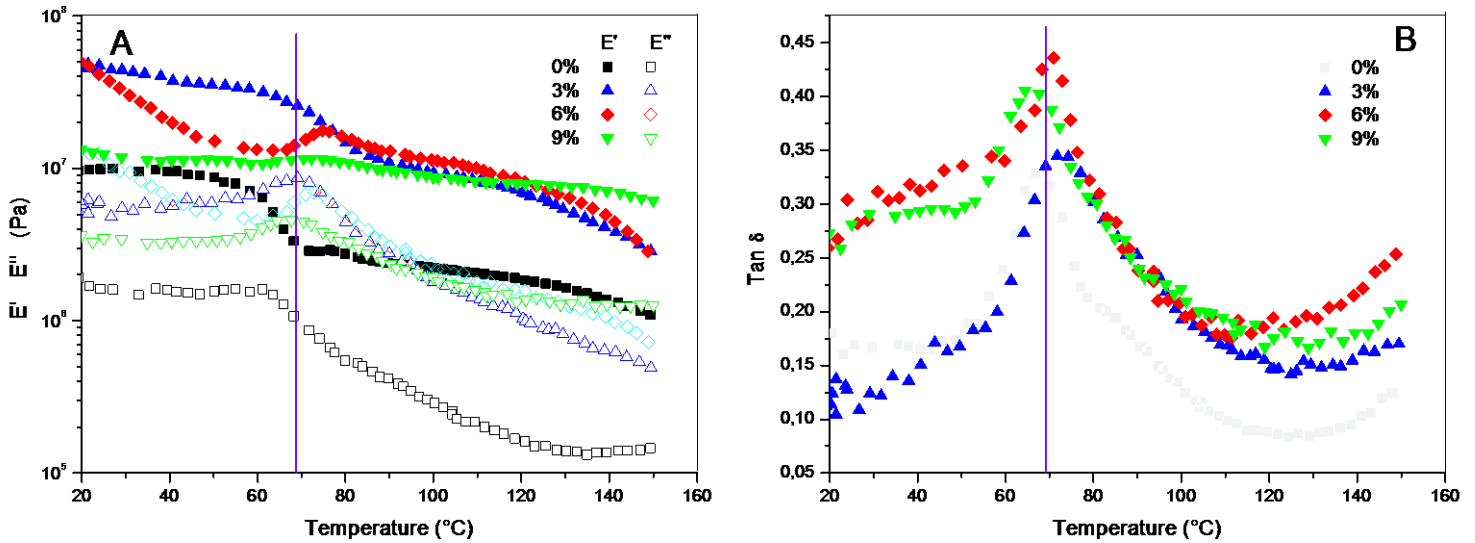


Figure 19: Temperature ramp for all systems: Evolution of (A) elastic and viscous moduli ( $E'$  and  $E''$ , respectively) and (B) loss tangent ( $\tan \delta$ ).

First of all, it is seen that the value of  $E'$  is always higher than  $E''$  value (so the values of  $\tan \delta$  is less than one) over the entire temperature range. Moreover, both viscoelastic moduli ( $E'$  and  $E''$ ) decrease with temperature although being less appreciable for 9%. Consequently, the complex viscosity also decreases such that the injection process is facilitated (data not shown). In any case, a difference of behaviour at 70 °C can be easily observed. For all blends, the thermal profile for  $E'$  passes through an inflection point, which may be related to a glass transition. This transition is related to a maximum of  $\tan \delta$ , which is closed to 70°C for all samples (Figure 19 B). Furthermore, the  $\tan \delta$  profile shows one peak, regardless of the proportion of protein used, which reveals a good compatibility between the protein and glycerol.

#### 4.2.4 INJECTION STAGE

Thanks to previous rheological analysis and previous studies (Félix et al, 2014), it is possible to select the conditions for the injection molding process.

Therefore, it was decided to choose 40°C of pre-injection temperature in order to facilitate the injection (drop of complex viscosity) and 70°C in the chamber corresponding to the glass transition. The temperature allows a good polymerization in the mold. The real conditions are shown in the table 4. It is noticed that there are two different injection pressures because it would be interesting to study the influence of processing conditions of the water absorption. The same concentration of 3% were kept but 500 or 900 bar for injection pressure

and 30 or 60 min for mixing time were used in order to evaluate the effect of injection pressure and mixing time on the final bioplastics properties.

	<b>Barrel Temperature (°C)</b>	<b>Mold Temperature (°C)</b>	<b>Injection pressure (bar)</b>	<b>Injection time (s)</b>	<b>Post injection Pressure (bar)</b>
<b>SPI</b>	40	70	500	20	200
<b>3% 500 30 min</b>	40	70	500	20	200
<b>3% 500 60min</b>	40	70	500	20	200
<b>3% 900 60min</b>	40	70	900	20	200
<b>6%</b>	40	70	500	20	200
<b>9%</b>	40	70	500	20	200

*Table 4: Selected injection molding process conditions*

### 4.3 BIOPLASTICS CHARACTERIZATION

#### 4.3.1 INFLUENCE OF CONCENTRATION

This part is focused on the study of the influence of additives incorporation. Dough containing 0, 3, 6 and 9 % of nanoclay were used to obtain bioplastics by injection molding.

##### 4.3.1.1 TENSILE STRENGTH MEASUREMENTS

Figure 20 shows tensile strength test results for each sample until break.

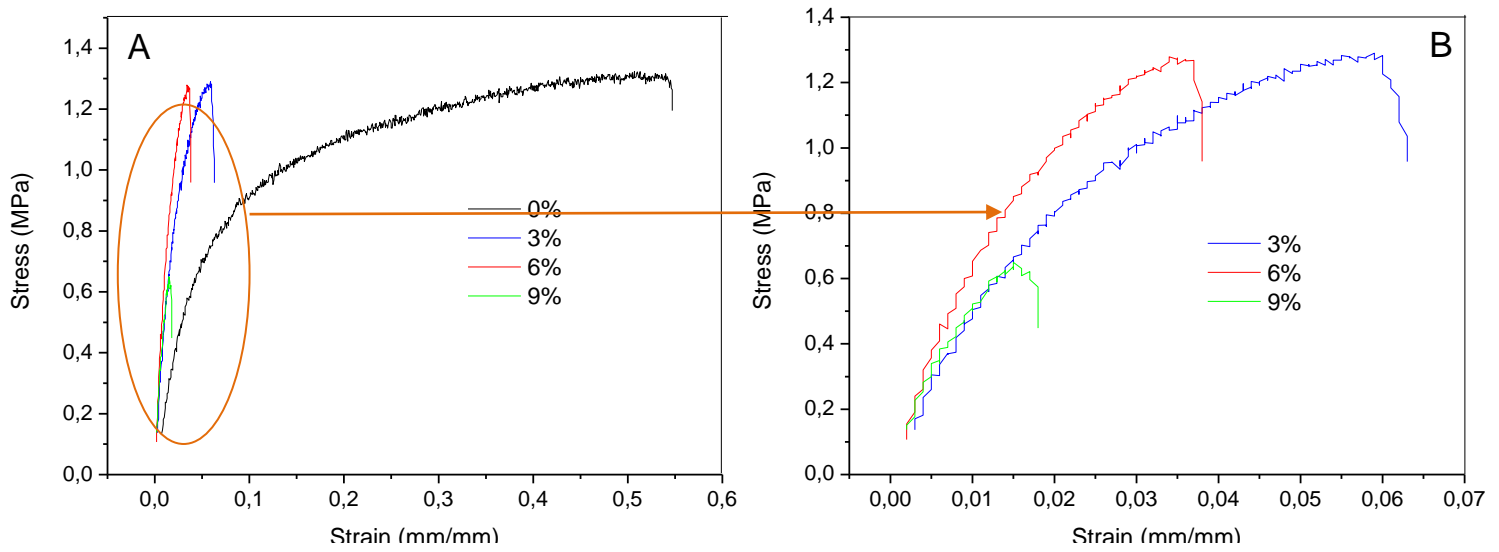


Figure 20: Stress-strain curves for (A) different concentration of nanoclay (B) bioplastics with 3, 6 and 9% of nanoclay

All the curves (Figure 20 A and 20 B) exhibit a similar behaviour which consist of an initial linear elastic behaviour of high constant stress-strain slope yielding high values for the Young's modulus (E), followed by a deformation stage with a continuous decrease in the stress-strain slope. A second constant slope tends to be reached at the end of the plastic deformation stage. All the curves eventually reach a break point, leading to values for the maximum stress ( $\sigma_{max}$ ) and the strain at break ( $\epsilon_{max}$ ). All values are summarized in the Figure 21.

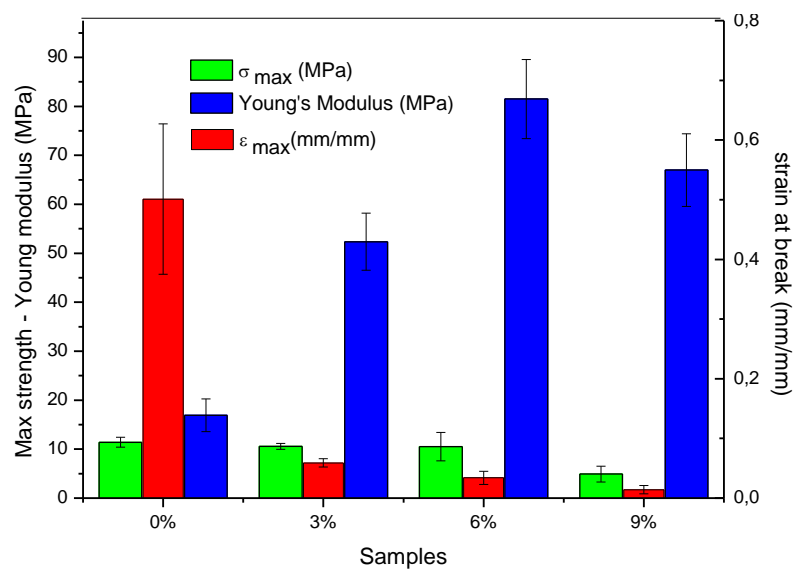


Figure 21: Different parameters of tensile strength measurements: max stress ( $\sigma_{max}$ ), strain at break ( $\epsilon_{max}$ ) and Young's modulus for all bioplastics materials (SPI, 3, 6, 9%).

The values of maximum force and strain at break tend to a decrease with the concentration of nanoclay, which is particularly dramatic for the later parameter. However, the value of Young's modulus increases significantly (except for the nanoclay concentration of 9%). This means that the resistance to tensile strain increases with the increase of nanoclay concentration but the material becomes more fragile. As a consequence, an increase in nanoclay concentration leads to bioplastic materials that are more rigid but show lower toughness or ability to absorb energy and plastically deform without fracturing.

#### 4.3.1.2 Dynamic Strain Sweep Tests

Strain sweep tests were carried out with two different temperatures, one at 20 °C and another at 80 °C (data not shown). However, this test allows doing a curve stress-strain (Figure 22) in order to determinate critical stress and critical strain ( $\gamma_c$ ). The results are below.

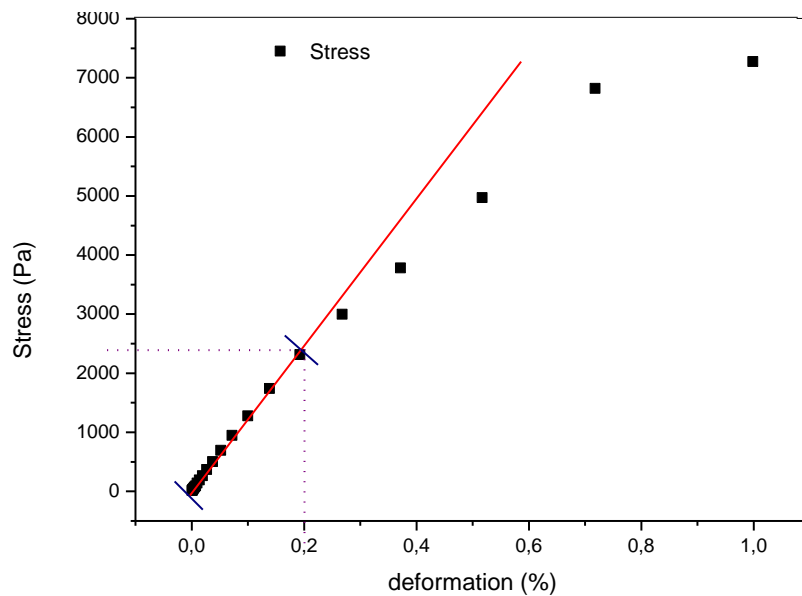


Figure 22: Strain-stress relation with linear regression from dynamic strain sweep test for system without nanoclay

Figure 22 represents the change of the strength in function of the strain. The first part of the curve shows a linear progression. The red line corresponds to the linear regression with correlation coefficient  $R^2 = 0.999$ . The last points of the linear regression are called critical stress and critical strain ( $\gamma_c$ ). The latter values are shown in the Table 5.

Results exhibit that when the concentration of nanoclay increases, the critical strain decreases. It is clear that increasing the concentration of nanoclay lead to more fragile

material. But when the temperature increases, better viscoelastic range is observed. As previously, 6% and 9% show similar properties

From those values, a strain has been selected below the critical strain in order to perform frequency sweeps and temperature ramp.

	$\gamma_c\%$ (20°C)	$\gamma_c\%$ (80°C)
0%	0.37	0.72
3%	0.27	0.37
6%	0.05	0.19
9%	0.05	0.14

Table 5: Critical strain at 20°C and 80°C for all bioplastics materials (SPI, 3, 6 and 9%)

#### 4.3.1.3 Dynamic Frequency Sweep Tests

Figure 23 shows results from frequency sweep tests for all injection-molded bioplastic systems (0%, 3%, 6% and 9% nanoclay).

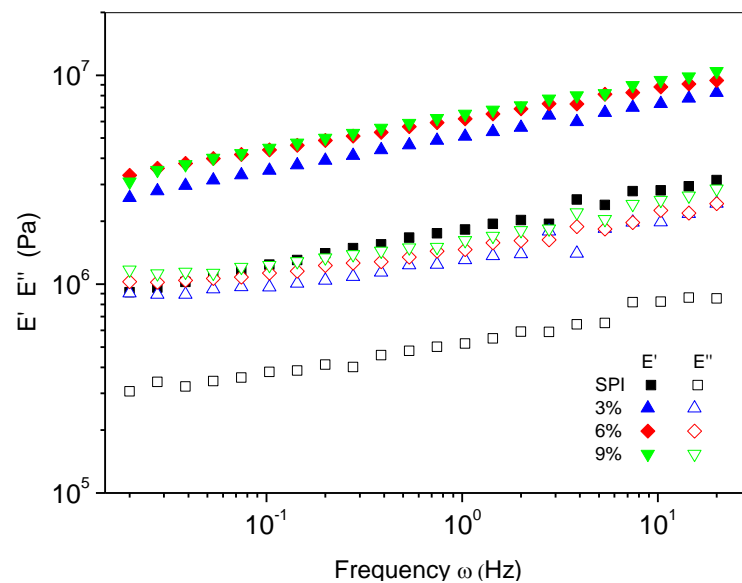


Figure 23: Dynamic frequency sweep test for all systems (0%, 3%, 6% and 9% of nanoclay).

It is possible to observe that  $E'$  is always superior than  $E''$  regardless of the frequency. This elastic predominant behavior takes place for all samples. Moreover, the values of  $E'$  and  $E''$  are constantly increasing with the raise of frequency.

It is interesting to observe that the addition of nanoclay (from 0 to 3%) yields a remarkable increase in both viscoelastic functions. A further but moderate increase takes place with increasing nanoclay content. However, there is a limit of concentration from which the properties are very similar without any change. This concentration corresponds to 6% since it is not possible to distinguish between 6% and 9% nanoclay samples with superimposed profiles.

The evolution described below can be observed in Table 6

	$E'$ (1 Hz) (MPa)	Tan $\delta$ (1 Hz)
0%	0.9	0.284
3%	2.0	0.256
6%	5.1	0.236
9%	5.5	0.240

Table 6:  $E'$  and Tan  $\delta$  values for frequency sweep tests for all systems (0, 3, 6 and 9% of nanoclay)

#### 4.3.1.4 Temperature ramp

Figure 24 shows the evolution of the elastic and viscous moduli ( $E'$  and  $E''$ ) as a function of temperature during bending tests.

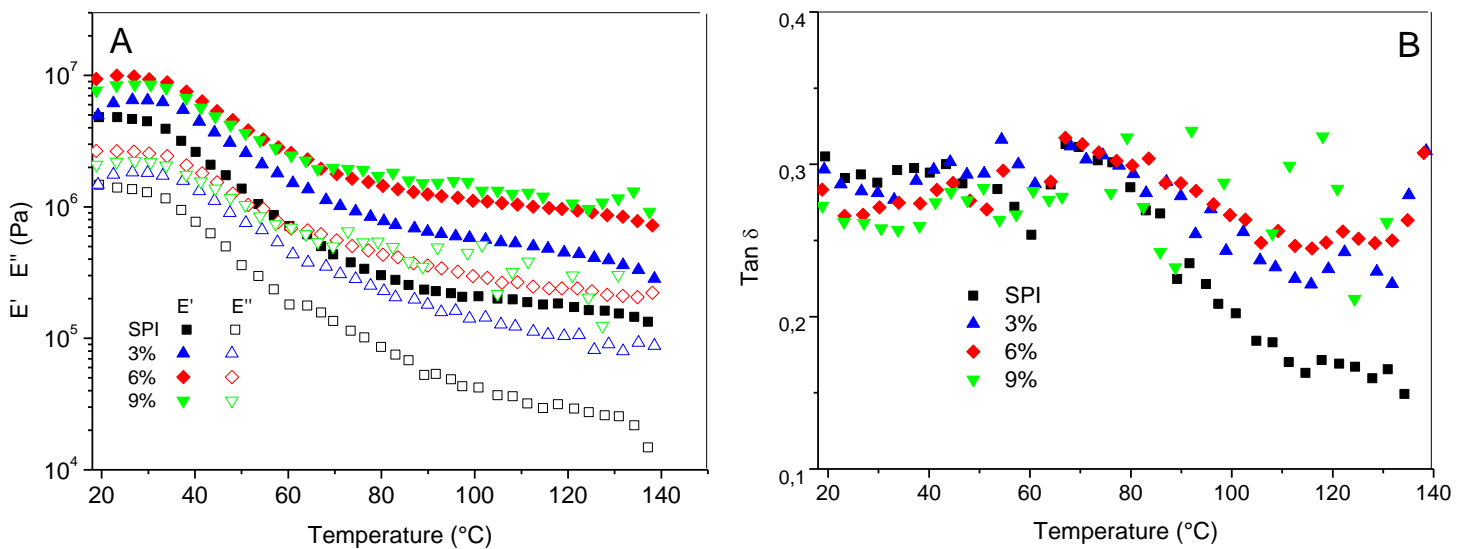


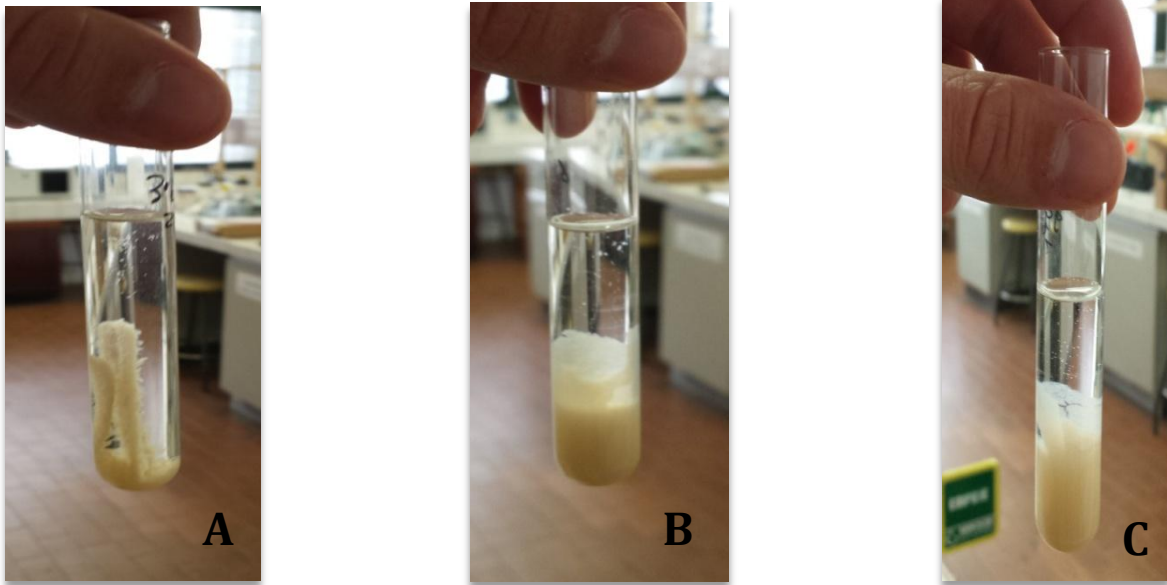
Figure 24: Temperature ramp for all systems: Evolution of (A) elastic and viscous modulus ( $E'$  and  $E''$ , respectively) and (B) loss tangent ( $\tan \delta$ ).

Figure 24 shows how the values of  $E'$  and  $E''$  change with temperature for the four bioplastic systems studied (0, 3, 6 and 9%). All systems exhibit the same behaviour, where the

elastic component ( $E'$ ) is always above the viscous one ( $E''$ ), as may be deduced from the values of  $\tan \delta$ , with both modulus decreasing markedly with temperature. However, 6% and 9% properties are very similar, because the two profiles are mingled. It is easy to separate the concentration in 3 categories: 0, 3% and 6-9%. Once again, it is confirmed that exists a limit concentration to improve material properties.

#### 4.3.1.5 Water uptake capacity

The result of material immersion in water is shown in figure 25.



*Figure 25: Pictures correspond to 3% (A), 6% (B) and 9% (C) bioplastics materials in water after 24h.*

It is observed that the disaggregation of material after immersion in water for 24h increases with the concentration of nanoclay due to its hydrophilic properties. The following Figure (Figure 26) represents all systems after freeze-drying except 0% because the material did not break down in water (Figure 27). Figure 28 reveals the water uptake capacity of all systems.

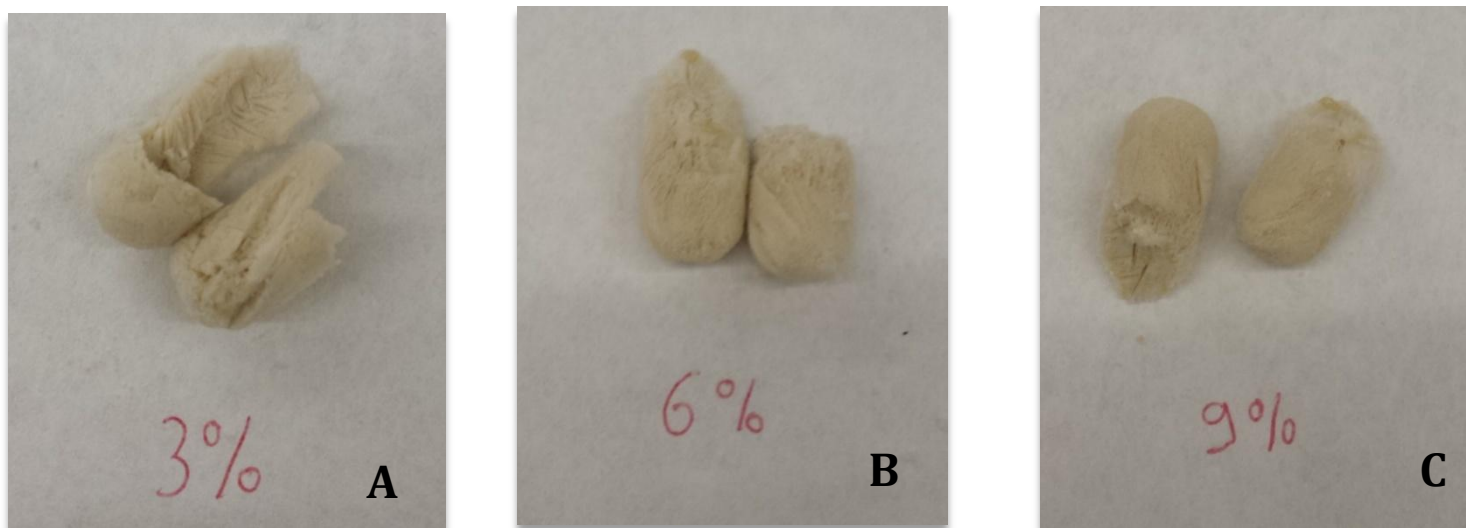


Figure 26: Pictures correspond to 3% (A), 6% (B) and 9% (C) bioplastics materials in water after freeze drying.



Figure 27: Picture corresponds to a nanoclay-free bioplastic in water after 24h.

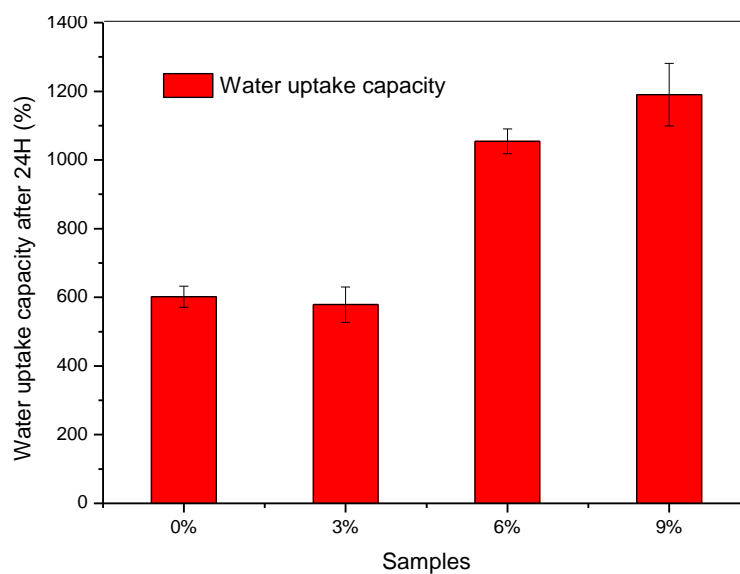


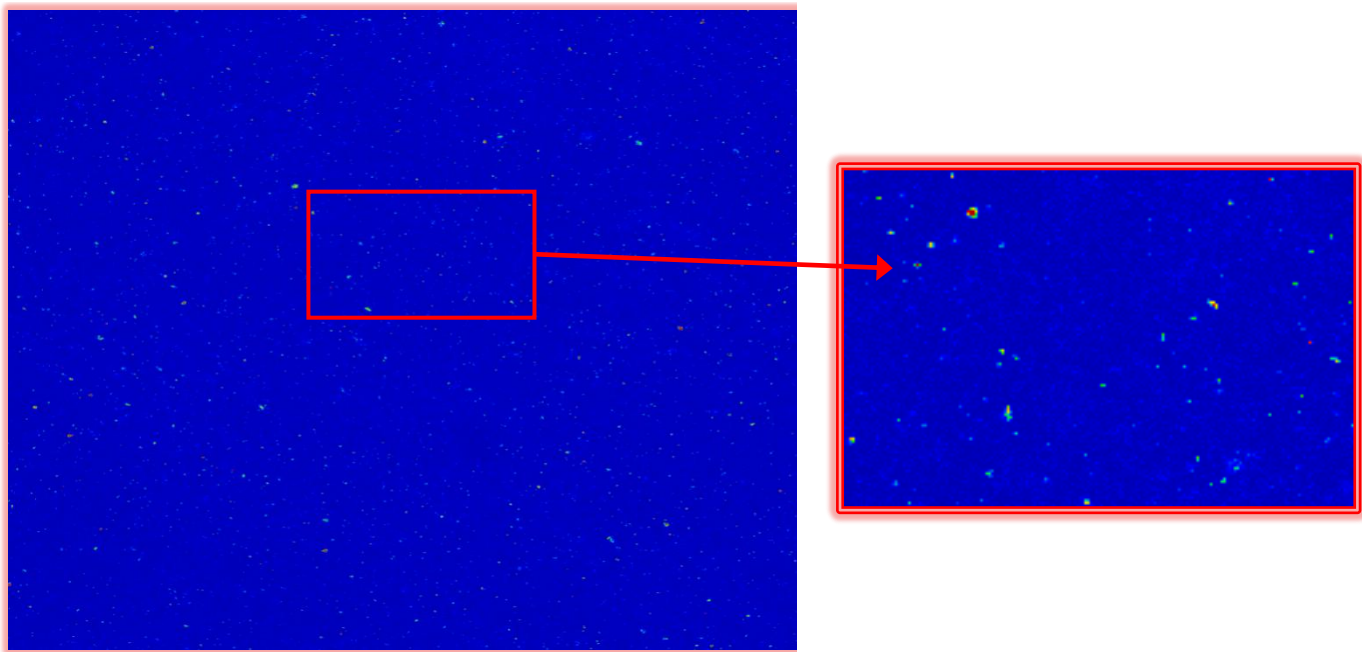
Figure 28: Water uptake capacity after 24h for bioplastics materials (0, 3, 6 and 9%).



Figure 28 shows that the water uptake generally increases with the concentration of nanoclay. But when the water uptake between 0 and 3% sample is compared, the values are similar, maybe because the glycerin is also hydrophilic and the concentration of nanoclay is too low compared to the quantity of plasticizer. The value can reach more than one thousand percent for 6 and 9% (1054% and 1190%, respectively). It is noticed that between 6% and 9% the improvement is moderate but significant, taking into account the standard deviation.

#### 4.3.1.6 CONFOCAL MICROSCOPY

Confocal microscopy has been done by reflection in order to analyze the material. The first picture (Figure 29) shows a dispersion of 9% nanoclay in water. The second one (Figure 30) corresponds to a bioplastic with 9% nanoclay.



*Figure 29: CLMS refraction images for 9% nanoclay dispersion in water  $\times 20$  (left)  $\times 40$  (right)*

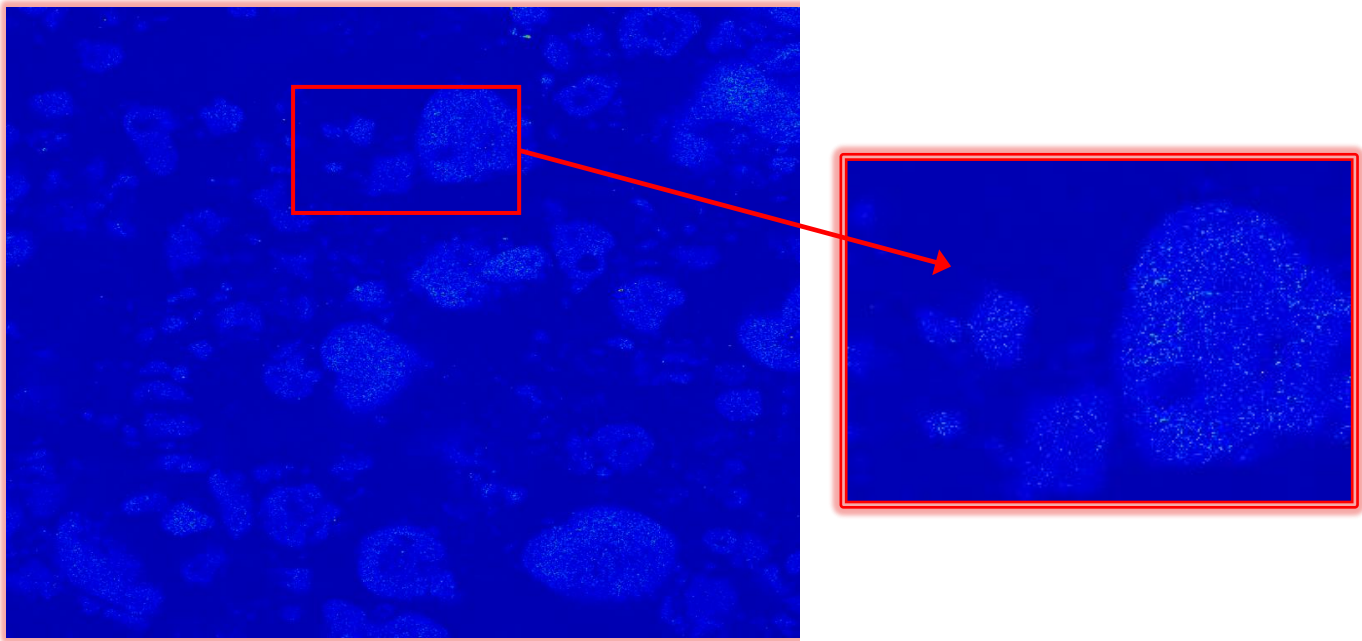


Figure 30: CLMS refraction images for bioplastics with 9% of nanoclay  $\times 20$ (left)  $\times 40$  (right)

Figure 29 shows transmission with a more or less homogeneous distribution and large particles compared to Figure 30 where particles appear smaller and integrated within the matrix.

#### 4.3.1.7 SEM Microscopy

Microscopy tests were performed in order to analyze the structure of the bioplastics. The following Figures (Figures 31-34) show the microstructures at different resolutions of the systems after spending 24 h in water and then freeze-dried.

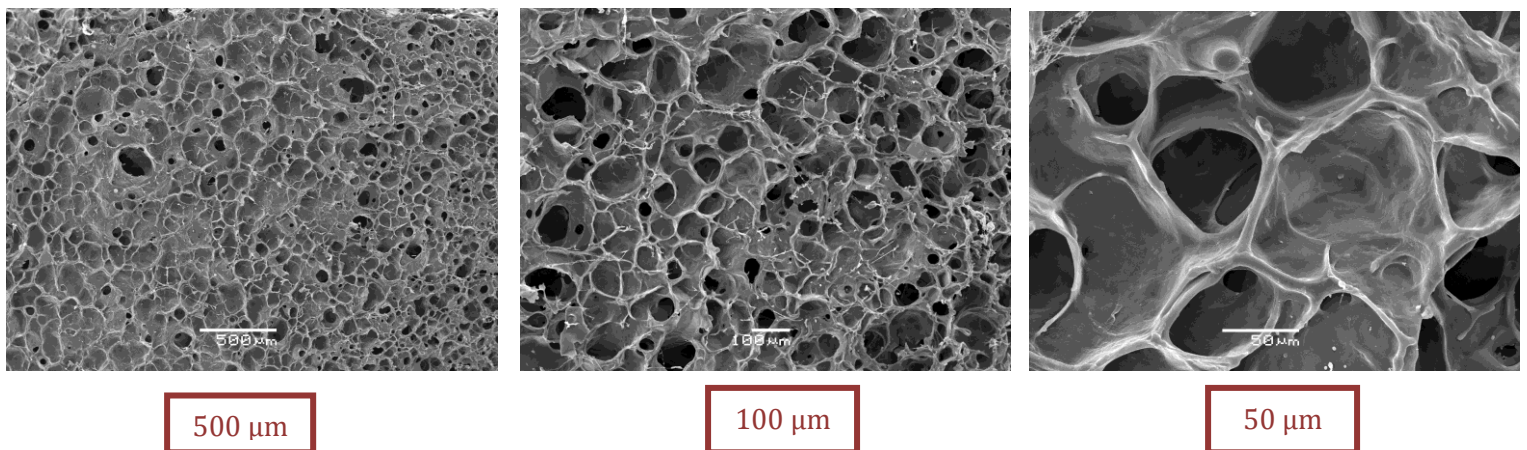


Figure 31: SEM images of nanoclay-free bioplastics

Figure 31 shows a structure with porous and quite different from other samples. At a small scale, it is seen that the protein forms a 3-dimensional matrix.

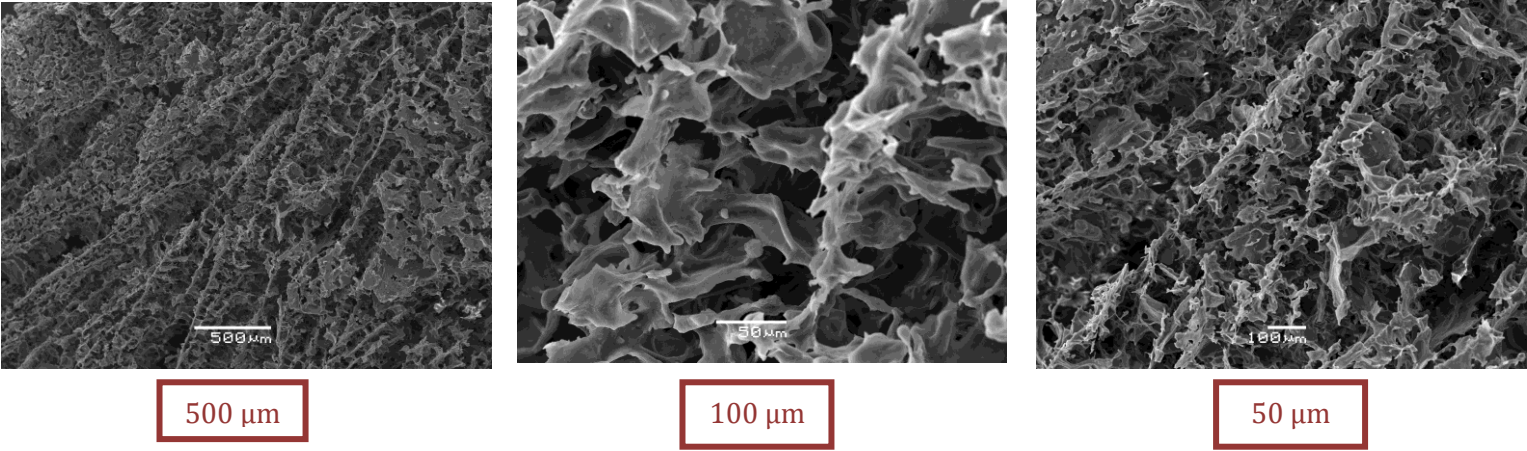


Figure 32: SEM images for bioplastics containing 3% nanoclay

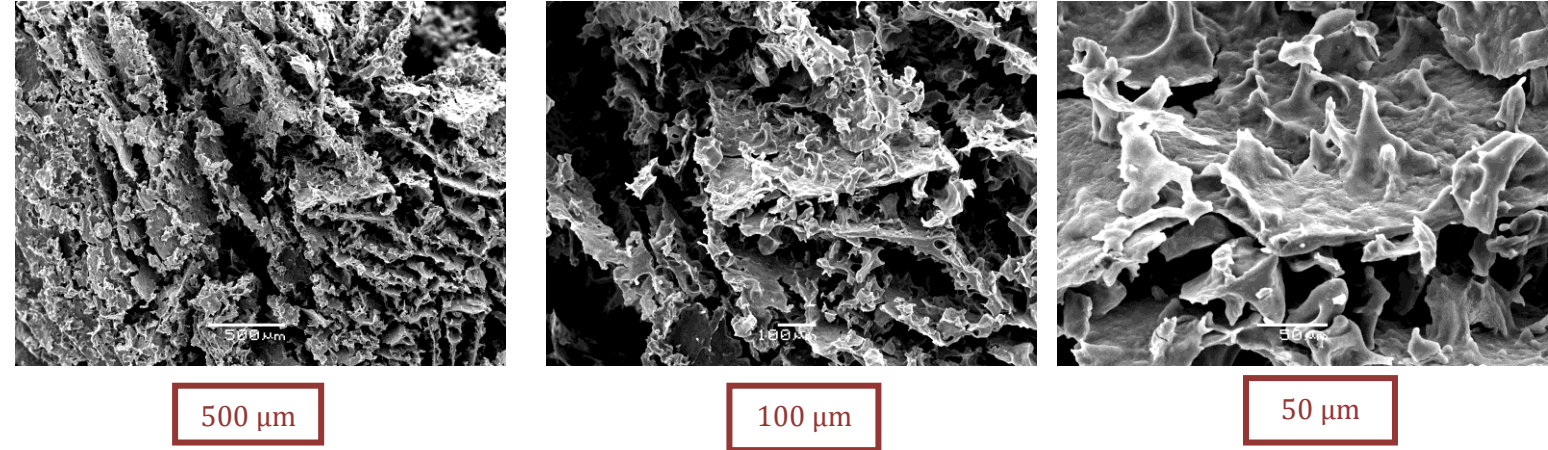


Figure 33: SEM images for bioplastics containing 6% nanoclay

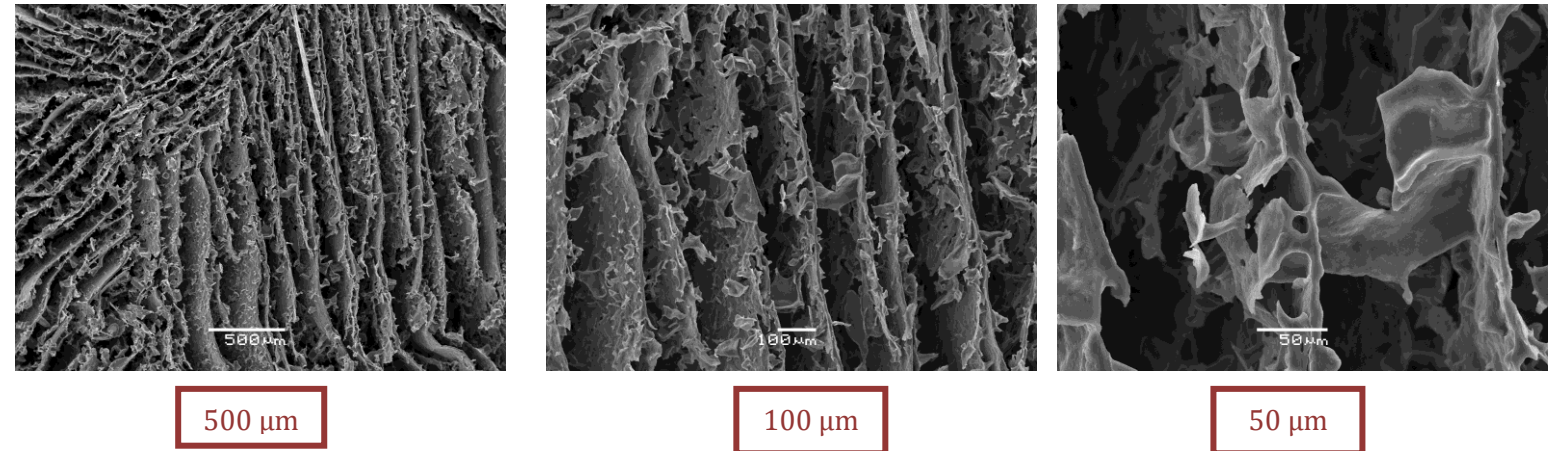


Figure 34: SEM images for bioplastics containing 9% nanoclay

Figures 32, 33 and 34 (bioplastic systems with 3%, 6% and 9% nanoclay, respectively) showed similar characteristics with laminar structure, although it is clearer when the concentration of nanoclay is the highest. In fact, the structural differences between the samples may explain some of the measured properties. Indeed, it is observed for the nanoclay-free system that the pores are able to deform easily without breaking when elongation tests are carried out. This is not the case for the samples containing nanoclay. The presence of nanoclay seems to promote the formation of a laminar structure.

#### 4.3.2 INFLUENCE OF PROCESS CONDITIONS

This part focuses on the difference in properties caused by different process conditions, especially mixing time and injection pressure which are key parameters.

##### 4.3.2.1 TENSILE STRENGTH MEASUREMENTS

Figure 35 shows the tensile strength test results for a system at 3% of nanoclay at different processing conditions evaluated for SPI.

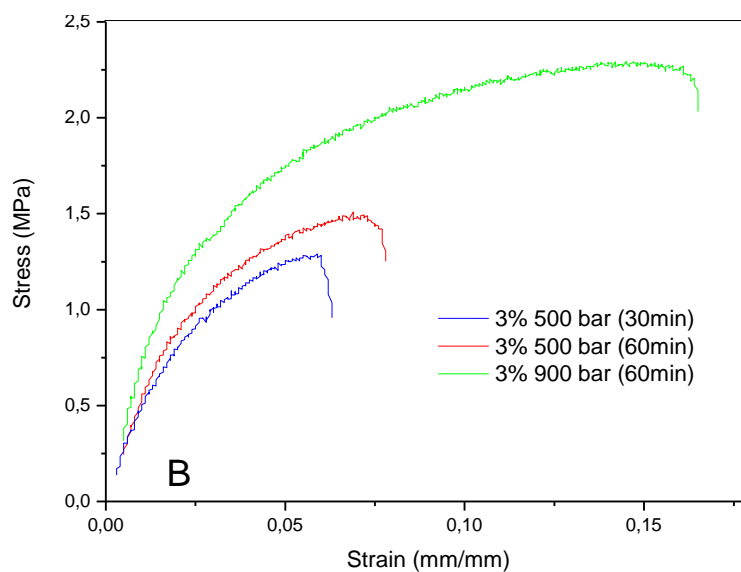


Figure 35: Stress-strain curves for different process conditions of mixing time and pressure for bioplastics containing 3% nanoclay

Figure 35 shows that both increasing mixing time and injection pressure clearly improves the tensile properties of bioplastic materials. Figure 36 represents the values obtained for different parameters of the test performed over bioplastic containing 3% nanoclay, compared with those obtained for the nanoclay-free bioplastic.

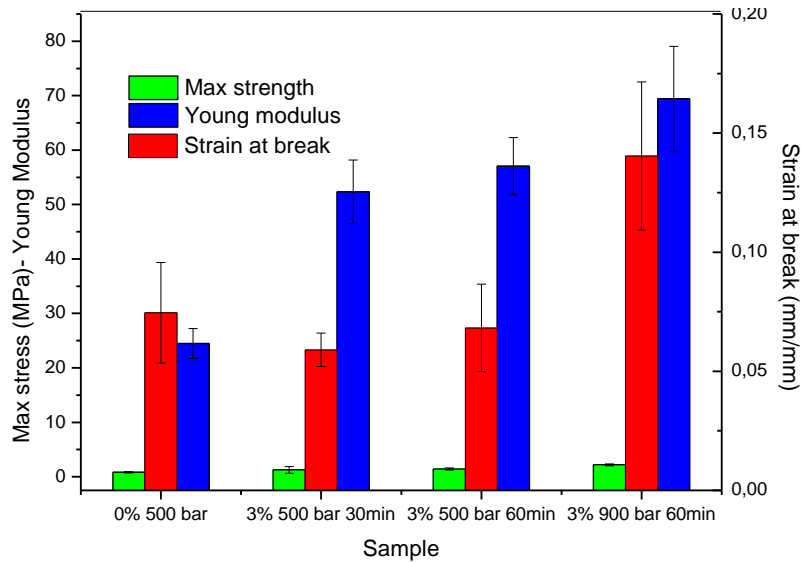


Figure 36: Different parameters of tensile strength measurements: max stress ( $\sigma_{max}$ ), strain at break ( $\epsilon_{max}$ ) and Young's modulus for all bioplastics materials (0% 500 bar, 3% 500 bar (30min), 3% 500 bar (60min), 3% 900 bar (60 min)).

Improving injection pressure from 500 to 900bar seems to have good consequences on tensile properties. Indeed, regarding Figure 36 exhibits that the sample injected at 900 bar shows values correspond to strain at break two times higher than 500 bar one. The strain at break increases by 102% and the maximum stress by 52%. The Young's modulus value is the same for the same mixing time but increases with the injection pressure. As matter of fact, the mixing time does not have any significant influence of tensile properties. However, the increase in the injection pressure shows better results. It is worth mentioning that it is rather difficult to find variables such as the injection pressure that are able to simultaneously improve the Young's Modulus and the strain at break.

#### 4.3.2.2 Dynamic Strain Sweep Test

Strain sweep test were carried out as previously with two different temperatures (20 and 80 °C) and results correspond to the critical strain ( $\gamma_c$ ) can be observed in the Table 7.

	$\gamma_c$ (20°C)	$\gamma_c$ (80°C)
SPI 500 bar	0.19	0.52
3% 500 bar 30min	0.27	0.37
3% 500 bar 60min	0.19	0.37
3% 900 bar 60min	0.37	0.37

Table 7: Critical strain ( $\gamma_c$ ) of bioplastics with 0% and 3% nanoclay subjected to different processing conditions (time and pressure) at two temperatures (20 and 80)

Results show that the critical strain at 20°C depends on the mixing and injection conditions. In fact, an increase in injection pressure tends to enhance the critical strain whereas mixing time tends to lower critical strain. Nevertheless, at 80°C the process condition does not seem to have any influence because the critical strain reaches the same value for any sample except for 0% nanoclay.

### 4.3.2.3 Dynamic Frequency Sweep Test

Figure 37 shows the dynamic frequency sweep for bioplastics with 0% and 3% nanoclay subjected to different processing conditions (mixing time and pressure).

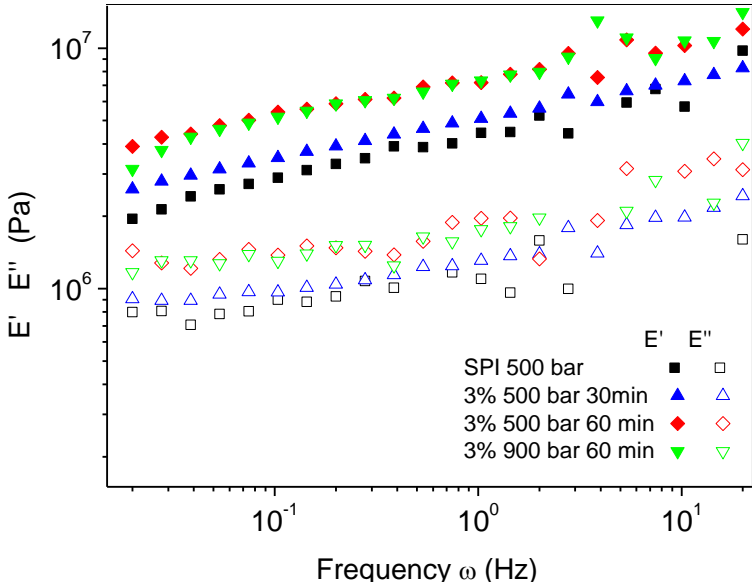


Figure 37: Dynamic frequency sweep test for of bioplastics with 0% and 3% nanoclay subjected to different processing conditions (time and pressure).

For each sample, the E' value is always higher than E'' and, consequently, the relation between both moduli ( $\tan \delta$ ) remains constant (data not shown). Bioplastics with longer mixing time lead to higher viscoelastic moduli. In addition, it is possible to make two categories, one with a mixing time of 30 min and other with a mixing time of 60 min. However, the injection pressure does not have any influence of the system. In fact, bioplastics containing 3% nanoclay injected at 500 bar behaves exactly as the system injected at 900 bar.

### 4.3.2.4 Temperature ramp

Figure 38 shows the evolution of elastic and viscous moduli (E' and E'') as a function of temperature during bending tests.

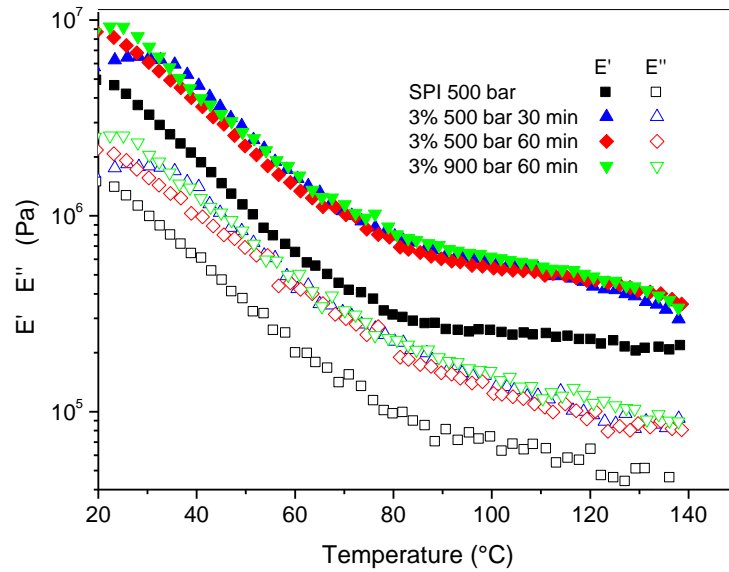
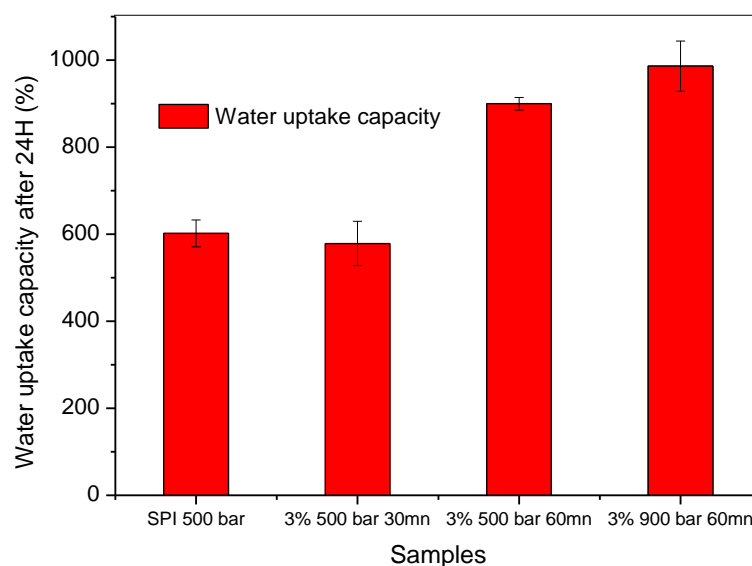


Figure 38: Temperature ramp for of bioplastics with 0% and 3% nanoclay subjected to different processing conditions (time and pressure).

$E'$  and  $E''$  modulus values decrease with the increase of temperature but  $E'$  exhibit still higher values than  $E''$ , preserving good elastic properties with  $\tan \delta$  always lower than 1 and a maximum corresponding to temperature transition at 70°C for all the systems (data not show). In addition, the temperature ramp tests show that neither the mixing time nor the injection pressure exerts any particular influences on viscoelastic properties.

#### 4.3.2.5 Water uptake capacity

Figure 39 shows the results of water uptake capacity for bioplastic systems with 0% and 3% nanoclay subjected to different processing conditions.



*Figure 39: Water uptake capacity after 24h results for bioplastics with 0% and 3% nanoclay subjected to different processing conditions (time and pressure).*

Regarding the mixing time effect, it shows that an increase of mixing time improves the water uptake capacity, which grows approximately by 50%. The water uptake value can reach 900% which means that those soy based bioplastics show really good water absorption.

By analyzing the injection pressure effect, there is also a change. However, this change is not so important. As a conclusion, mixing time is the variable showing the most apparent impact on water absorption capacity.



## 5- CONCLUSION

### 5.1 GENERAL CONCLUSION

Soy protein based bioplastics has been performed by means of thermomechanical process which consists of a mixing process with plasticizer and an injection molding stage by using the most appropriate pressure, temperature and time. The study reveals that nanoparticles were well integrated into the polymer matrix and consequently increase water absorption properties.

### 5.2 SPECIFIC CONCLUSION

This study shows how mixing stage conditions are important to get appropriate and homogenous dough. Indeed, self-heating need to be avoided in order to prevent cross linking reactions and structuring of the material inside the equipment. The mixing time is a key factor because, if the time is too small, the dough will be not homogenous, if it is too high, the protein will start to polymerize and create a polymer matrix. It will be not possible to inject the dough under such conditions.

Optimum injection molding process has been determined by using previous studies and rheological studies. The injection temperature was selected by analyzing the material with temperature ramp. A temperature of 70 °C was chosen in the chamber corresponding to the glass transition which enables polymerization in the mold.

It is noticed that the water absorption of those bioplastics is very high (more than 1000% for some of them). This water uptake capacity is due to the presence of glycerin and nanoclay which shows excellent hydrophilic properties. Maybe, they can be used as potential sources of absorbent material in food industry for example.

Confocal microscopy reveals the good integration of the nanoclay inside the polymer matrix whereas Scanning Electron Microscopy shows the different structure of the sample which can explain the different behaviour between polymer with and without nanoclay.

In a nutshell, the different processing conditions influence a lot the final result. Indeed, choosing different concentration of nanoclay, mixing time or injection pressure leads to bioplastics with different physicochemical, mechanical and rheological properties. It can target different applications

Influence of concentration: Increasing the proportion in nanoclay inside the protein, increase the hydrophilic character and shows water absorption properties close to superabsorbent. Moreover increasing the nanoclay concentration reduces tensile properties (lower maximum stress and strain at break values), but creates a material more rigid (with higher Young's Modulus). No difference of water absorption and mechanical properties was observed from 6% of nanoclay concentration.

Influence of injection pressure: an increase of injection pressure (here from 500 bar to 900 bar) induces an increase in tensile properties (strain at break enhanced by 100% and maximum stress by 52%). However, the injection pressure exerts no influence on the bending properties and only slight differences on water absorption properties.

Influence of mixing time: Contrary to expectations, changing the mixing time has a huge impact on the final bioplastics. Water uptake capacity is greatly improved. Moreover, even if the tensile properties are not really improved (Young's modulus is similar, strain at break and max strength increased by 15%), it leads to an increase in the bending properties ( $E'$ ) when the frequency increases.

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